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Folic Acid Fortification of Double Fortified Salt

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1 **Abstract**

2 The addition of folic acid to Double Fortified Salt aims to ameliorate iron, iodine, and folic acid
3 deficiencies in vulnerable populations. Folic acid was added either to the iodine spray solution or
4 to the iron premix used to prepare salt fortified with iron and iodine. When added through the
5 solution, sodium carbonate was needed to dissolve folic acid and to adjust pH. Alternately, folic
6 acid was added either to the iron core or sandwiched between the core and the TiO₂ layer of the
7 ferrous fumarate premix. Folic acid and iodine were stable in all three cases, retaining more than
8 70% of the added micronutrients after six months at 45 °C/ 60-70% RH. Adding folic acid to the
9 premix's iron core is preferred as folic acid retention was slightly higher, and the added folic acid
10 did not impact a yellow colour to the salt. Folic acid in this salt was stable in cooking and did not
11 affect selected cooked foods' sensory properties. The technology is a cost-effective approach for
12 simultaneously combating iron, iodine, and folic acid deficiencies.

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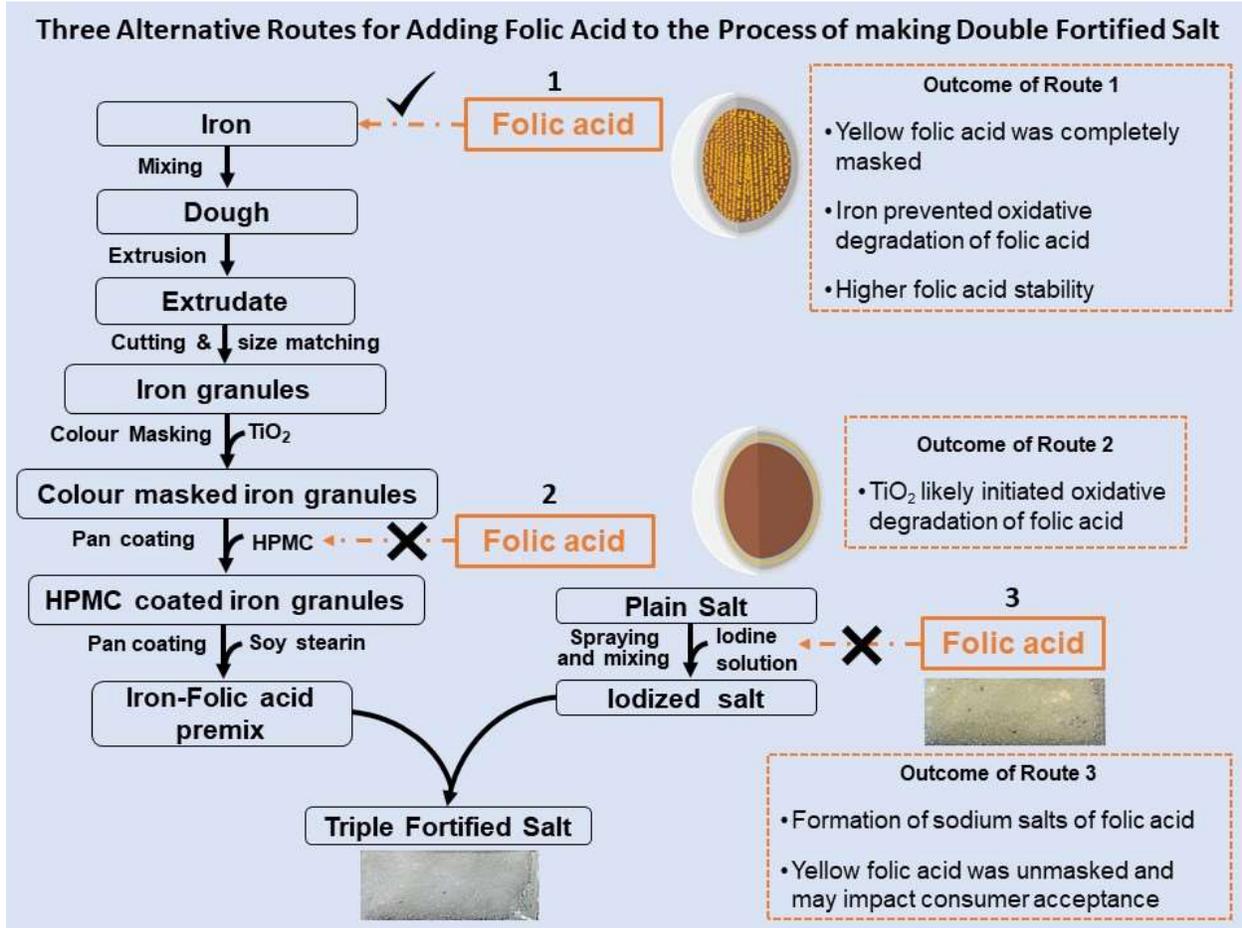
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17 **Keywords:** Folic acid; Iron; Iodine; Salt fortification

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19 **Graphic Abstract**



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24 INTRODUCTION

25 Vitamin B₉ plays a vital role in the metabolism of nucleic acids and proteins (Institute of Medicine
26 Standing Committee on the Scientific Evaluation of Dietary Reference, 1998; Mahmood, 2014).
27 Its deficiency is a major cause of neural tube defects (Guilland and Aimone-Gastin, 2013). The
28 deficiency of vitamin B₉ also causes cancer, cardiovascular diseases, and impaired cognitive
29 function. Folic acid is the synthetic form of the naturally existing folate (vitamin B₉). It only differs
30 from folate in that it has only one glutamic acid moiety. This difference makes folic acid more
31 stable and readily bioavailable (Scaglione and Panzavolta, 2014).

32 The increasing prevalence of neural tube defects, a direct consequence of vitamin B₉ deficiency in
33 pregnancy, led many developed countries, including Canada and the USA, to mandate the
34 fortification of flour with folic acid (Williams *et al.*, 2015). This action has resulted in a drastic
35 reduction in the prevalence of neural tube defects in those countries (Jägerstad, 2012).
36 Unfortunately, large rural populations in developing countries do not have access to or cannot
37 afford these fortified foods. Therefore adding folic acid to table salt that is ubiquitously consumed
38 has an immense potential public health benefit.

39 The addition of folic acid to the Double Fortified Salt will be more economical and allow
40 simultaneous amelioration of iron, iodine, and folic acid deficiencies that can prospectively impact
41 the health and or growth of reproductive women and children. The existing technology for adding
42 micronutrients to salt involves adding some micronutrients as a premix, while others are sprayed
43 as a solution on salt (Rao, 1994; Zimmermann *et al.*, 2004b; Zimmermann *et al.*, 2004a; Diosady,
44 2007). With the technology developed at the University of Toronto for the double fortification of
45 salt with iron and iodine, ferrous fumarate, as the iron source, was added to salt as an extruded
46 and microencapsulated particle that matches the size and colour of salt; a solution of potassium

47 iodate, the iodine source, was sprayed unto the salt (Li, Diosady and Wesley, 2010). This
48 technology provides two potential paths for which folic acid can be added to salt - either through
49 the extruded iron microcapsule or the iodine spray solution.

50 Folic acid can impart a bright yellow colour to salt if added directly with the iodine solution
51 (Modupe, Krishnaswamy and Diosady, 2019). Additionally, since folic acid is only sparingly
52 soluble in water, a solvent system is thus required to dissolve a high concentration of folic acid.
53 Folic acid is susceptible to oxidative degradation (Gazzali *et al.*, 2016; Modupe, Krishnaswamy
54 and Diosady, 2019). While potassium iodate has oxidative potential, ferrous fumarate has
55 reductive potential. It was crucial to investigate how these constituents of Double Fortified Salt
56 would affect folic acid stability and, ultimately, establish how folic acid is best added to salt.

57 McGee *et al.* (McGee, Sangakkara and Diosady, 2017) described a process for adding iodine and
58 folic acid to salt, where 1-3g folic acid and iodine were dissolved in a 100 mL bicarbonate buffer
59 solution adjusted to pH 9. The solution was sprayed on the salt so that the concentrations of iodine
60 and folic acid in the salt were 50ppm each. Modupe *et al.* (2019) also described the addition of
61 iron, iodine, and folic acid to salt. While iodine and folic acid were added as a solution sprayed
62 unto salt, iron was added as an extruded and encapsulated ferrous fumarate. A buffer solution was
63 not used; instead, 1% folic acid and 2% iodine were dissolved in 0.1M sodium carbonate solution.
64 The solution (5 mL) was sprayed on 2kg salt mixed with a ribbon blender. The salt was air-dried
65 and mixed with the microencapsulated ferrous fumarate (10g).

66 The present study was carried out to evaluate the methods of adding folic acid to Double Fortified
67 Salt. The study's objective was to develop Triple Fortified Salt (TFS) that retains at least 70% of
68 the added micronutrients for at least six months, without significantly impacting salt or cooked
69 foods' sensory properties.

70 **MATERIALS AND METHODS**

71 **Materials**

72 Ferrous fumarate (food grade) was obtained from *Dr. Paul Lohmann Chemicals (Emmerthal,*
73 *Germany)*; soy stearin (SS) was obtained from *JVS Food Pvt, (India)*; hydroxypropyl
74 methylcellulose (HPMC) was obtained from *Dow Chemical Company (Midland, Michigan USA)*;
75 titanium (IV) oxide was obtained from *ACROS Organics (Fair Lawn, New Jersey, USA)*; folic acid
76 was obtained from *Bulk Pharmaceuticals Inc., (Toronto, Ontario, Canada)*; potassium iodate was
77 obtained from *Sigma–Aldrich Chem (Oakville, Ontario, Canada)*; absolute ethanol and
78 dichloromethane were obtained from *Thermo Fischer Scientific (Mississauga, Ontario, Canada)*;
79 sodium carbonate, potassium iodide and starch indicator were obtained from *Caledon Laboratory*
80 *Ltd (Georgetown, Ontario, Canada)*; sulfuric acid was obtained from *EMD Chemicals Inc.*
81 *(Oakville, Ontario, Canada)*; and 1.0% sodium thiosulfate solution was obtained from *VWR*
82 *International, (Mississauga, Ontario, Canada)*; vegetable shortening, semolina, rice grains and
83 ingredients used for cooking were obtained from *Walmart (Toronto, Ontario, Canada)*.

84 All chemicals used for the fortification of salt were food-grade, while those used for analysis were
85 ACS grade.

86 **Formulation of Spray Solutions**

87 **Iodine Solution**

88 A potassium iodate solution (3.37 % w/v), containing 2% w/v iodine, was prepared in a 100mL
89 volumetric flask with RO water.

90 **Folic acid and Iodine Solution**

91 Solutions of folic acid and iodine (1-3 % w/v) were prepared with sodium carbonate buffers (0.1-
92 0.3M); the impact of sodium carbonate buffer concentration on folic acid solubility was

93 investigated. The second set of three solutions contained 1% folic acid + 1% iodine, 2% folic acid
94 + 2% iodine and 1.8% folic acid + 3% iodine. The solutions were formulated with 0.2M sodium
95 carbonate buffer, and the stability of iodine and folic acid was monitored for 2 months. The third
96 set of solutions containing 0.5-1% w/v, folic acid, and 2% w/v iodine was made using sodium
97 carbonate solution (0.1M) instead of the carbonate buffer. The sodium carbonate solution was used
98 to adjusted the pH of the solution from 7-10. The pH of the solutions was measured with a VWR
99 Scientific Model 8000 pH meter.

100 The spray solutions were tightly secured in scintillation vials and were stored in temperature-
101 controlled incubators (25, 35, 45 °C). The stability of iodine and folic acid in the spray solutions
102 were monitored for two months. The stability of iodine and folic acid in the solution was expressed
103 as a percentage of the micronutrients in the freshly prepared solution. Given the precipitation of
104 folic acid from some higher folic acid concentration solutions, the 2% iodine and 1% folic acid
105 solution adjusted to pH 9 with sodium carbonate solution was used for salt formulation.

106 **Formulation of Micronutrient Premix**

107 The iron premix was formulated as described by Li et al. (Li *et al.*, 2011) and Modupe et al. (2019).
108 Ferrous fumarate (800g) was thoroughly mixed with semolina (200g), vegetable shortening (25g),
109 and water with a Kitchen Aid Mixer. The dough was preconditioned for 2-3 hours. The dough was
110 extruded with a La Monferrina P12 pasta extruder. The extrudate was cut, and size screened to
111 match the size of salt (300-600 µm). The extrudate was colour masked by dusting titanium dioxide
112 (15% w/w) on the surface of the extrudate and finally coated with 5% (w/w) HPMC and 5% (w/w)
113 soy stearin, respectively.

114 Two models were designed for the iron-folic acid premix: (a) the coextrusion of iron and folic acid
115 such that both iron and folic acid were in the core of the premix, and (b) the sandwiching of folic

116 acid between titanium dioxide layers such that the iron core was separated from the folic acid. For
117 iron and folic acid coextrusion, the same process described for making iron premix was followed.
118 For this purpose, 7g of folic acid was added and mixed with the dough before preconditioning and
119 extrusion. In the second design, the process of making iron premix was followed until the colour
120 masking step. There were two colour masking steps before the coating steps - the first colour
121 masking step created a thin layer between iron extrudate and the folic acid layer, and the second
122 colour masking step masked the yellow colour of folic acid. After the first colour masking step,
123 either a homogenous suspension of folic acid (0.52% w/v) in a 2.5% w/v HPMC (dissolved in a 1:1
124 absolute ethanol: dichloromethane solvent) was sprayed on the TiO₂ masked iron extrudate
125 tumbling in a pan coater or a suspension of folic acid in water was mixed thoroughly with colour
126 masked iron extrudate. The iron-folic acid particles obtained from the two approaches were again
127 colour-masked and coated with HPMC and soy stearin, as described earlier.

128 **Formulation of Fortified Salt**

129 Non-iodized refined salt (about 400 µm diameter), obtained from *Sifto Canada Corp*, was mixed
130 in a ribbon blender to break any salt lumps. A micronutrient(s) solution, either of iodine or iodine
131 and folic acid (2.5mL/ kg salt), was sprayed onto the salt inside the ribbon blender and mixed. This
132 corresponds to 50ppm iodine and or 12.5-25ppm folic acid in the salt. The mixing operation was
133 stopped intermittently to remove any solution stuck to the blade of the blender. The salt was mixed
134 with the solution for about 20 minutes. The salt was then collected and spread on aluminum foil
135 for air drying.

136 The air-dried salt was mixed with iron or iron-folic acid premix. For a 2kg batch, 10g of the premix
137 was mixed with 1990g of salt in the ribbon blender. The mixing ensured uniform dispersion of

138 0.5% w/w premix in the salt. The iron premix concentration in the iron premix was about 20% w/w,
139 such that the formulated salt contained 1000ppm iron, 50ppm iodine, and 12.5-25 ppm folic acid.

140 **Handling and Storage of Fortified Salt**

141 The salt samples were divided first into four portions by a glass divider; the fourth portion was
142 shared over the other three portions. The glass divider ensured a randomized division of the salt
143 into the three portions. They were packed in Zip-Lock™ polyethylene freezer bags and stored at
144 incubators with controlled temperature and humidity (25, 35, and 45 °C; 60-70% RH). Also, the
145 incubator was in complete darkness so that the impact of light was minimized.

146 **Stability Study of Fortified Salt**

147 The stability of folic acid and iodine in some of the premix samples and each salt sample were
148 evaluated at 0, 0.5, 1, 2, 4, 6, and 12 months. The amount of iodine and folic acid in the salt was
149 reported as the percentage of the micronutrients in the freshly prepared samples. Iodine and folic
150 acid in the salt were quantified as described by Modupe et al. (2019) and Modupe et al. (2020),
151 respectively.

152 **Evaluation of the Colour of the Premix**

153 The colour of iron-folic acid premix was used to judge the homogenous distribution of folic acid
154 in the premix. The colour was observed from the image obtained with an iPhone 8 camera.

155 **Elucidation of the Chemistry Folic Acid in Spray Solution**

156 The mass spectrum of folic acid in sodium carbonate solution was obtained from Thermo Q-
157 Exactive Mass Spectrometer. The mass spectrum was obtained for folic acid in solutions that
158 contained just folic acid or folic acid and potassium iodate.

159

160

161 **Kinetic Study of the Degradation of Folic Acid and Iodine in Triple Fortified Salt**

162 For some salt samples, the amounts of micronutrients were determined at 0.5, 1, 2, 4, and 6 months
 163 of storage. The data obtained from these studies were used to extrapolate kinetic data of the
 164 degradation of micronutrients in the salt over the period. The expression of zero (Equation 1) and
 165 first-order kinetics (Equation 2) were used. The best fit was selected. The kinetic data obtained
 166 were validated with data obtained from a 12-month stability study. The same data was used to
 167 extrapolate the equation to predict micronutrients' long-term stability in the salt.

168 $[A]_t = -kt + [A]_0$ Equation 1

169 $\ln[A]_t = -kt + \ln[A]_0$ Equation 2

170 **Effect of Cooking on the Stability of Folic Acid**

171 The degradation of folic acid during cooking was evaluated. Boiled rice and Bondi raita (a yogurt-
 172 based Indian dish) were selected. A known amount (~ 4-5 g per kg food) of unfortified and fortified
 173 salt was used. The rice was boiled for 20 minutes while the fermentation of Bondi raita was allowed
 174 to continue for 30 minutes. Folic acid was extracted from the food and quantified by HPLC, as
 175 described in Table 1.

176 Table 1:HPLC Conditioned for Folic Acid and Vitamin B₁₂ Analysis

| | | | | |
|-------------------------|---|------------|--------|--------|
| Column | Kinetex, 2.6 μm, C18, 100 Å, LC column 100 x 4.6 mm | | | |
| Dimensions | 100 x 4.6 mm ID | | | |
| Elution Type | Gradient | | | |
| | Acetonitrile | | | |
| Elution B: | 20mM Phosphate buffer pH 3 | | | |
| Gradient Profile | Step No. | Time (min) | Sol. A | Sol. B |
| | 0 | 0.5 | 5 | 95 |
| | 1 | 5.0 | 25 | 75 |
| | 2 | 5.00 | 5 | 95 |
| | 3 | 5.00 | 75 | 25 |
| | 4 | 5.00 | 5 | 95 |
| Flow Rate | 1 mL/min | | | |
| Col. Temp | Ambient | | | |

| | |
|-------------------------|--|
| Detection | UV-Vis Abs.-Variable Wave.(UV) @ 278 & 360 nm (22°C) |
| Run time | 20 min |
| Injection volume | 20µL |

177

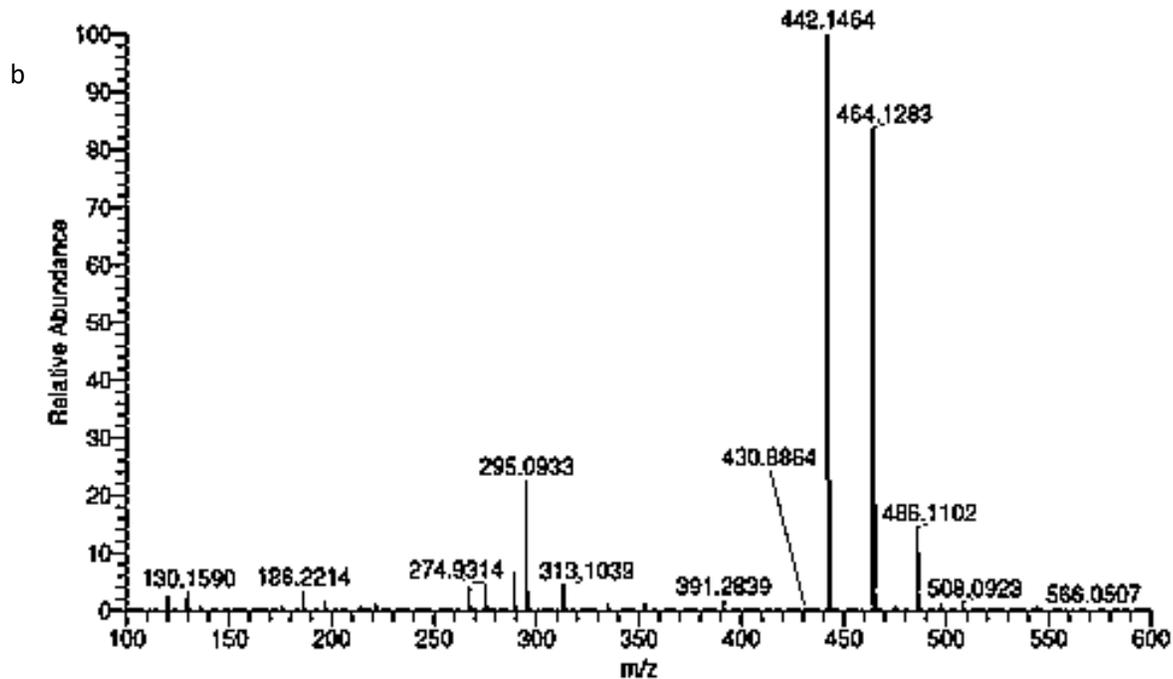
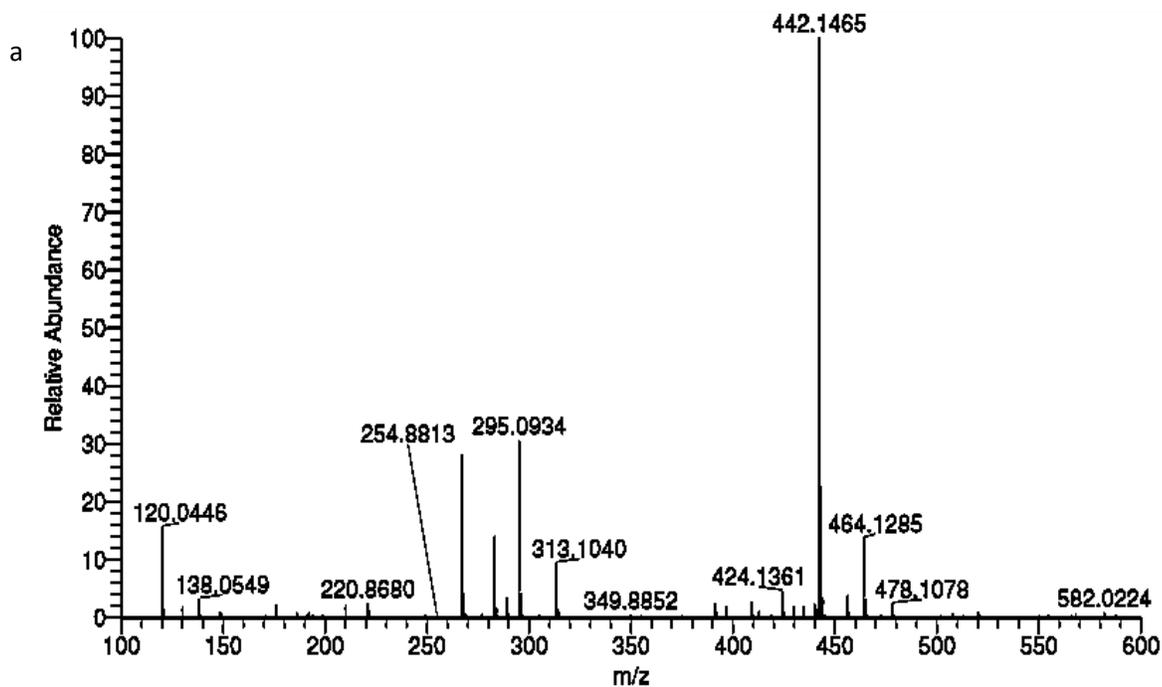
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179 **RESULTS AND DISCUSSION**

180 In the process described by Li et al. (Li, Diosady and Wesley, 2010) for the double fortification of
 181 salt, iodine as a solution of potassium iodate was sprayed onto the salt, and iron was added as
 182 extruded and microencapsulated ferrous fumarate. The addition of folic acid through the iodine
 183 solution was thought to be the easiest path for adding folic acid to salt. Hence, in a first step to
 184 making salt fortified with folic acid, a solution of folic acid and iodine was added to salt (McGee,
 185 2012). However, the salt's high moisture content (2.9%) due to the low concentration of folic acid
 186 and iodine (0.35%, each) in the spray solution accelerated iodine loss in the salt. Therefore, a
 187 higher concentration of folic acid and iodine (at least 1%, each) was formulated. With this spray
 188 solution, less solution was added to salt, resulting in low moisture content (0.06% or less).

189 **Preliminary Formulation of Spray Solution**

190 The impact of sodium carbonate buffer concentration on folic acid solubility in the spray solution
 191 was evaluated. The solubility of folic acid in the solution increased as the carbonate buffer
 192 concentration increased; at least 0.2M sodium carbonate buffer was required to dissolve 3% folic
 193 acid. The MS spectra of folic acid in the solution showed the formation of mono- and disodium
 194 salts of folic acid. The relative abundance of the disodium salt was higher in the solution that
 195 contained potassium iodate (Figure 1). The formation of sodium salt was responsible for folic acid
 196 solubility and explained why a higher concentration of sodium carbonate buffer is required to
 197 dissolve 3% folic acid.



198 Figure 1: Mass spectrum of folic acid in spray solution (a) folic acid in sodium carbonate solution
 199 (b) folic acid and potassium iodate in sodium carbonate solution

200 The stability studies carried out on the second set of solutions (that contained 1% folic acid + 1%
 201 iodine, 2% folic acid + 2% iodine, and 1.8% folic acid + 3% iodine) showed that both iodine and

202 folic acid were very stable in the solution. Less than 20% of the added micronutrients were lost
203 after 2-month storage, even at 45 °C. This result was consistent with those reported by McGee et
204 al. (2012; McGee, Sangakkara and Diosady, 2017). While increasing the iodine concentration
205 resulted in a significant increase in iodine and folic acid stability, increasing folic acid
206 concentration did not impact iodine and folic acid stability in any particular pattern. The relatively
207 higher folic acid stability observed in the solution that contained higher iodine (3%) may be due
208 to the formation of more disodium salt of folic acid. At the point of preparation, 0.2M sodium
209 carbonate buffer dissolved folic acid, irrespective of the amount of folic acid added (1, 1.8, or 2%
210 folic acid). However, in the solutions that contained more than 1% ^{w/v} folic acid, some of the folic
211 acids precipitated out from the solution, which can be a significant problem for small salt plants
212 that store their spray solutions for up to a month.

213

214 **Stability of Iodine and Folic Acid in the Optimized Spray Solution**

215 To prevent folic acid precipitation, the spray solution was reformulated, reducing the folic acid
216 concentration to 0.5-1%, and the buffer solution used in the previous method was replaced with a
217 sodium carbonate solution (0.1M). The solution of sodium carbonate itself is a buffer as it
218 dissociates to sodium and bicarbonate ions. The iodine concentration was maintained at 2% in the
219 spray solution to be consistent with industrial practice. The change from the buffer (prepared with
220 sodium carbonate and sodium bicarbonate) reduced the number of steps required for making the
221 spray solution. In all the samples, 70-100% of folic acid and iodine were retained after two months
222 of storage (

223

224 Table 2).

225

226

227 Table 2: Stability of Folic Acid and Iodine in the Optimized Spray Solution

| Types of Solution | Folic Acid | | | Iodine | | |
|-------------------|------------|----------|----------|-----------|----------|----------|
| | 25 °C | 35 °C | 45 °C | 25 °C | 35 °C | 45 °C |
| 0.5% FA | 82.4±3.4 | 82.0±1.9 | 68.8±6.7 | | | |
| 1% FA | 92.1±2.3 | 84.4±6.9 | 71.5±3.4 | | | |
| 0.5% FA + 2% I | 91.1±2.1 | 88.4±4.9 | 74.4±2.2 | 94.7±4.7 | 92.7±0.6 | 89.4±4.6 |
| 1% FA + 2% I | 88.7±4.7 | 89.3±2.6 | 80.2±5.2 | 96.5±4.0 | 94.4±1.0 | 88.6±2.7 |
| 2% I | | | | 100.0±1.0 | 96.5±5.1 | 93.1±1.2 |

228 FA=folic acid, I= iodine; the pH of the solutions were adjusted to pH 9

229

230 The concentration of folic acid in the spray solution did not significantly affect folic acid and
231 iodine stability in the spray solutions, except at 25 °C (

232

233 Table 2). The percentage of folic acid retained in the spray solution, initially containing 0.5% folic
234 acid, was significantly lower than other solutions. This trend was also reported by McGee et
235 al. (2017). As shown earlier, potassium iodate accelerated the formation of sodium salts of folic
236 acid in the solution. This may be responsible for the improved stability of folic acid in solutions
237 that contained potassium iodate.

238 pH had a significant effect on the solubility of folic acid in the KIO₃ solutions. After a few weeks,
239 folic acid precipitated out of the solutions at pH 7 and 8. This observation was consistent with the
240 study of Taub and Lieberman (1953), who found that folic acid solution at pH 6 turned cloudy
241 after a few days. Folic acid in solutions adjusted to pH 9 and 10 did not precipitate even after a
242 few months. Hence, the pH of subsequent solutions was maintained at ≥pH 9. In order to obtain
243 pH 9, 0.742g sodium carbonate, 3.37 g potassium iodate, and 1 g folic acid were dissolved in 100
244 mL of water.

245 **Stability of Iodine and Folic Acid in Triple Fortified Salt (TFS) after 6-Months storage**

246 The first attempt on triple fortification of salt was reported by McGee (2012), who formulated TFS
247 by spraying 0.35% iodine and folic acid on salt and adding iron premix prepared by a one-step
248 agglomeration and encapsulation using spray drying (Romita, Cheng and Diosady Levente, 2011).
249 The idea was that the relatively smaller iron premix particles would adhere to the salt particles'
250 surface. The lower concentration of folic acid and iodine in the spray solution led to adding more
251 liquid to the salt (30 mL/kg); hence, increasing the salt's moisture content. The high moisture
252 content of the salt accelerated the rate of loss of iodine. The iron premix colour was not acceptable
253 as it formed grey spots in the salt (Romita, Cheng and Diosady Levente, 2011).

254 In contrast to the technology developed by McGee (2012), a higher concentration of folic acid and
255 iodine (0.5-1% and 2%, respectively) was used. The spray solution volume was drastically reduced
256 from 30 mL/kg salt to 2.5mL/kg salt. The newly fortified salt's moisture content was 0.06%
257 compared to 2.9% of the fortified salt formulated by McGee(2012). The premix, prepared by
258 forming extrusion and microencapsulation similar to that developed by Li et al. (2011), was used
259 in place of the premix formulated by spray drying. The similar particle size and density of the
260 premix and salt were produced ensured that the iron premix would not segregate from the salt.

261 Table salt stays in the distribution channels for an average of 2 months (Diosady *et al.*, 2006).
262 Also, the target population buys a small amount of salt, typically consumed within two months.
263 Hence, the goal is to have at least 70% of the micronutrients retained in the fortified salt after 6-
264 month storage. This was achieved; after 6-month storage, 70-85% of folic acid and 85-95% of
265 iodine were retained in all the samples, even at 45°C and 60-70% RH (Table 3). This result
266 confirmed that the technology could deliver iron, iodine, and folic acid simultaneously through
267 salt. Given the traditional distribution channels of salt, Triple Fortified Salt has the potential of

268 reaching millions of vulnerable households that otherwise may not have access to diets with
 269 sufficient iron, iodine, and folic acid.

270 The direct addition of folic acid on the surface of the salt resulted in bright yellow salt. Although
 271 the reduction in folic acid concentration in the salt (12.5 ppm) significantly reduced the salt's
 272 yellow colour, it was still visibly yellow. The colour may reduce the end-users' acceptance of the
 273 salt since colour is an essential factor for selecting food products by consumers (Dias *et al.*, 2012).
 274 Hence, technology to eliminate this colour change was investigated.

275 Table 3: Stability of Folic Acid and Iodine in TFS after 6-Month storage

| Types of | | Retention of Folic Acid (%) | | | Retention of Iodine (%) | | |
|----------|-------|-----------------------------|----------|----------|-------------------------|----------|----------|
| Sol. | Prem. | 25 °C | 35 °C | 45 °C | 25 °C | 35 °C | 45 °C |
| I | H&S | - | - | - | 93.2±2.9 | 90.3±3.9 | 92.3±6.8 |
| C | - | 82.3±6.1 | 80.4±5.9 | 78.9±3.5 | 95.3±3.4 | 92.9±3.6 | 89.2±6.2 |
| D | - | 81.1±1.2 | 77.3±0.8 | 72.9±3.3 | 92.2±1.5 | 89.8±2.1 | 86.8±1.4 |
| C | H&S | 83.5±0.5 | 82.2±2.9 | 83.5±0.5 | 89.6±3.2 | 89.7±3.9 | 87.4±1.8 |
| D | H&S | 80.6±1.5 | 77.8±1.2 | 73.2±1.5 | 93.1±1.5 | 94.0±4.3 | 93.0±1.4 |

276 Sol.=solution: C=0.5% folic acid + 2% iodine, D=1% folic acid + 2% iodine, and I= 2% iodine. Prem.=iron premix:
 277 H&S= iron premix coated with 5% HPMC and 5% soy stearin

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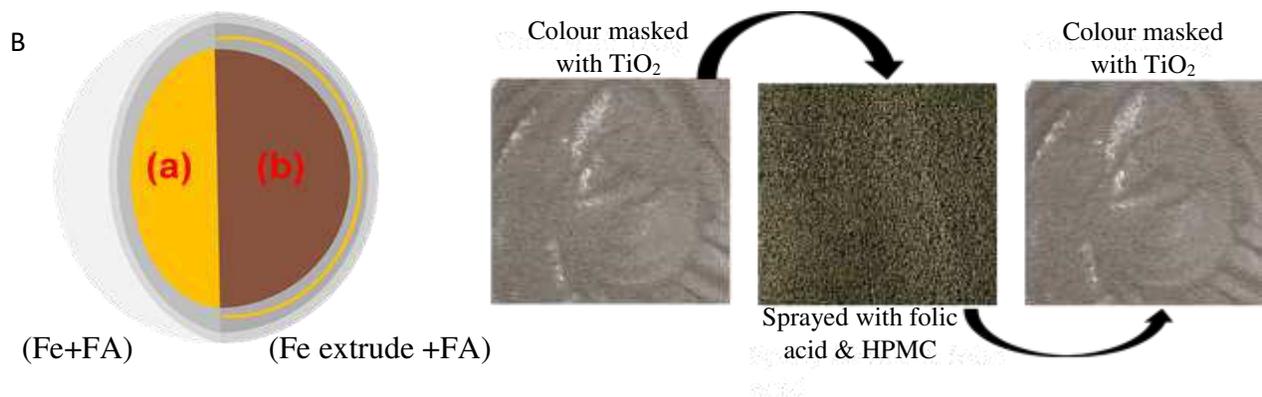
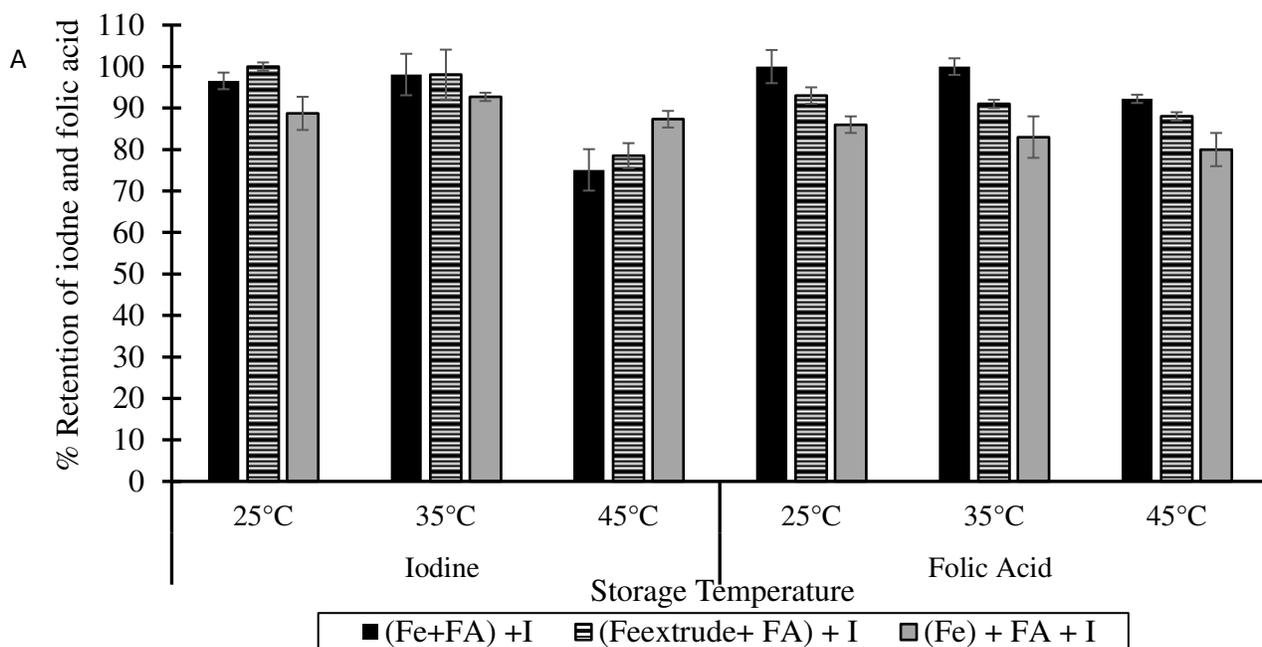
279 **Optimizing the Process of Triple Fortification of Salt**

280 Folic acid was added to the iron premix to form a Fe-FA premix. There were two designs for Fe-
 281 FA premix - either iron and folic acid were added to the core of the premix (Fe +FA) or folic acid
 282 was separated from the iron in the core by a thin layer of TiO₂ (Fe_{extrudate} + FA), as illustrated in
 283 Figure 2B. Iron and folic were coextruded to have both micronutrients in the core of the premix.
 284 Coextruding iron and folic acid was a straight forward process; it only involved adding folic acid
 285 to ferrous fumarate before extrusion. Separating folic acid from the core was achieved by two
 286 different routes:

287 a) adding folic acid as a uniform suspension in water to colour masked iron extrudate, or

288 b) spraying a folic acid suspension in 2.5% HPMC (in a 1:1 ethanol and dichloromethane
 289 solvent system) on color-masked iron extrudate tumbled in a pan coater.

290 The solvents' ratio that made up the solvent system is vital to having a uniform suspension of folic
 291 acid that is volatile enough for pan coating. A 1:1 dichloromethane: ethanol solvent system was
 292 used. More dichloromethane caused folic acid to settle at the bottom of the spray flask, while more
 293 ethanol caused the colour masked iron extrudate to clump inside the pan coater. Spraying folic



294 acid suspension in 2.5% HPMC (Route b) resulted in a more uniform distribution of folic acid than
 295 the other method (Route a).

296 Figure 2: (A) Stability of iodine and folic acid in TFS formulated with Fe-FA premix.
297 (B) Schematic of the two designs for iron-folic acid premix and how one of the premix
298 samples (Fe extrude + FA) was made
299 (Fe+FA) has both iron and folic acid in the core of the premix; (Fe_{extrude} + FA) has folic acid separated by a tiny layer
300 of TiO₂ in the premix; (Fe) has only iron in the premix, and folic acid and iodine was added as a solution
301

302 The iron-folic acid premix (Fe_{extrude} + FA) with uniform distribution of folic acid (made by Route
303 b) and the coextruded iron and folic acid premix (Fe-FA) were subsequently used to formulate
304 TFS. In the optimized process, folic acid will no longer impact the TFS color, as folic acid was
305 hidden with iron by the colour masking and coating agents of the premix. Over 75% of the added
306 folic acid and iodine were retained after 6-month storage. The loss of iodine in the salt did not
307 follow any particular trend (Figure 2A). Folic acid was more stable in the (Fe+FA) premix than
308 the (Fe_{extrude} + FA) premix. TiO₂ being in contact with folic acid may have initiated photocatalytic
309 degradation, which led to the significant loss of folic acid. Putting folic acid in the opaque core of
310 the ferrous fumarate (Fe + FA) prevented photocatalytic degradation. Folic acid was more stable
311 in the TFS formulated with Fe-FA premix than in TFS formulated by spraying folic acid and iodine
312 solution on salt. Iron seems to have enhanced the stability of folic acid in the salt. The same pattern
313 was shown by Li et al. (2011) in fortified rice that contained folic acid and iron. McGee et al.
314 (2017) suggested that folic acid loss in a salt fortified with iodine and folic acid is due to oxidative
315 stress. The stability of folic acid in the TFS confirms the oxidative degradative pathway of folic
316 acid in salt postulated by Modupe et al. (2019). The reductive potential of ferrous iron may have
317 prevented the oxidative degradation of folic acid in the salt. Aside from the enhanced stability of
318 folic acid in the (Fe+FA) premix, it is easier to make than the (Fe_{extrude} + FA) premix. Going
319 forward, TFS should be formulated by adding folic acid and the iron source (usually ferrous
320 fumarate) as microencapsulated coextrudate, and iodine added by the traditional method of

321 spraying potassium iodate solution. The TFS will deliver 200% iodine's RDA, 56% iron RDA and
322 100% folic acid RDA based on the consumption of 10g of salt per day (Vinodkumar and
323 Rajagopalan, 2009).

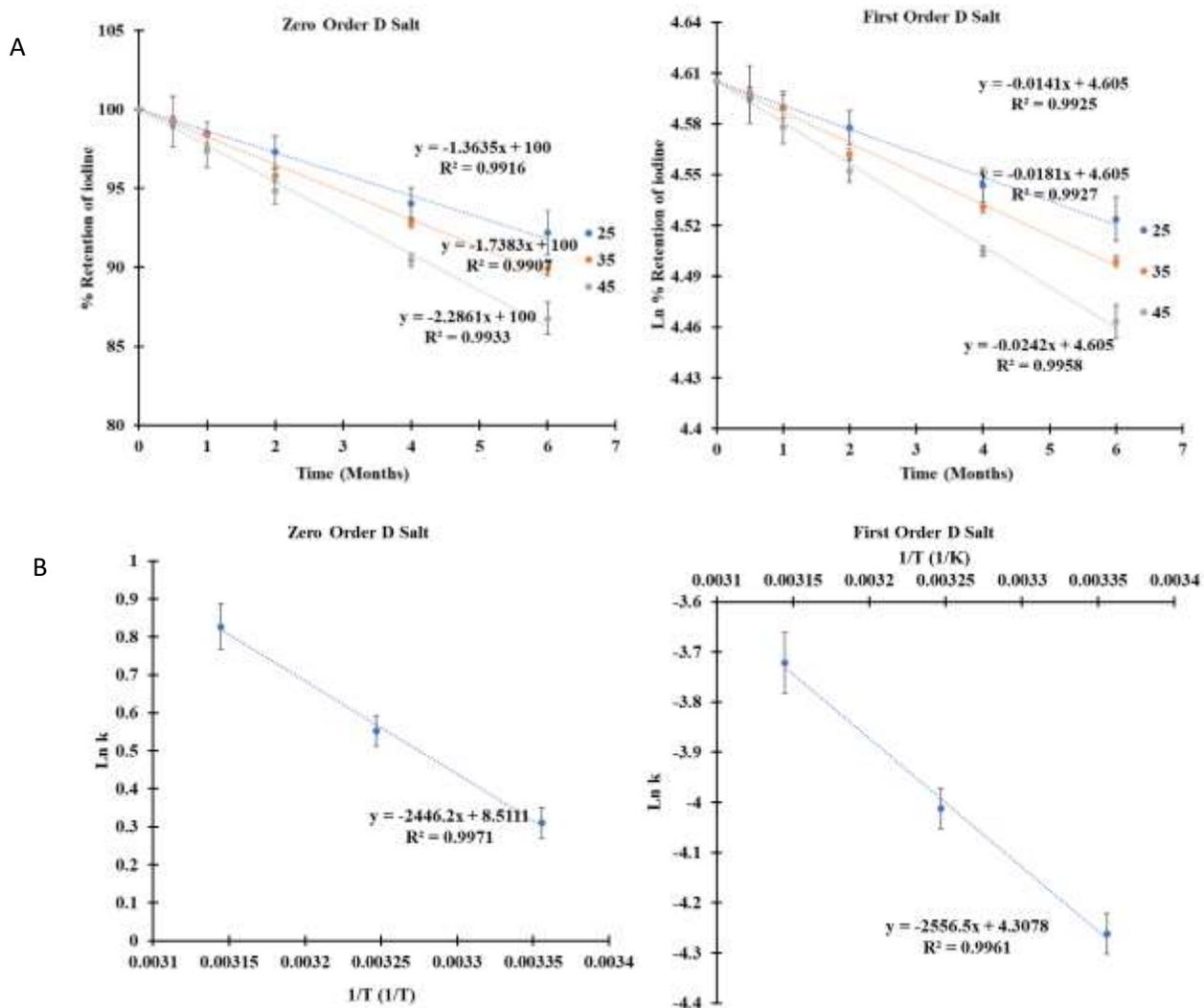
324 Other microencapsulation technologies could have been considered for folic acid in this study, for
325 instance, the electrohydrodynamic technology, described by Bakhshi et al. (2011), and the spray
326 drying technology described by Assadpour et al. (2016). These techniques cannot produce the
327 microcapsule's desired size, and their introduction into the fortification process will increase the
328 plant's capital cost as none of these technologies are used in salt fortification. The technology
329 developed can be used in the existing setup of plants used for making Double Fortified Salt. Also,
330 it is uncertain whether the technology described by Bakhshi et al. (2011) or Assadpour et al.
331 (2016) can effectively mask the colour of ferrous fumarate or folic acid. Romita et al. (2011)
332 clearly showed that spray drying technology could not effectively mask the ferrous fumarate's dark
333 brown color.

334 **Calculation and Validation of the Kinetic Parameters for the Stability of Iodine and Folic** 335 **Acid in TFS** 336 **Acid in TFS**

337 Kinetic tools, such as degradation constant and Gibb free energy, are vital to predicting
338 micronutrients' stability. The data obtained from the 6-month stability study were used to calculate
339 the kinetic parameters for micronutrient stability in salt (Figure 3A). The linear regression (R^2) of
340 the different rate laws of degradation of the micronutrients was used to predict the order of
341 degradation.

342 The correlation coefficient (R^2) of the first-order rate was slightly higher than that of the zero-
343 order (Figure 3A) but very much higher than that of the second-order rate. The slight difference
344 between zero and first-order rate may be due to the two likely mechanisms of degradation of the

345 micronutrient in the salt- diffusion through the coat of the premix and chemical interaction among
 346 the micronutrients. While the diffusion is a zero-order rate, the chemical interaction is a first-order
 347 rate. The first-order rate of degradation was chosen because chemical interaction is likely
 348 prominent of the two mechanisms. Although the activation energy (obtained from Figure 3,



349 Arrhenius plot, and Equation 3) for the first-order degradation was higher than that of the zero-
 350 order, they showed similar trends (
 351
 352 Table 4).

353 Figure 3: (A) Sample of the zero- and first-order degradation kinetics of iodine in a fortified salt;
 354 (B) Sample of the Arrhenius plot for the zero- and first-order degradation kinetics of
 355 iodine in a fortified salt
 356

357

358

359 Table 4: Kinetic Parameters of the Degradation of Folic Acid and Iodine in DFS* and TFS

| Salt Samples | Zero Order | | | | First Order | | | |
|-------------------|--------------------------------|---------|---------|-----------------------------|---|---------|---------|-----------------------------|
| | k (g/ml. month ⁻¹) | | | Activation Energy (kJ/mole) | k (10 ⁻² month ⁻¹) | | | Activation Energy (kJ/mole) |
| | 25 °C | 35 °C | 45 °C | | 25 °C | 35 °C | 45 °C | |
| Folic Acid | | | | | | | | |
| C | 1.8±0.1 | 2.1±0.1 | 2.6±0.1 | 14.1±0.7 | 2.0±0.1 | 2.3±0.1 | 3.2±0.1 | 14.1±0.7 |
| D | 2.7±0.2 | 3.1±0.1 | 3.7±0.2 | 12.8±0.6 | 3.2±0.2 | 3.9±0.1 | 4.6±0.2 | 15.7±0.7 |
| CA | 2.0±0.1 | 3.0±0.2 | 3.7±0.1 | 27.6±1.1 | 1.9±0.1 | 3.2±0.2 | 4.1±0.1 | 30.1±1.2 |
| DA | 2.3±0.1 | 2.8±0.2 | 3.4±0.2 | 16.2±0.9 | 2.8±0.1 | 3.6±0.3 | 4.6±0.2 | 19.7±1.1 |
| Iodine | | | | | | | | |
| C | 0.8±0 | 1.2±0 | 1.6±0 | 28.5±0.8 | 0.8±0 | 1.2±0 | 1.9±0.1 | 33.5±0.8 |
| D | 1.4±0 | 1.7±0 | 2.3±0.1 | 20.3±0.5 | 1.4±0 | 1.8±0 | 2.4±0.1 | 21.3±0.5 |
| CA | 1.0±0 | 1.5±0.1 | 2.0±0.1 | 26.4±1.0 | 1.1±0 | 1.8±0.1 | 2.2±0.1 | 26.3±1.0 |
| DA | 1.0±0 | 1.2±0 | 1.5±0 | 15.4±0.4 | 1.1±0 | 1.3±0 | 1.9±0.1 | 16.4±0.4 |

360 The C and D salt (DFS*) are fortified by spraying folic acid and iodine solution on salt. They differ in folic acid
 361 concentration; the C salt had 12.5ppm of folic acid + 50ppm iodine, while the D salt had 25ppm folic acid + 50ppm
 362 iodine. The CA and DA salt samples had iron at 1000ppm and their corresponding concentration of folic acid and
 363 iodine concentrations.
 364

365 $E_a = -(slope\ of\ Arrhenius\ plot \times R)$ Equation 3

366 Where E_a = activation energy (J/mole)

367 R= gas constant (8.314 J/K·mole)

368

369 The activation energies of the C salts were higher than those of the D salts. The iron premix's
370 presence increased the activation energies for folic acid in the salt but reduced iodine's activation
371 energies in the salt (C vs. CA and D vs. DA,

372
373 Table 4). These observations imply that folic acid and iodine were more stable in the C salts than
374 in D salts and that iron seems to improve folic acid stability while decreasing the stability of iodine
375 in the salts. As stated earlier, the reducing power of the ferrous fumarate may have played a role
376 in this. While it reduces the iodate to iodine, which is then lost by sublimation, the iron may have
377 reduced folic acid's oxidative degradation. These deductions are consistent with the trend of folic
378 acid and iodine stability in TFS samples.

379 The kinetic parameters of the degradation of micronutrients in the TFS were derived based on a 6-
380 month study which was validated with the stability of micronutrients in the salt in a 12-month study (

381
382 Table 4). Equation 4 was derived given that the degradation of iodine and folic acid in the TFS
383 primarily obeyed the first-order rate law. Value $t_{(R, T)}$ being approximately equal to 12 months,
384 obtained when $R_{(T)}$ from twelve-month stability study and corresponding $k_{(T)}$ from six-month
385 stability was put into Equation 4, validates TFS's degradation constants and Equation 4. Using
386 Equation 4, the time it will take to lose 25% of the iodine and folic acid ($R_{(T)} = 75%$) in TFS
387 samples was calculated. From the calculation, it will take 15, 9, and 7 months to lose 25% folic
388 acid in TFS with the best outcome at 25, 35, and 45 °C, respectively. For iodine, it will take 26,
389 16, and 13 months, respectively. In all samples, the micronutrients can be projected to retain at
390 least 75% of the added micronutrients for more than 6 months.

391

$$t_{(R,T)} = \frac{\ln\left(\frac{1}{R_{(T)}}\right)}{k_{(T)}}$$

Equation 4

392

393 Where;

394 $t_{(R, T)}$ = time (in months) required to retain micronutrient (%) in fortified salt at a given temperature

395 $R_{(T)}$ = retention of micronutrients in fortified salts (%) for a given temperature; values were
396 obtained from the 12-month stability study

397 $k_{(T)}$ = degradation constant obtained from the 6-month stability study

398

399 **Impact of Cooking on the Stability of Folic Acid**

400 The impact of boiling and fermentation on the folic acid was evaluated in cooked rice and Bondi
401 raita. There were several failed attempts for cooked rice because of the difficulty in extracting the
402 micronutrients from cooked rice. The extraction involves sieving, and the high content of
403 amylopectin in rice made sieving impossible. The use of sodium carbonate as a flocculant did not
404 solve the problem. After several failed attempts, cooking rice with excess water (1:9, rice: water)
405 helped resolve this problem. Even then, the filtration was nearly impossible. The extraction of folic
406 acid from Bondi raita did not cause much of a problem. The fortified salt contributed significantly
407 to the folic acid content of the two foods. Given the amount of salt added to the foods and
408 concentration of folic acid in the unfortified and fortified cooked foods, over 70% of the folic acid
409 due to added fortified salt was retained in the cooked rice. There was no observed sensory
410 difference between the cooked foods with or without the fortified salt.

411 **Cost Analysis**

412 Since the poor and vulnerable populations, who cannot afford or do not have access to processed
413 fortified foods, are the primary targets of this technology, there was a need to evaluate the

414 technology's cost implication to assess if the targeted population can afford the fortified salt. The
415 formulation of premix is the additional unit operation to the traditional process of making iodized
416 salt. Based on the average daily consumption of 10g salt (Kalra, Sahay and Baruah, 2013), the
417 additional costs of triple fortification is less than 27¢/person per year. It is assumed that this cost
418 can be reduced further by large-scale production and be covered by government or philanthropic
419 contributions for the needy.

420

421

422 **CONCLUSION**

423 The chemistry and colour of folic acid guided the development of the technology for adding folic
424 acid to the established process of double fortification of salt. Folic acid that is susceptible to
425 oxidative degradation was more stable when coextruded with iron. Also, the coextrusion of iron
426 and folic acid limits folic acid's impacts on the salt's sensory properties. Based on the kinetic model
427 developed, folic acid and iodine stability in the salt are predicted to exceed six months for retention
428 of over 75% of the micronutrients added to salt at 45 °C 60-70% RH. Folic acid in the salt was
429 stable in boiling and fermentation in cooked rice and Bondi raita, respectively. The described
430 technology is potentially a cost-effective approach for simultaneously ameliorating iron, iodine,
431 and folic acid deficiencies.

432

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434 manuscript. Oluwasegun Modupe, Juveria Siddiqui, and Akhila Jonnalagadda carried out the

435 experiments. Diosady Levente was the Principal Investigator who supervised the study and edited
436 the manuscript.

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438

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442

443

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Figures

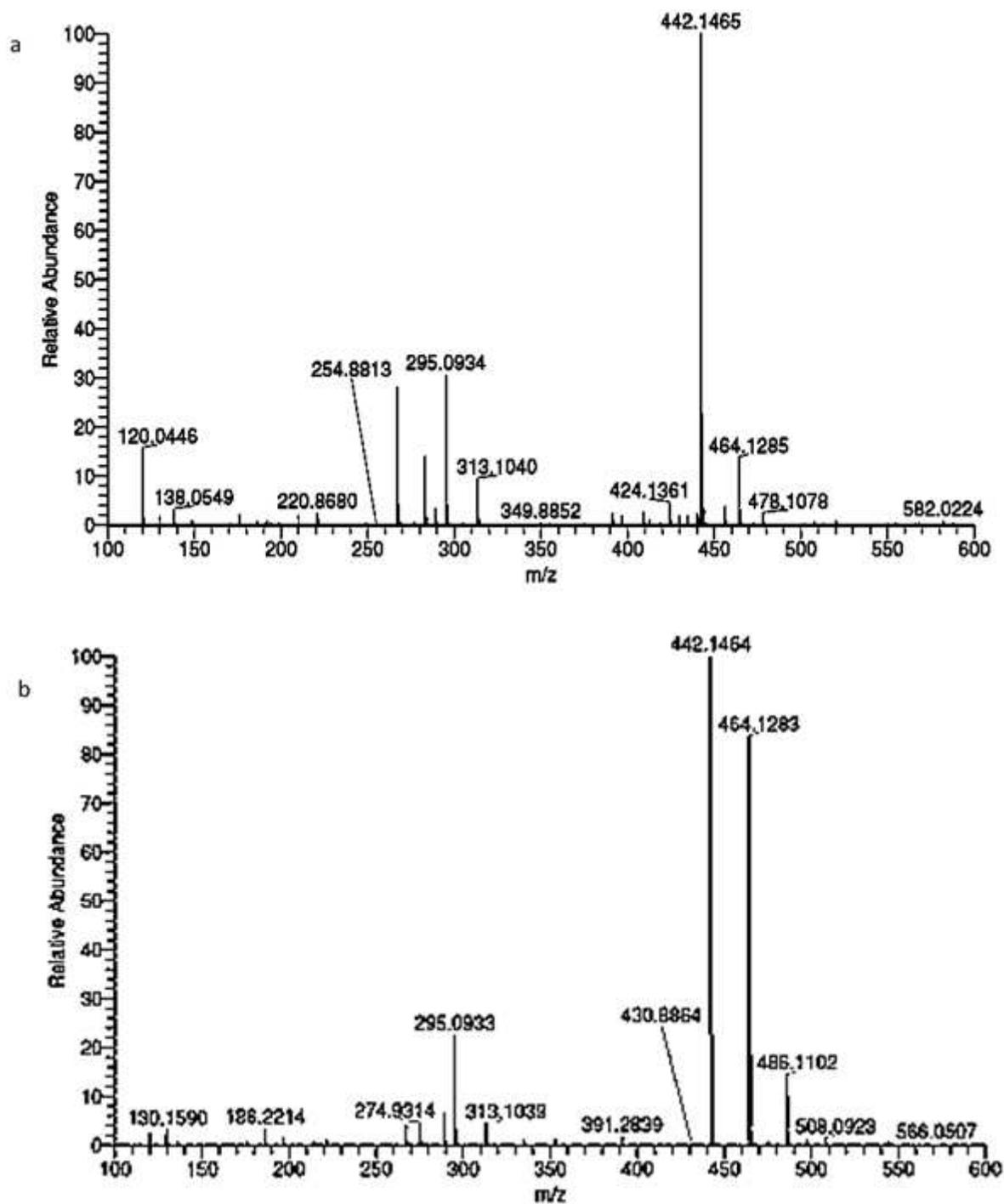


Figure 1

Mass spectrum of folic acid in spray solution (a) folic acid in sodium carbonate solution (b) folic acid and potassium iodate in sodium carbonate solution

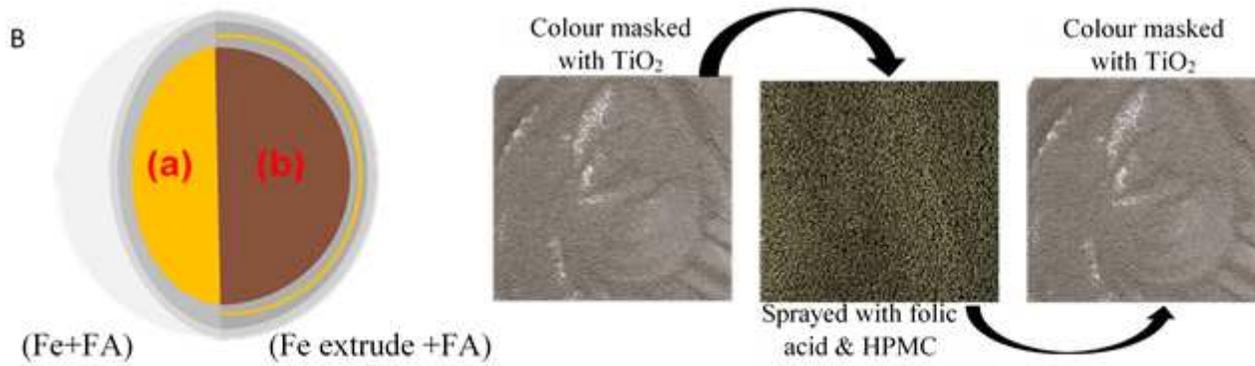
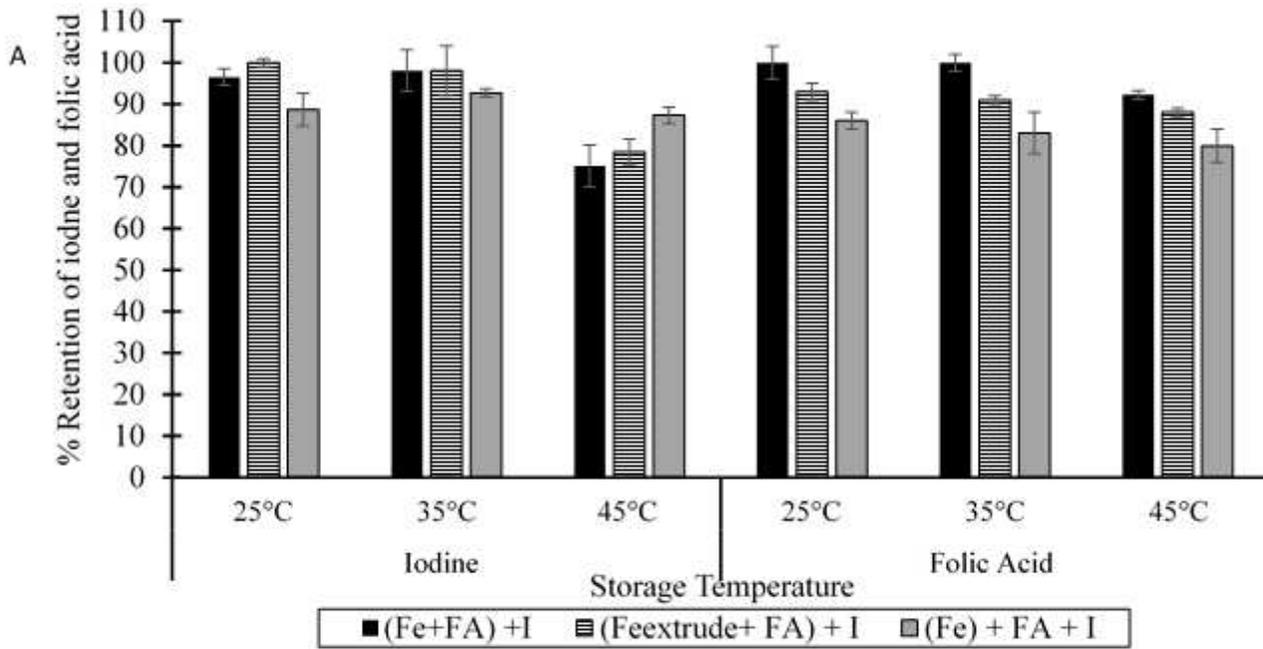


Figure 2

(A) Stability of iodine and folic acid in TFS formulated with Fe-FA premix. (B) Schematic of the two designs for iron-folic acid premix and how one of the premix samples (Fe extrude + FA) was made

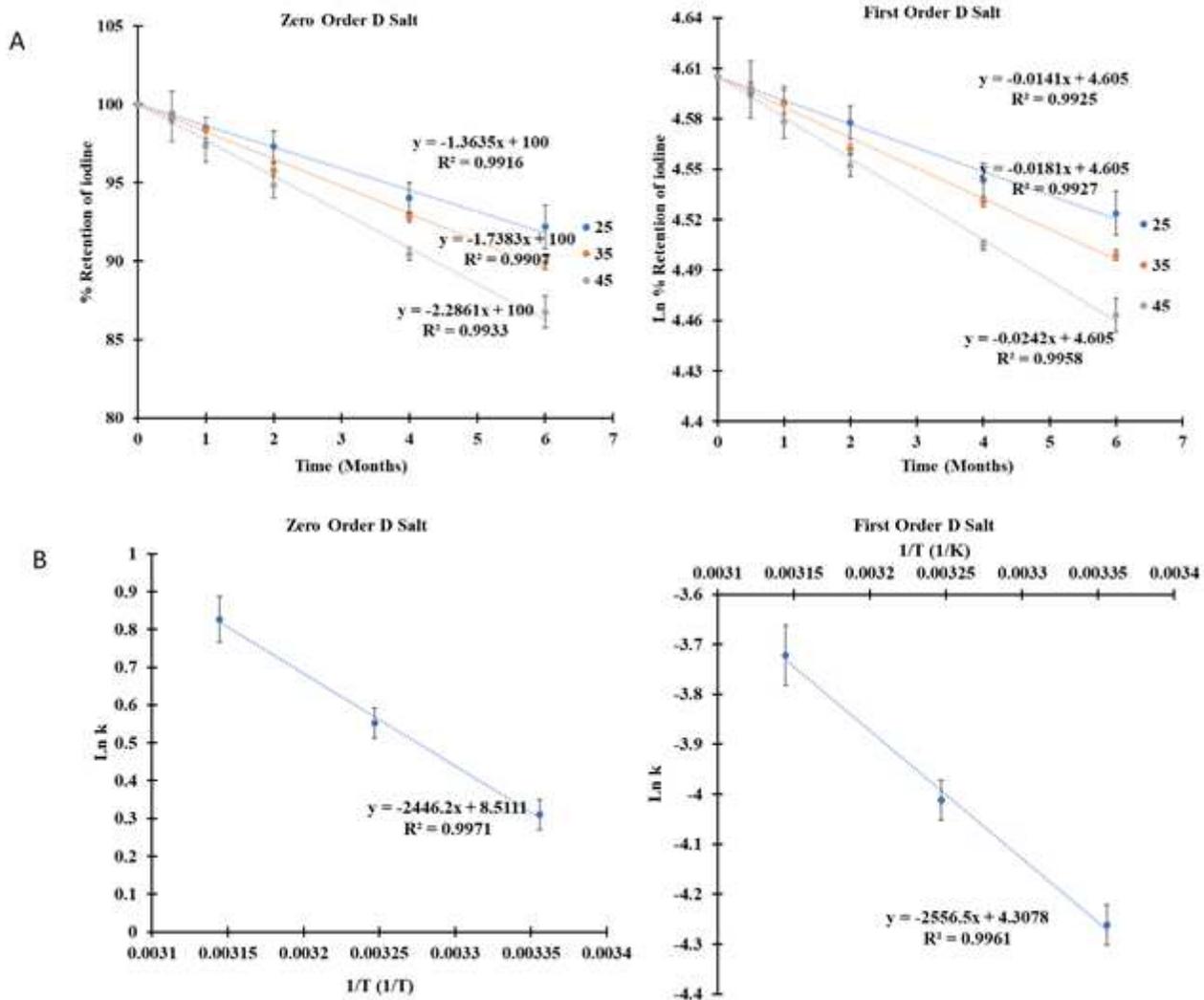


Figure 3

(A) Sample of the zero- and first-order degradation kinetics of iodine in a fortified salt; (B) Sample of the Arrhenius plot for the zero- and first-order degradation kinetics of iodine in a fortified salt

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