

## Research Article

# Predicting the Stability of Double Fortified Salt by Determining the Coating Quality of the Encapsulated Iron Premix

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The technology to simultaneously fortify salt with iron and iodine was developed in Canada and transferred and scaled up in India. The double fortified salt has reached more than 60 million consumers so far. Double fortification of salt is a cost-effective and reliable means of improving iron and iodine deficiencies at a population level. However, high-quality iron premix is essential for the stability of iodine and the program's success. Therefore, we developed a reliable and cost-effective method for premix coating quality evaluation in the field, especially in low-income settings. The integrity and chemical composition of the coating and exposure of iron at the surface (~10 μm deep) were determined using scanning electron microscopy and energy-dispersive X-ray spectroscopy to predict the stability of the fortified salt. The phenanthroline colour dropper test was used to test the quality of the double fortified salt by reaction with ferrous iron present on the premix surface. Five iron premix samples were compared. Based on the iron release, coating composition, and the reaction with phenanthroline, Premix-3, and its corresponding DFS, obtained from a local shop in India had the lowest quality among all samples tested. The results of the dropper test corresponded with the analysis using sophisticated analytical tools, confirming it as a simple, reliable, and cost-effective test for iron premix coating quality and integrity. This simple test would be crucial for a successful double fortification program, especially in low-income countries, in predicting iron premix quality, a critical determinant of iodine stability during storage, distribution, and retail. These study results can help governments and NGOs to establish quality standards for iron premix used for salt fortification programs.

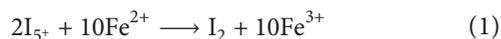
## 1. Introduction

Despite numerous attempts to end global malnutrition, more than one-fourth of the world's population suffers from micronutrient deficiencies. According to the report on the status of global malnutrition presented in the 2017 *Global Nutrition Report*, anemia in women and children remains the most prevalent nutritional disorder in middle and low-income countries [1]. Anemia hinders the well-being, growth and performance, cognitive and physical development, and overall work performance of women and their children as they grow into adults. It also augments the overall disease burden and mortality rate [2, 3]. Anemia levels across large parts of South Asia and sub-Saharan

Africa have remained flat over several decades despite significant increases in their GDP [4].

Studies demonstrated that iron fortification is a successful approach to significantly improve serum iron concentration and hemoglobin level in children and women of reproductive age [5–7]. The crucial step in the fortification strategy is selecting inexpensive and uniformly processed food carriers that the target population must consume at predictable rates [8, 9]. The worldwide success of salt iodization and its impact on reducing and controlling iodine deficiency disorders provides an excellent motivation to fortify salt with iodine and iron [10–12]. The challenge of salt fortification with iron and iodine is the interaction of ferrous iron with iodate. This

results in the formation of iodine and oxidation of ferrous to ferric form.



This interaction allows elemental iodine to sublime and eventually lose while converting iron to a less bioavailable ferric form and eliminating the beneficial health effects of salt iodization [11]. The encapsulation of ferrous iron in double fortified salt prevents this interaction, and the iodized salt can be stable for extended storage [9]. Additionally, the oxidation of exposed iron can change white salt crystals' colour and reduce their acceptability by consumers [13]. A field-based investigation conducted by Modupe et al. [14] detailed on the stability of iodine and iron double fortified salt in Kenya's coastal region. A sample of fortified salt sample developed pink and purple spots. The blue or purple colour formation is due to a redox reaction with exposed iron in the presence of starch in the coating formulation, while developing reddish brown or yellow colour can be due to ferrous oxidation to ferric fumarate [15]. Improving the coating quality and developing a moisture-resistant physical barrier can prevent the interaction of added iron and iodine in iodized salt [16]. Moreover, the encapsulation of iron prevents undesirable organoleptic changes in food where exposed iron can contribute to undesirable organoleptic properties and impart metallic taste by iron-mediated chelation-redox reactions [14, 17]. Consumer education in ensuring consumer acceptance of double fortified salt for health benefits is also considered essential.

The Food Engineering Group of the Department of Chemical Engineering and Applied Chemistry at the University of Toronto developed a technology for fortifying salt with iron and iodine by preparing a ferrous fumarate premix that approximates the salt in size and colour. The process has been tested commercially and has proved effective in improving the iron status of children and tea pickers and other efficacy studies [9, 18, 19]. The technology was transferred to JVS Foods Pvt, Jaipur, India and scaled up. The prevention of iodine loss in double fortified salt during the storage and distribution cycle is a crucial and a measure of the success of the technology. Fortified salt produced by this technology was stable for more than a year, retaining more than 80% of the added iodine [20].

From a consumer's acceptability standpoint, a good quality premix should not look very different from salt crystal and not keep apart. To avoid the segregation of the iron premix and salt and for higher customer acceptability, the encapsulated iron should be granulated and the colour masked to match the size and colour of salt grains. Challenges including floating of the premix particles during cooking, washing away of the coating from the extruded premix, and darkening of premix particles were observed and identified during the scaling up in India with great risk of consumer rejection. The improved coating formulations were suggested and implemented in the pilot and commercial-scale production [20]. Deficiencies of DFS formulation standards and regulatory monitoring in addition to organoleptic challenges were identified in a comprehensive review of global double fortification programs in countries

including *Argentina*, Cote d'Ivoire, Kenya, Morocco, Nigeria, Philippines, Sri Lanka, and India [21]. India is presently the only commercial producer of iron premix and double fortified salt and the only country with a DFS quality standard issued by the Bureau of India Standards (BIS). Shields and Ansari reported 24 registered DFS producers, and four registered out of six listed producers of encapsulated iron premix in India [22]. Although the final DFS and input salt standards are established, salt producers and distributors have issued no standards for encapsulated premix. In the absence of sufficient low-cost quality tests, producers, distributors, and implementing agencies find it challenging to ensure premix quality. The lack of standardization and production of some low-quality premixes resulted in very high or complete loss of iodine in the double fortified salt after only a few months in the distribution system. Therefore, it is critical to design and implement simple analytical techniques that can reliably predict the stability of iodine in the fortified food and the quality and level of iron fortificant.

There are no reports currently available on the evaluation of coating integrity of commercial DFS and iron premixes. The goal is to verify a simple colour test readily used in the field or at a consumer level to complement tests performed in standardized laboratories using sophisticated equipment. We conducted the coating integrity analysis of commercial samples of iron premixes and respective DFS obtained from three different processing plants in India along with the encapsulated premix and DFS prepared in the UofT laboratory as the positive control. Semiquantitative and qualitative methods were modified and used for encapsulated ferrous fumarate in DFS and iron premixes. We expect that our results will form the basis of future analytical standards for the quality of encapsulated premix and salt fortified with iodine and iron in the field and low-income settings.

## 2. Materials and Methods

**2.1. Materials.** Food-grade non-iodized salt was purchased from Toronto Salt Chemicals Co., Toronto, Canada. The commercial fortified salt samples (DFS-1, 2, and 3) used in the study were collected from three Indian states, namely *Uttar Pradesh*, *Madhya Pradesh*, and *Jharkhand*. The respective iron premixes (Premix-1, 2, and 3) were acquired directly from local premix producers in these states. Iron premix and DFS produced in our laboratory were used as the positive control. ACS grade 1,10-phenanthroline ( $\text{C}_{12}\text{H}_8\text{N}_2$ ,  $\text{H}_2\text{O} \geq 99\%$ ), nitric acid ( $\text{HNO}_3 \geq 70.0\%$ ), and hydrochloric acid ( $\text{HCl} \geq 37\%$ ) were purchased from Millipore Sigma. We purchased ACS reagent grade sodium acetate ( $\text{CH}_3\text{COONa} \geq 99\%$ ), hydroxylamine hydrochloride ( $\text{NH}_2\text{OH HCl} \geq 96\%$ ), and a multi-element standard solution in dilute nitric acid for elemental analysis from Sigma-Aldrich Canada Co.

**2.2. Visual Examination of Iron Premix Coating Integrity.** The first criterion of good quality premix is the visual appearance, including colour, size, size distribution, and shape

of the particle. Excellent quality iron premix particles should be well coated and present a smooth surface, with no dark areas, indicating that all iron is protected. The colour and morphology of premix samples were observed. Surface smoothness, presence of rupture, or dents on iron premixes were visualized using the QX7 microscope (Model DB12020) and photographed. Premixes' colour ( $L * a * b$ ) values were measured as described by Modupe et al. [20].

**2.3. Surface Morphology and Elemental Mapping by Electron Microscopy (EDS-SEM).** The visualization of premix morphology, coating quality, and the evaluation of exposed iron on the premix surface was determined using a sophisticated scanning electron microscopy technique coupled with element mapping by using energy-dispersive X-ray spectroscopy (SU-3500 VP-SEM, EDX, Oxford Instruments) [23]. In this test, premix particles were carefully sprinkled onto double-sided conductive adhesive carbon tape, which glued to aluminum SEM stubs. Loose particles were removed with an air blower. After placing the stub on the specimen holder and inserting it in the observation chamber, the examination was carried out using variable pressure secondary electron (VP-SEM) and backscattering electron (BSE) mode to start three-dimensional image and composition collection. The

beam voltage and the spot intensity were adjusted to 20 kV and 40, respectively. In the EDS-SEM mode, the EDXZ detector was used to map the surface by selecting the area from the SEM image, scanning, and acquiring spectra.

**2.4. Total Iron Content in the Encapsulated Iron Premix.** Total iron content in the premix samples was quantified by microwave-assisted acid digestion followed by inductively coupled plasma optical emission spectrometer [23]. Briefly, 0.5 g of premix was weighed into microwave digestion vessels, and 15 mL of concentrated nitric acid was added and secured the vessel contents with a screwed cap. The temperature was raised to 200–210°C via microwave irradiation. The samples were digested using a microwave digester (MARS 6, John Morris Scientific Pvt., Ltd.) for 0.5 hours. The resulting solutions were poured into 25-mL volumetric flasks, and the volume was made up to the mark using Milli-Q water. The iron content in the samples was analyzed after appropriate dilution using an inductively coupled plasma optical emission spectrometry ICP (Optima 7300 DV ICP-AES). The concentrations of elements in the premixes were calculated by constructing a calibration curve from a multimineral standard.

$$\text{Iron Content} \left( \% \frac{w}{w} \right) = \frac{\text{ICP Conc (mg/L)} \times \text{Dilution Factor} \times \text{Vol (L)}}{\text{Wt. of the sample (mg)}} \times 100. \quad (2)$$

**2.5. Coating Integrity Evaluation by the Release of Iron at pH 4.** The coating integrity of iron premixes was determined by measuring the iron release in a slightly acidic (pH 4) solution to evaluate the dissolution of ferrous fumarate only. The fat layer is unaffected at this pH, but ferrous fumarate is soluble [14]. Briefly, 0.1 g of iron premix was carefully weighed into 50-mL glass tubes, and 40 mL of 0.0001 M HCl solution was added and placed in a water bath at 37°C with constant stirring. 1 mL aliquots were collected from the glass tube after 2 hours, filtered (0.45 µm), and diluted to 10 mL volume with 5% nitric acid. The iron released was measured by aspirating the sample into ICP-AES, as described above, and the results were reported as a weight percentage.

**2.6. Coating Integrity of Premix and Fortified Salt; Field Test Method.** The coating integrity of premixes in fortified salt samples was analyzed by the method developed by Yuan et al., for field-based qualitative testing for iron in fortified salt with minor modification [24].

Briefly, a reacting solution was prepared by mixing 0.3% 1,10-phenanthroline, 2.5% sodium acetate, and 10% hydroxylamine hydrochloride. Iodized salt as negative control and uncoated iron as positive control were used. The removal of the coating and exposure of iron by mild pretreatment from the surface of the premix was done by (i) reaction with mild acid (0.5 M sulfuric acid), (ii) extraction of the fat coating layer with tetrachloroethylene, and (iii)

physical removal of the coating by grinding. A small sample cup was filled with approximately 5 g of DFS salt and levelled, ensuring a consistent sample quantity. 1–2 ml of reacting solution was added after each pretreatment. Colour change was observed, and photographs were taken at different time intervals. All tests were repeated at least three times for confirmation.

**2.7. Statistical Analysis.** The experiments were performed at least in triplicate. The data were analyzed and expressed as means ± standard deviation for each measurement. The data were subjected to one-way ANOVA using SPSS, and the differences between means were considered significant at  $P < 0.05$ .

### 3. Results and Discussion

Salt is an ideal vehicle for fortification as it is universally consumed in low and uniform quantities. The success of salt iodization technology forms the basis for fortification with other micronutrients. It has been proved that the double fortification of salt with iron and iodine is an effective intervention to reduce the rate of iron deficiency anemia in a vulnerable population [18, 25]. Iron is highly reactive and can induce the alterations in sensory properties in food [26]. The integrity of the premix coating is therefore essential to sustain the overall success of the fortification intervention.

TABLE 1: ( $L^* a^* b^*$ ) colour space value of all premixes and uncoated extruded ferrous fumarate. A higher  $L$  value indicates whiteness, and a lower  $L$  indicates darkness.

Sample	$L^*$	$a^*$	$b^*$
Premix-1	90.71 ± 1.95	-0.58 ± 0.23	2.4 ± 0.64
Premix-2	78.82 ± 1.31	0.60 ± 0.15	-1.55 ± 0.13
Premix-3	80.18 ± 1.20	-1.14 ± 0.10	-1.03 ± 0.08
Premix-4	93.45 ± 1.12	-0.84 ± 0.21	1.56 ± 0.80
Uncoated premix	30.17 ± 0.4	12.63 ± 0.05	18.06 ± 0.16

$L^*$  (+ = lighter; - = blacker);  $a^*$  (+ = red; - = green);  $b^*$  (+ = yellow; - = blue); the  $L^*$  values of Premix-2 and Premix-3 are not significantly different from each other, but are significantly lower than the values for Premix-1 and Premix-4, which are not significantly different from each other.

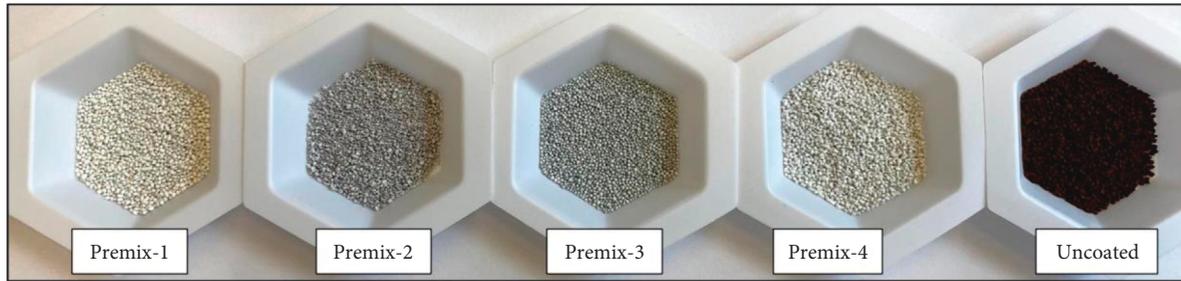


FIGURE 1: Indian premixes 1–3, UoT positive control 4, and uncoated ferrous fumarate.

The standard iron premix coating developed at the University of Toronto contained titanium dioxide and hydroxypropyl methylcellulose as primary colour masking agents, sodium hexametaphosphate as a colour stabilizer, and soy stearin as a hydrophobic surface overlay [20]. The dusting of fumarate particles with white titanium dioxide hid the brown-coloured fumarate, while coating layers with soy stearin and modified cellulose mixture improved surface properties. The amount used for dusting-rubbing-forming titanium dioxide for colour masking is estimated to be around or below 0.6–0.7 mg-per-g of salt or a consumption level of 7.5 mg per day per person. Although there are concerns about using titanium dioxide nanoparticles, no studies have yet reported on the general or reproductive and developmental toxicity of food-grade  $\text{TiO}_2$  up to 1000 mg/kg body weight per day containing fractions of nanoparticles [27].

The quality of the premix in DFS depends mainly on the coating integrity, which should not be impacted by the processes of blending with granular salt, storing at high moisture and temperature conditions, and using in food preparation. The analytical methods developed for coating integrity are expensive and can only be performed by trained professionals using sophisticated instruments and are unsuitable for analysis in the field by untrained personnel. To vindicate the earlier developed cost-effective field test kit-based method for coating integrity [24], we tested both commercial encapsulated iron premix and DFS samples and compared the results with those obtained by exacting laboratory methods.

**3.1. Coating Integrity by Premix Surface Analysis.** The most important sensory attribute is the visual appearance of premix particles. The premix particle size and colour should

match that of salt grains for consumers' acceptability. In an inappropriate coating, the dark ferrous fumarate will appear, and DFS will be visually unacceptable. The quality of  $\text{TiO}_2$  and the ratio of  $\text{TiO}_2$  to other binders in coating formulations also play a key role in the whiteness of premix. The ( $L^* a^* b^*$ ) colour space value was used to guide the appropriate colour masking of the iron premix. Table 1 shows the ( $L^* a^* b^*$ ) values of all three commercial premixes compared with positive and negative control samples. Premix-1 had the highest  $L$  value (90.71 ± 1.95), closest to the laboratory standard (93.45 ± 1.12), and Premix-2 with the lowest (78.82 ± 1.31)  $L$  value was the darkest sample (Figure 1).

The visualization of iron premixes produced in different commercial settings revealed a great wealth of information under light microscopy and scanning electron microscopy. All three commercial iron premixes had a wide range of size distribution, and the range of sizes within each sample was small (Figure 2). The size of the premix particles ranged from 580 to 800  $\mu\text{m}$ . Premix-2 had the biggest particles (770.0 ± 5.4  $\mu\text{m}$ ) while Premix-3 had the smallest (589.0 ± 9.0  $\mu\text{m}$ ), slightly smaller than the laboratory-made positive control sample (623.0 ± 17.3  $\mu\text{m}$ ). Due to the highly irregular shape of Premix-2, as revealed by light microscopy and SEM images (Figures 3 and 4), size analysis was difficult to evaluate. The average size (diameter in  $\mu\text{m}$ ) of the coated particles was found greater compared to uncoated particles (550 ± 13  $\mu\text{m}$ ) in the range of 40–200  $\mu\text{m}$ .

All three commercial premixes were different in shape, surface morphology, and smoothness. Premix-1 had a visually smooth and thick layer of coating with minimum dark spots on the surface. It was evident from the visual appearance as well as the size difference from similar uncoated particles as discussed earlier (Figures 1 and 2). In contrast, Premix-3 had a thinner coating and regular darker areas on

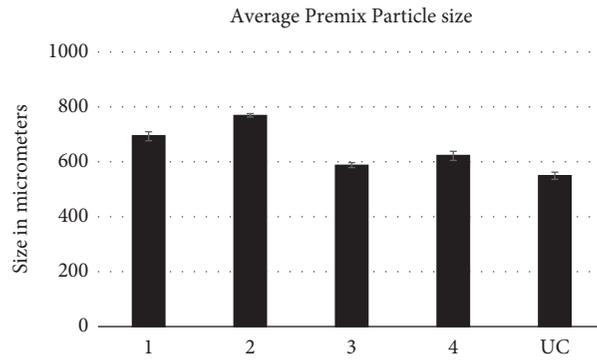


FIGURE 2: Average particle size in micrometres. Indian premixes 1–3, UofT positive control 4, and uncoated ferrous fumarate particles UC.

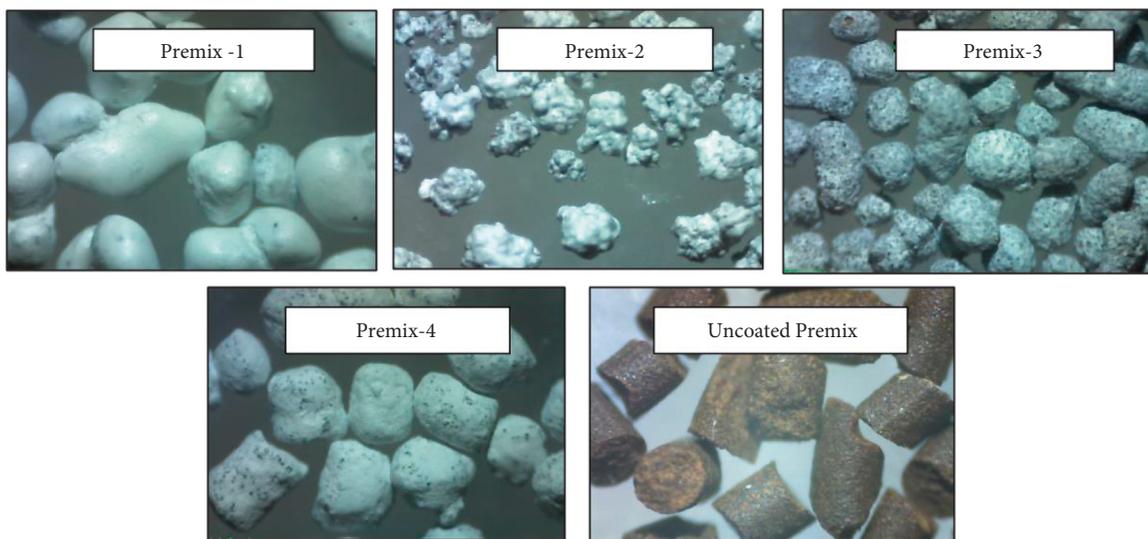


FIGURE 3: Light microscopic images of Indian iron premixes 1–3, UofT positive control 4, and uncoated extruded ferrous fumarate.

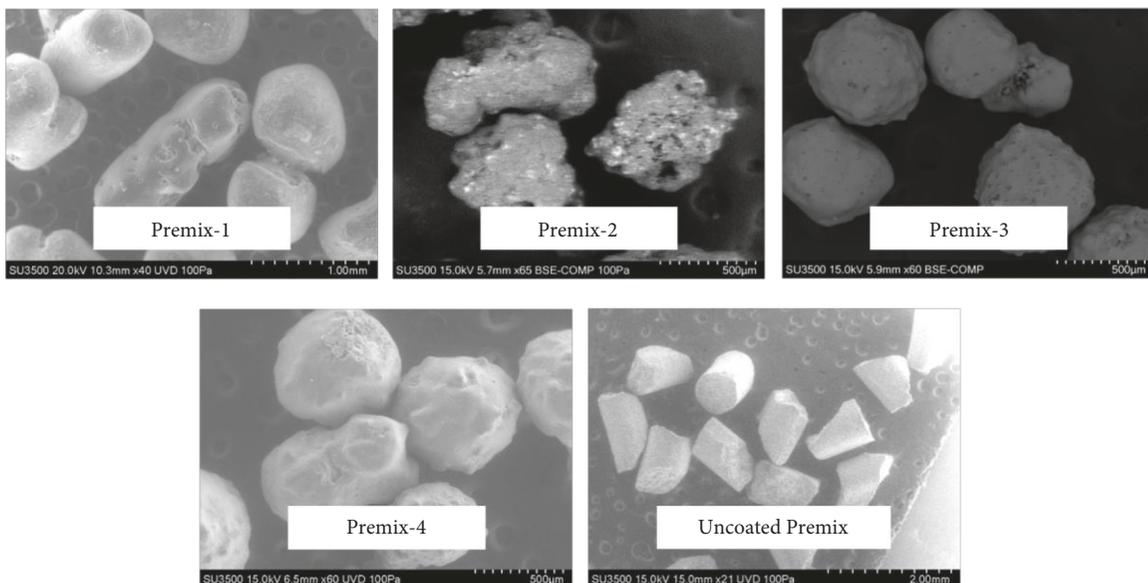


FIGURE 4: SEM images of Indian premixes 1–3, UofT positive control 4, and uncoated extruded ferrous fumarate.

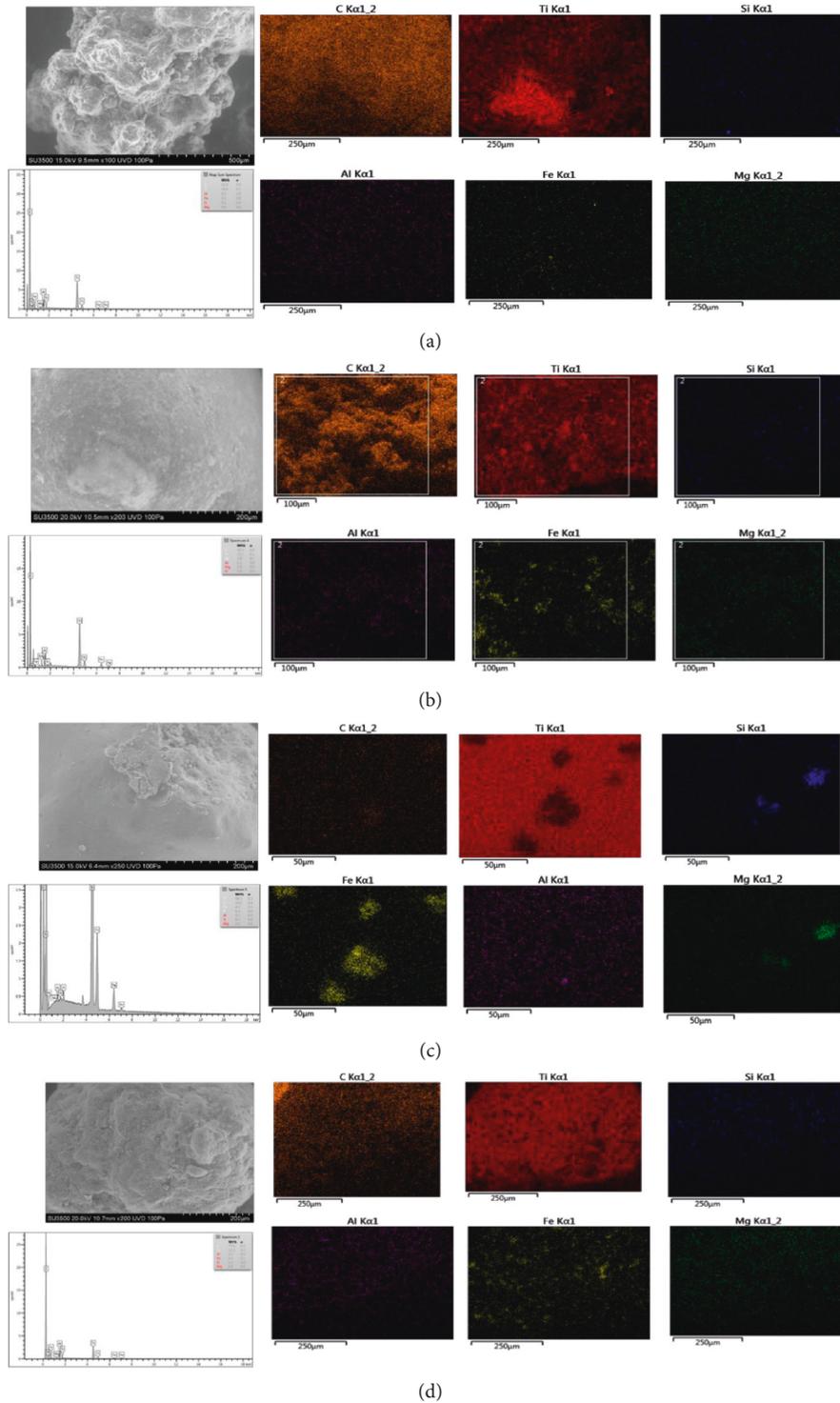


FIGURE 5: (a) Elemental composition of Premix-1. (b) Elemental composition of Premix-2. (c) Elemental composition of Premix-3. (d) Elemental composition of positive control 4.

its surface, which indicated that the premix was insufficiently coated. The coating was easily wiped by gentle rubbing of the particles by hand. Microscopic observation revealed that Premix-2 was unique in shape and resembles popcorn's structure instead of the solid cylindrical shape of the more commonly extruded ferrous fumarate particles. The particles of Premix-2 may have been produced using fluidized bed

technology instead of the extrusion method as reported and described previously by Oshinowo et al. [13]. The SEM images provided more detailed information and confirmed the observations using visual appearance and light microscopy (Figures 1 and 3). SEM images showed that the shape and size of Premix-3 particles were closely related to the premix produced in the laboratory that was used as the

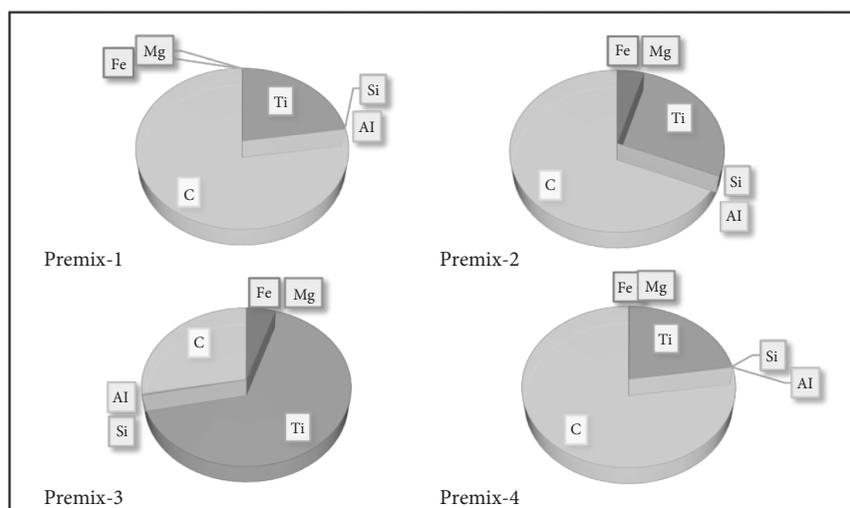


FIGURE 6: Relative abundances of selected element at the targeted surface area of Indian premixes 1–3 and UoFT positive control 4.

TABLE 2: Total iron content and iron release at pH 4 within 2 hrs.

Sample	(%) Iron (W/W)	Iron release at pH 4 (%)
Premix-1	15.0 ± 0.415	5.3 ± 0.7
Premix-2	20.5 ± 0.705	5.4 ± 0.5
Premix-3	23.3 ± 1.585	65 ± 1.1
Positive control	22.7 ± 0.675	ND

positive control (Figure 4). All premixes had light spots on their surface, indicating the presence of some exposed iron at the surface of the particles. All three Indian premix particles had some irregular surfaces and shapes.

**3.2. Elemental Mapping and Surface Analysis by Electron Microscopy (EDS-SEM).** The comprehensive surface analysis of iron premixes quantified the exposed iron by determining elemental surface composition. The coupling of SEM and EDX helped to quantify the relative concentration of elements of interest and the most abundant elements at the surface, including C, Ti, Fe, Al, Si, Zn, and Mg. Each element was colour-coded to visualize the concentration of metals across the inspected area (Figures 5(a)–5(d)). The relative concentrations of the selected, most abundant elements are presented in Figure 6. As expected, carbon formed a considerable proportion in the observed area of the positive control since the final coating consists primarily of stearin and modified starch. The result was similar in Premix-1, and Premix-2 indicated comparable coating composition. However, titanium was mainly present at the surface of Premix-3, indicating that the water-impervious final coat did not adequately overcoat the colour masking agent. Premix-2 and Premix-3 had higher iron at the surface than Premix-1 and the positive control, indicating higher levels of the exposed iron core.

**3.3. Coating Integrity and the Release of Iron from Premixes.** The encapsulated premix, in addition to desirable surface properties, shape, size, and colour, should contain around

17–20% w/w iron. The premix is blended with salt at a 1:175 or 1:200 mass ratio so that the final DFS contains 900–1000 ppm iron. The percentage of the release of iron from encapsulated premixes at pH 4 was calculated based on the analyzed iron content in each sample.

Low release at pH 4 is indicative of good coating integrity since ferrous fumarate is soluble at this pH, and it will readily dissolve. On the other hand, fat and cellulosic coating material will not be soluble at this pH and should prevent iron release into the dissolution medium. The commercial samples showed slight variations when tested for total iron content and release at pH 4 (Table 2). Premix-1 had the lowest iron content at 15 ± 0.415% w/w, while Premix-3 had the highest iron content, 23.3 ± 1.585% w/w. Premix-1 and Premix-2 had the least iron release (5.3 ± 0.7% and 5.4 ± 0.5%, respectively), while Premix-3 (65.0 ± 1.1%) lost the most iron (Table 2). This trend is compatible with the surface composition analysis, where Premix-3 had the lowest surface carbon indicating insufficient coating.

**3.4. Field Test Method for Premix Coating Integrity.** O-phenanthroline reacts with iron to form a yellow-orange-coloured complex. If the iron in the DFS is adequately encapsulated, the test should show minimal to no colour formation. Intense orange-red colour will form if the coating is not intact or was removed by physical or chemical means. Figure 7 represents the results of the colour indicator dropper test. No colour appeared until 4 minutes in all samples; the intense orange colour became visible in DFS-3 after 5 minutes. DFS-1, DFS-2, and control DFS did not develop any colour after several minutes with the reagent in salt. The positive control, ferrous fumarate mixed with salt, immediately changed colour, and its intensity was enhanced with time. The chemical and physical impact on the coating by adding mild acid, organic solvent, and grinding of particles were tested. Drops of diluted sulfuric acid were added to the solid DFS samples and allowed it to react with the premix for 30 seconds, and then a few drops of phenanthroline reagent were added. All samples except DFS-1

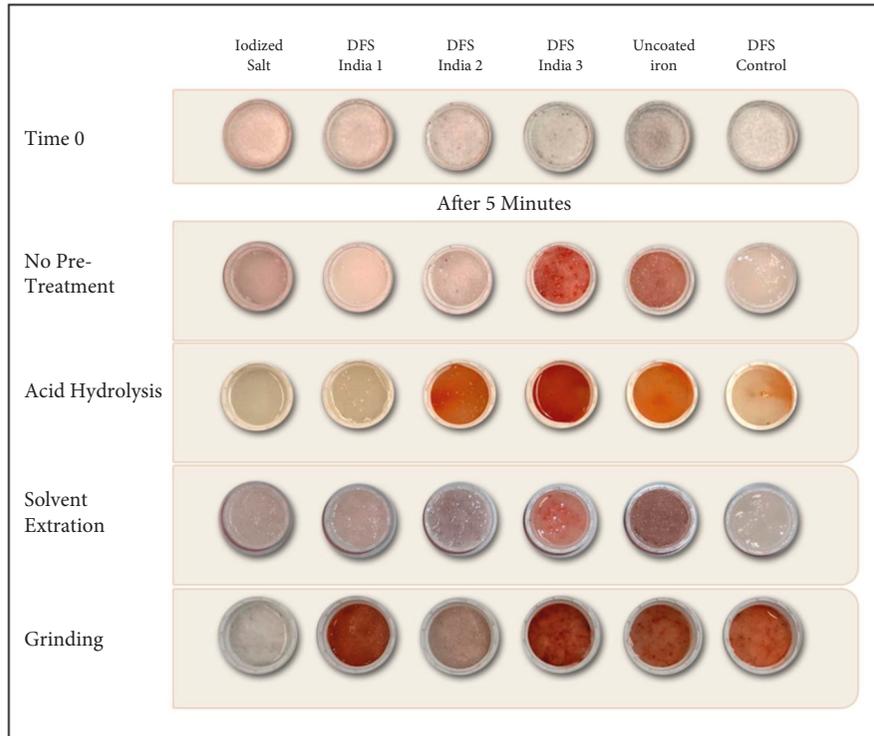


FIGURE 7: Qualitative DFS coating integrity test using O-phenanthroline and three different pretreatments and exposed iron from premix. Intense red-orange colour formation indicates the interaction of ferrous iron with phenanthroline and poor surface coating.

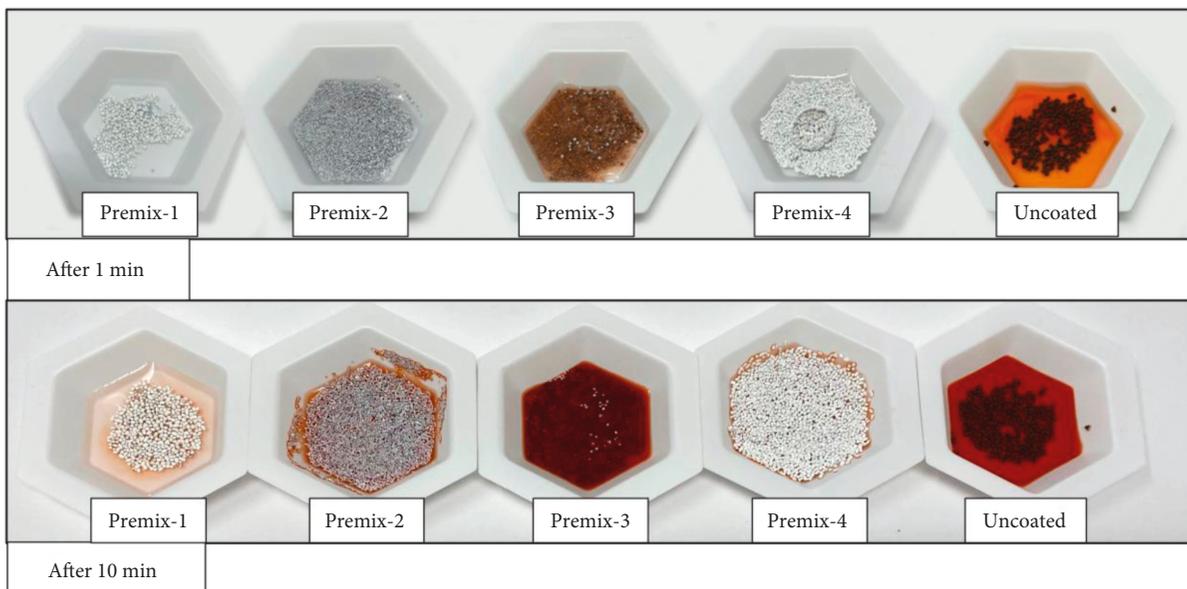


FIGURE 8: Qualitative iron test after 1 and 10 minutes of addition of phenanthroline colour reagent on Indian premixes 1-3, UofT positive control 4, and uncoated extruded ferrous fumarate. The intense red-orange colour formation of Premix-3 indicates the poor quality of the coating.

started to develop orange colour within 5 minutes. To investigate the impact of fat layer removal, TCA was added dropwise. Initially, when the test samples were still filled

with solvent, no colour formation was observed. Colours began to form immediately in DFS-3. Others showed intense orange colour after the solvent evaporated. Premix-1, which

TABLE 3: Comparison of encapsulated premix surface properties, iron release, and qualitative iron colour test in the premix and double fortified samples.

Sample	Surface	Encapsulated premix surface properties		
		Shape	Elemental abundances (%)	Colour test
Premix-1	Smooth	Cylindrical	C, Ti, Mg	No
Premix-2	Rough	Irregular	C, Ti, Fe	No
Premix-3	Rough	Round	Ti, C, Fe Mg,	Yes
Premix-4	Smooth	Cylindrical	C, Ti, Fe, Mg	No

Sample	DFS colour test			
	No pretreatment	Acid hydrolysis (effect of acid)	Fat removal (effect of solvent)	Physical abrasion (grinding)
DFS-1	No	No	No	Yes
DFS-2	No	Yes	No	No
DFS-3	Yes	Yes	Yes	Yes
DFS-4	No	Slight	No	Yes

did not react immediately in all previous chemical treatments, showed strong colour formation after the physical removal of the coating by mild abrasive treatment. Interestingly, DFS-2 did not show any colour formation within 5 minutes.

The same dropper test was also conducted on the respective premixes, as shown in Figure 8. The direct application of phenanthroline to the respective premixes was consistent with the surface analysis and iron release test observations. It was observed that Premix-1 and the positive control samples resisted colour formation for 7 to 8 minutes, while Premix-3 immediately turned dark orange, like uncoated ferrous fumarate, indicating poor coating (Figure 8). The laboratory-prepared control sample also remained white, showing sufficient coating with a hydrophobic fat layer. Table 3 summarizes the surface properties in terms of morphology and elemental abundance and the reaction to the colour test of each premix and respective DFS. The composition and quality of premix greatly influence the stability of DFS. Premix-1 with lower iron release at pH 4, smooth surface and regular shape, and high abundance of carbon at the surface produce a very light colour when reacted with phenanthroline reagent. The DFS-1 made from the same premix also resisted until the coating was physically removed by grinding. Premix-2 had a rough surface, larger particle size, and irregular shape, revealing that the production method differed from the others. The low iron release at pH 4 and the high abundance of carbon indicate cellulosic and fat coating. The DFS-2 reacted with an acid, showing the quick dissolution of a coating layer in a mild acid environment. Premix-3, with the highest analyzed iron content by weight, lower coating percentage by weight, and higher abundance of titanium, indicated the absence of an appropriate cellulosic and fat layer. The fortified salt made from this coating instantly reacted with phenanthroline, indicating higher exposure of iron at the surface and the poor coating quality. This makes the Premix-3 the lowest quality premix, and DFS with low stability can be expected.

It is evident that double fortification of salt effectively provides beneficial health outcomes and improves anemia in the target population [28, 29]. There are still some technical challenges related to premix coating integrity, standardization, and sensory attributes of food prepared with fortified

salt [30]. The underlying challenge of the lack of quality standards and monitoring protocol of DFS globally needs to be addressed to ensure the nutritional benefits of the fortification program. Food fortification intervention targets mainly marginalized populations who cannot afford the additional cost of process and standardization. The commercial production of DFS in India is covered by the social safety net program and the additional cost of fortification is covered by the state governments [31]. Effective monitoring and regularization of the program at state, national, and global levels are essential to achieving the fortification intervention's targets and the program's success.

#### 4. Conclusions

The iron premix encapsulation quality and integrity are essential for preventing iodine loss from fortified salt and the sensory properties of food prepared with it. We tested three commercial iron premixes and their respective salts and compared them with laboratory-made samples. The commercial samples tested in this study showed differences in terms of colour, appearance, surface morphology, and coating integrity. The results indicate that the commercially produced samples differ in coating quality, and this results in compromised salt stability in at least one tested sample. The simple colour test can be used for determining both premix quality and predicting DFS stability. The qualitative colour test may be used by minimally trained personnel to test DFS quality in the field confirming the stability of salt during storage and distribution. The phenanthroline colour test is a promising cost-effective field test that can be used in low-income settings and produces results that are consistent with results obtained by using high-quality imaging and iron release methods.

#### Data Availability

The data (SEM-EDX) and other analytical data used to support the findings of this study are included in the article. Previously reported analytical data were used to support this study and are available online and identified with DOI. These prior studies are cited at relevant places within the text as references [1–19].

## Additional Points

(i) Double fortified salt ensures the delivery of the adequate level of iron and iodine to the target consumer. (ii) The cost-effective coating quality and integration evaluation of premix are essential for double fortified salt storage stability in low-income settings. (iii) We developed and verified that cost-effective, qualitative field tests are adequate for monitoring the effective delivery of iron and iodine to the consumer.

## Conflicts of Interest

The authors declare that there are no conflicts of interest.

## Authors' Contributions

J. Siddiqui designed the experiments, carried out the analysis (O. Modupe and A. Vatandoust carried out the other parts), and wrote the manuscript. Levente. L. Diosady supervised the experiments and edited the manuscript. All authors reviewed the manuscript.

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## References

- [1] WHO, *Global Nutrition Report 2017: Nourishing the SDGs*, WHO, Geneva, Switzerland, 2017.
- [2] R. E. Black, L. H. Allen, Z. A. Bhutta et al., "Maternal and child undernutrition: global and regional exposures and health consequences," *The Lancet*, vol. 371, no. 9608, pp. 243–260, 2008.
- [3] H. P. S. Sachdev and T. Gera, "Preventing childhood anemia in India: iron supplementation and beyond," *European Journal of Clinical Nutrition*, vol. 67, 2013.
- [4] G. Story, "India development update, July 2020," 2018, <https://elibrary.worldbank.org/doi/abs/10.1596/34367>.
- [5] J. K. Das, R. A. Salam, R. Kumar, and Z. A. Bhutta, "Micronutrient fortification of food and its impact on woman and child health: a systematic review," *Systematic Reviews*, vol. 2, no. 1, p. 67, 2013.
- [6] L. S. Jackson and K. Lee, "Microencapsulated iron for food fortification," *Journal of Food Science*, vol. 56, no. 4, pp. 1047–1050, 1991.
- [7] P. Liu, R. Bhatia, and H. Pachón, "Food fortification in India: a literature review," *Indian Journal of Community Health*, vol. 26, pp. 59–74, 2014.
- [8] L. Allen, B. de Benoist, O. Dary, and R. Hurrell, *Guidelines on Food Fortification with Micronutrients*, WHO, Geneva, Switzerland, 2006.
- [9] K. M. Nair, G. N. V. Brahmam, S. Ranganathan, K. Vijayaraghavan, B. Sivakumar, and K. Krishnaswamy, "Impact evaluation of iron and iodine fortified salt," *Indian Journal of Medical Research*, vol. 108, 1998.
- [10] B. Sivakumar, G. N. V. Brahmam, K. M. Nair et al., "Prospects of fortification of salt with iron and iodine," *British Journal of Nutrition*, vol. 85, no. S2, 2001.
- [11] B. S. N. Rao, "Fortification of salt with iron and iodine to control anaemia and goitre: development of a new formula with good stability and bioavailability of iron and iodine," *Food and Nutrition Bulletin*, vol. 15, 1994.
- [12] K. Madhavan Nair, B. Sesikeran, S. Ranganathan, and B. Sivakumar, "Bioeffect and safety of long-term feeding of common salt fortified with iron and iodine (double fortified salt) in rat," *Nutrition Research*, vol. 18, no. 1, 1998.
- [13] T. Oshinowo, L. L. Diosady, R. Yusufali, and A. S. Wesley, "Production of iron premix for the fortification of table salt," *International Journal of Food Engineering*, vol. 8, no. 3, 2012.
- [14] O. Modupe, Y. O. Li, and L. L. Diosady, "Optimization of the color masking and coating unit operations for microencapsulating ferrous fumarate for double fortification of salt," *Journal of Food Science and Technology*, 2022.
- [15] L. Diosady, R. Yusufali, T. Oshinowo, and L. Laleye, "A study of storage and distribution of double fortified salts in Kenya," *Journal of Food Engineering*, vol. 76, no. 4, 2006.
- [16] L. Diosady and M. G. V. Mannar, "Technology development and scaling up for double fortification of salt with iodine and iron," *Annals of Nutrition and Metabolism*, vol. 71, 2017.
- [17] H. Mehansho, "Iron fortification technology development: new approaches," *Journal of Nutrition*, vol. 136, no. 4, pp. 1059–1063, 2006.
- [18] M. Andersson, P. Thankachan, S. Muthayya et al., "Dual fortification of salt with iodine and iron: a randomized, double-blind, controlled trial of micronized ferric pyrophosphate and encapsulated ferrous fumarate in southern India," *American Journal of Clinical Nutrition*, vol. 88, 2008.
- [19] J. Hammons, S. Venkatramanan, S. Mehta, and J. Haas, "Preventing iron deficiency: results of a randomized controlled trial of double-fortified salt in female Indian tea pluckers," *The FASEB Journal*, vol. 27, no. S1, 2013.
- [20] O. Modupe, K. Krishnaswamy, Y. O. Li, and L. Diosady, "Optimization of unit operations for microencapsulating ferrous fumarate during scale-up of double fortification of salt with iron and iodine," *Food Quality and Safety*, vol. 5, 2021.
- [21] D. Moorthy and L. Rowe, "Evaluation of global experiences in large-scale double-fortified salt programs," *Journal of Nutrition*, vol. 151, 2021.
- [22] A. Shields and M. A. Ansari, "Review of experience of the production of salt fortified with iron and iodine," *Journal of Nutrition*, vol. 151, no. Supplement\_1, pp. 29S–37S, 2021.
- [23] A. Pratap Singh, J. Siddiqui, and L. L. Diosady, "Characterizing the PH-dependent release kinetics of food-grade spray drying encapsulated iron microcapsules for food fortification," *Food and Bioprocess Technology*, vol. 11, 2018.
- [24] J. S. Yuan, Y. O. Li, J. W. Ue, A. S. Wesley, and L. L. Diosady, "Development of field test kits for determination of microencapsulated iron in double-fortified salt," *Food and Nutrition Bulletin*, vol. 29, no. 4, pp. 288–296, 2008.
- [25] E. Asibey-Berko, S. H. Zlotkin, G. S. Yeung et al., "Dual fortification of salt with iron and iodine in women and children in rural Ghana," *East African Medical Journal*, vol. 84, no. 10, 2007.
- [26] E. Habeych, V. van Kogelenberg, L. Sagalowicz, M. Michel, and N. Galaffu, "Strategies to limit colour changes when fortifying food products with iron," *Food Research International*, vol. 88, pp. 122–128, 2016.

- [27] M. Younes, G. Aquilina, L. Castle et al., "Safety assessment of titanium dioxide (E171) as a food additive," *EFSA Journal*, vol. 19, no. 5, 2021.
- [28] S. Sultan, F. M. Anjum, M. S. Butt, N. Huma, and H. A. R. Suleria, "Concept of double salt fortification; a tool to curtail micronutrient deficiencies and improve human health status," *Journal of the Science of Food and Agriculture*, vol. 94, 2014.
- [29] J. A. B. Baxter, M. Kamali, M. F. Gaffey, S. H. Zlotkin, and Z. A. Bhutta, "Fortification of salt with iron and iodine versus fortification of salt with iodine for improving iron and iodine status (protocol)," *Cochrane Database of Systematic Reviews*, vol. 2019, no. 10, 2019.
- [30] A. Drewnowski, G. S. Garrett, R. Kansagra et al., "Key considerations for policymakers - iodized salt as a vehicle for iron fortification: current evidence, challenges, and knowledge gaps," *Journal of Nutrition*, vol. 151, 2021.
- [31] S. Makkar, S. Minocha, K. G. Bhat et al., "Iron fortification through universal distribution of double-fortified salt can increase wages and be cost-effective: an ex-ante modeling study in India," *Journal of Nutrition*, vol. 152, no. 2, pp. 597–611, 2022.