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Research Article

Spray Encapsulation of Iron in Chitosan Biopolymer for Tea Fortification

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Aim. Tea was studied as a carrier for iron in a fortification strategy to reduce iron deficiency. Iron forms insoluble coloured complexes with tea polyphenols which lower consumer acceptability. Complexation of iron by polyphenols and quinones derived from tea inhibits iron absorption in the first segment of the small intestine. Spray-dried chitosan-iron microcapsules were prepared to prevent iron-polyphenol interaction before the beverage is consumed. A competing chelating agent (EDTA) or antioxidant (sodium ascorbate) was added to prevent interactions and help improve iron bioavailability. *Methods*. The effect of concentration of chitosan (0.2–1.5%w/w), iron loading (10–60% w/w $FeSO_4$), addition of secondary coatings on particle morphology, surface iron exposure and release, and bioaccessibility were evaluated. Tea-containing chitosan microcapsules and chelating agents to enable iron absorption were evaluated for sensory acceptability. *Results*. The iron release profile at pH 1 and pH 7 exhibited reverse enteric behaviour of non-cross-linked chitosan microcapsules. Increasing the iron content leads to more iron exposure on the surface due to a high core to coat ratio. Cross-linked chitosan effectively encapsulated iron, and its release in tea was inhibited, as indicated by lower delta *E* values in comparison with untreated tea and positive sensory testing scores. The use of maltodextrin as secondary coating slightly improved the spray process and produced larger particles, with less exposed iron on the surface. However, it did not improve the colour performance in milk tea. *Conclusions*. Tea fortified with encapsulated iron and a chelating agent providing 40% of the daily iron requirement of an adult, prepared in a traditional South Asian manner, i.e., with milk and water, resulted in tea with acceptable colour and taste. However, further research is required to develop an encapsulation formulation for stable iron encapsulation in hot tea and exploration of equivalent plant-based chitosan sources to address concerns of consumers with dietary restrictions.

1. Introduction

Micronutrient deficiency is the most widespread risk to human health. Iron deficiency anemia affects an estimated 2 billion people worldwide. Although the world has progressed in reducing infant mortality, millions of children die before the age of five because of preventable diseases for which malnutrition is the prominent cause [1]. Iron deficiency combined with decreased absorption of iodine and vitamin A leads to additional nutritional disorders including mental retardation, brain damage, early childhood blindness, and increased severity of infectious diseases. Nutritional deficiencies are preventable by simple modifications in dietary intake; for example, consumption of iron-rich food, nutritional supplements, and iron-fortified food can make a significant difference. Women of childbearing age (15–44 years) are most vulnerable, due to iron loss in menstruation and pregnancy. Food fortification is a process of adding vitamins and minerals to staple foods or beverages. It makes frequently eaten foods more nutritious without relying on consumers to change their habits [2–4]. Next to water, tea is the most popular beverage in the world

irrespective of race, gender, or socioeconomic status. Over the past decade, global per capita tea consumption has risen by 2.5 percent, primarily driven by the significant growth in tea-producing nations. Developing and emerging economies, particularly in East Asia, Africa, Latin America, the Caribbean, and the Near East, have been at the forefront of this expansion in demand [5–7]. This hot beverage is particularly common in developing countries including India, Turkey Bangladesh, China, Pakistan, Iran, and Sri Lanka, where on average people consume 120 mL of tea every day [6, 8]. This regular and increasing consumption presents an opportunity to utilize tea as a safe and effective iron fortification vehicle. Research data on micronutrient fortification, specifically iron in black and milk tea, are limited. The food engineering research group at the University of Toronto made a significant contribution to exploring micronutrient fortification of black tea and provided valuable insights for further investigations and advancements in the field of fortification of tea products [9–11].

Tea is rich in polyphenolic compounds that are valuable for their antioxidant activities that can scavenge free radicals and chelate metals [12, 13]. Tea polyphenols with catechol or gallol substituents form stable-coloured complexes with iron. At the low pH of the stomach, these polyphenols may be converted into quinones which also form stable complexes with iron. This makes tea a challenging vehicle for iron fortification, as it reduces iron bioavailability and the antioxidant capacity of tea [10, 14]. The target populations for the present project are developing nations in South Asia, where tea is most often prepared with milk. This adds an additional technical challenge to the overall iron fortification of tea because it raises pH, encouraging iron-polyphenol complex formation. The resulting discolouration becomes a barrier to consumer acceptance.

Ferrous iron (Fe^{++}) is the most bioavailable form of iron. It is converted to the ferric form (Fe^{+++}) by oxidation, which can be triggered by alkaline conditions, oxidizing agents such as those present in the air, high humidity, and phenolic compounds [15]. Generally, food fortified with ferrous compounds that undergo oxidation exhibit low iron bioavailability, poor taste, and discolouration reducing consumer acceptability [16].

A study conducted by Dueik et al. found that the chelating agent EDTA can compete with tea polyphenols, and at 1 : 2 molar ratio of iron to EDTA, it prevents iron-polyphenol complex formation at pH 5 [17]. The iron-EDTA complex is highly bioavailable despite iron being present in the ferric form [18]. The 1 : 2 ratio of iron to EDTA in tea was shown to maintain iron solubility after undergoing a pH adjustment similar to digestion, suggesting that iron bioavailability is preserved [11]. The reaction is dependent on pH, polyphenol concentration, and brewing time. When combined with milk, the infusion of tea exhibits a striking hue of milky orange, which is visually captivating and highly appealing to consumers [19]. Unfortunately, in the presence of milk, due to higher pH and possibly increased calcium, iron reacts with polyphenols even in the presence of EDTA to form a dark coloured complex that is unappetizing to some consumers [20–22].

Because the formation of stable iron-polyphenol complexes depends on the oxidation of iron, antioxidants may be an alternative to EDTA to prevent iron-polyphenol complex formation [23]. Reference [24] conducted a study investigating the manner in which reducing agents interfere with iron-polyphenol reaction in food. Catechol, gallic acid, catechin, caffeic acid, and chlorogenic acid were all tested with ferric sulphate and reducing agents (ascorbic acid, sodium bisulphite, and hydroxylamine). It was found that for all of these phenolic compounds, ascorbic acid and sodium bisulphate eliminated colour formation. Also, the addition of ascorbic acid to commercial products including black tea, green tea, coffee, hot chocolate, and banana baby cereal was shown to reduce colour formation due to added ferrous iron [24]. Furthermore, ascorbic acid (vitamin C) is well known to increase iron absorption in general. However, in the presence of milk, sodium ascorbate (selected over ascorbic acid to minimize pH shift) resulted in the same colour issue as EDTA.

Therefore, there are two chemical strategies that may be employed to increase iron bioavailability and reduce, but not eliminate, colour formation in tea prepared with milk: use of a completing chelating agent that forms a bioavailable complex with iron (e.g., EDTA) and optionally a reducing agent/antioxidant added to prevent the oxidation necessary to form stable iron-polyphenol complexes (e.g., ascorbate).

Although additives are needed to increase iron bioavailability in the presence of tea, microencapsulation provides an opportunity to form a physical barrier between iron and polyphenols to prevent discolouration in the beverage. In this way, iron may remain unnoticeable to the consumer while delivering 30–100% of RDI of iron for a healthy adult [25].

Chitosan has gained significant attention in recent years mainly because of its novel industrial applications in food, pharmaceutical drug delivery, and medical products [26]. It is a pseudonatural, cationic, hydrophilic, nontoxic, biodegradable, polyaminosaccharide, which is structurally similar to cellulose. It is chemically derived from partial deacetylation of chitin, which is a natural animal polysaccharide obtained from the hard outer skeleton of shrimp. Chitosan is biodegradable by enzymes, including chitosanase, papain, cellulase, and acid protease [27]. Its molecular weight (MW) and number of deacetylation (DA) units vary in different chitosan polymers. It is insoluble in water, organic solvents, and aqueous bases but soluble in acids such as acetic, nitric, hydrochloric, perchloric, and phosphoric acid. Its solubility depends on the amount of protonated amino groups in the polymeric chain [27–29]. The positively charged free amino groups of chitosan can form chemical complexes with many negatively charged polyanionic polymers and small molecules. Cross-linking of chitosan has been utilized for producing microcapsules with improved functional properties and controlled release properties.

Sodium tripolyphosphate (TPP) is a nontoxic food additive, used to prepare cross-linked chitosan microparticles. The positively charged amino group (NH_3^+, protonated in acidic solution) of chitosan reacts with negatively charged phosphate groups (PO_4^{-3}) in TPP [30]. This leads to the

formation of biocompatible cross-linked chitosan with improved functionality [30–32]. Spray drying based chitosan-iron microcapsules have been previously prepared by our research group [33].

The present research focuses on the prevention of off-colour formation while maintaining iron bioavailability in iron-fortified tea prepared with milk. Optimization of chitosan microcapsule production and the effects of additional secondary coating, hydrophobic surface overcoating, and cross-linking in improving iron-loading capacity, iron release profile, morphology of microparticles, and their suitability in milk tea were investigated. Iron-fortified tea samples were made by adding iron microcapsules to tea, and the resultant colour of tea was observed. Final formulations combining iron microcapsule absorption enhancers, EDTA, and sodium citrate, as well as chitosan microcapsules, were tested for sensory attributes.

2. Materials and Methods

2.1. Materials. Medium molecular weight chitosan (240–270 kD, Brookfield viscosity 200.00 cps in dilute acid), iron (II) sulphate heptahydrate, sodium tripolyphosphate 85%, acetic acid 99.7%, and AAS iron standard (TraceCERT® 1000 mg/L Fe in nitric acid) were purchased from Sigma Aldrich, St. Louis, USA. Reagent grade disodium EDTA and sodium ascorbate were purchased from Supelco® Inc., and dichloromethane was purchased from Caledon Laboratories Ltd., Ontario, Canada. Maltodextrin (DE 7–10) was purchased from Cerestar USA, Inc. JVS Foods India provided soy stearin and black tea samples. Milli Q water was used throughout the experiments.

2.2. Spray-Drying Parameters. Microcapsules were produced using a B-290 mini-spray dryer (Buchi, Switzerland). Spray-drying parameters were optimized with some modification of our earlier published method [33]. Briefly, an atomizing gas flow rate of 667 std L/h at 618 kPa (90 psi) and an aspirator operating at 5.5 Pa were kept constant throughout the experiments using a standard 0.7 mm diameter nozzle tip. The inlet feed flow rate was controlled by varying the peristaltic pump speed depending on solution viscosity. The inlet air temperature (135–160°C) was controlled in each experiment to keep the outlet temperature below 80°C.

2.3. Microcapsule Preparation. The microcapsules were collected at the bottom of the cyclone separator from a sample collection vessel. At the end of each experiment, samples were transferred into clean sample bottles and weighed to determine the yield. Process yield % (equation (1)) was calculated by dividing the amount of the resultant powder from the collection vessel by the total solid content in the initial feed solution:

$$\text{Yield } (\%) = \frac{\text{weight of premix (g)}}{\text{weight of solids in feed (g)}} * 100. \quad (1)$$

2.3.1. Primary Chitosan Microcapsule Production. Chitosan flakes were dissolved in a 1% w/w aqueous solution of acetic acid. Complete solubility was achieved by keeping the system at room temperature overnight. Chitosan produces a highly viscous solution even at a low concentration; therefore, it is useful to determine the highest possible chitosan concentration for a better solid yield of the powder with spray drying. Chitosan concentrations of 0.1%, 0.2%, 0.5%, 1.0%, 1.5%, and 2.0% w/w were used. Iron salt was added at concentrations of 15, 20, 30, 40, 50, and 60% by weight of total solids in 1% chitosan solution. Ferrous sulphate was mixed with constant stirring at 1500 rpm for about 15 min before spray drying.

2.3.2. Cross-Linked Chitosan Preparation. Chitosan was crossed-linked using sodium tripolyphosphate. After mixing the chitosan solution with the iron salt (50% and 60% by wt.) for 10 min, sodium triphosphate (10–15 mL per 100 mL chitosan as 1% w/v solution) was added dropwise to the chitosan solution with constant stirring using a laboratory mixer (Silverson Machine Ltd., UK). An opaque suspension of cross-linked chitosan was spray-dried to obtain encapsulated microparticles.

2.3.3. Microcapsules with Added Maltodextrin. A clear solution of maltodextrin (10% w/v) was prepared and added (1 : 1) to the 1% w/w chitosan solution containing ferrous sulphate (30 and 40% wt.) under mild stirring for 10–15 min. The final solution was spray-dried. While added maltodextrin increased the solid content of the chitosan solution, it also reduced solution viscosity.

2.3.4. Microcapsule with Hydrophobic Surface Coating. With the intention of stabilizing these microcapsules in an aqueous medium and improving the surface properties and increasing the hydrophobicity of the microcapsules, a hydrophobic surface coating was applied.

Approximately, 20 g of chitosan microcapsules was placed on a rotating pan, inclined at 45° and rotating speed to ~50 rpm. In a 250 mL spraying flask, 3.5 g of melted soy stearin dissolved in a 1 : 4 mixture of water and dichloromethane was kept warm on a hot plate. The solution was sprayed onto the free-flowing particles in the pan to ensure uniform coating.

2.4. Characterization of Microcapsules

2.4.1. Total Iron Content and Loading Efficiency. Total iron content in microcapsules was analyzed to determine iron loading capacity and iron release from the capsules. The microcapsules were digested by the addition of 10 mL conc. HNO_3 to 0.1 g of the powder in a microwave-assisted acid digestion system (MARS 6, John Morris Scientific Pty Ltd.) with the temperature raised to 200–210°C via microwave irradiation. The solution obtained was quantitatively transferred to a 25 mL flask and brought to volume with deionized water. As needed, this was further diluted to

a known volume (1 : 10) with 5% w/v nitric acid. The samples were then analyzed by using ICP-AES (Optima 7300 DV ICP AES), calibrated with a 1000 mg/L Fe standard (Merck) solution. Iron concentration was calculated using the following equation:

$$\textbf{Iron content}\left(\%\,\frac{\mathbf{w}}{\mathbf{w}}\right) = \frac{\text{ICP conc (mg/}L\text{)} * \text{dilution Factor} * \text{Vol }(L)}{\text{Wt. of sample (mg)}} * 100. \quad (2)$$

The success of overall spray-drying process parameters was determined by calculating iron-loading efficiency using the following equation:

$$\textbf{Loading effeciency}\left(\%\,\frac{\mathbf{w}}{\mathbf{w}}\right) = \frac{\text{analyzed iron in microcapsules}}{\text{added iron in feed solution}} * 100. \quad (3)$$

2.4.2. Iron Release Profile and In Vitro Iron Bioaccessibility. The iron release from microcapsules was evaluated under three distinct conditions: 1. The rate of dissolution of iron in 0.1 N HCl solution (pH 1) resembling acids in gastric juices was utilized to predict the bioaccessibility of iron. The amount of iron released at pH 1 within two hours related the approximate amount available for absorption from fortified tea. 2. To test the reverse enteric behaviour of chitosan microcapsules, release of iron at pH 7 (phosphate buffer solution) was measured. 3. A stress test to mimic the tea-brewing conditions was conducted by determining the iron release profile in constantly boiling water ($95 \pm 5^{\circ}$C). In all release kinetic experiments, 50 mg of each spray-dried powder was weighed and dispersed in 100 mL of either pH 1 HCl or pH 7 phosphate buffer in triplicates. The tubes were placed into a shaking water bath set at 37°C for two hours. To test release in boiling water, flasks were placed directly on a hotplate. A thermometer was placed inside the flask to monitor the temperature. 1 mL aliquots from each flask were taken, filtered, and diluted to 10 mL in a volumetric flask at time intervals 15, 30, 60, 90, and 120 min. The iron content in the samples was measured using ICP-AES as described above. The amount of iron was analyzed, and percent release was calculated by dividing the weight of the released iron by the total weight of analyzed iron present in the microcapsules using the following equation:

$$\textbf{Iron release}\left(\%\,\frac{\mathbf{w}}{\mathbf{w}}\right) = \frac{\text{iron in aliquot at specified interval}}{\text{total iron in the premix}} * 100. \quad (4)$$

2.4.3. Scanning Electron Microscopy. The morphology of microcapsules was determined by examining scanning electron microscope images (SU-3500 VP SEM, Hitachi High-Technologies). Microcapsules were attached on SEM stubs by carbon conductive double-coated adhesive tape and blasted with air to remove loose particles. Samples were examined, and micrographs were recorded at an acceleration voltage of 1.5 kV, with a working distance of 51 mm, under high vacuum. The size of the microcapsules was evaluated using image analysis of the micrographs using *ImageJ* (an open-source image processing software).

2.4.4. Surface Analysis by X-Ray Photoelectron Spectroscopy (XPS). The spray-dried particles produced using a two-fluid nozzle system result in a matrix structure, with a possibility of uncoated particles on the microcapsule surface. To quantify the elements present on the sample surface at nanometer depth, X-ray photoelectron spectroscopy (XPS) or electron spectroscopy was employed. The selected chitosan microcapsules were analyzed using the Thermo Scientific™ K-Alpha™ XPS system at the Surface Interface Ontario Facility at the University of Toronto, Canada.

2.5. Sensory Analysis of Iron-Fortified Indian-Style Tea. Tea was brewed in RO water and/or milk to represent common tea preparation methods used in India. Briefly, 250 g of water, skim milk, or whole milk was brought to boil. Then, tea leaves (1% or 3% w/w Indian black tea) and iron-containing chitosan microcapsules (delivering at least 4 mg iron per cup) were added together with Na_2EDTA at a 1 : 2 molar ratio [17]. The tea was boiled for further 4–6 min after the addition of the iron microcapsules and chelating agent. Water loss due to boiling was measured after cooling the tea to 35°C. Pictures of different tea preparations were

taken in a white light box. The Hunter colour (L^*a^* and b^*) of tea samples at 40°C was determined, and $\triangle E$ values of selected samples were calculated using an NR series precision colorimeter (3 nH). Delta E was measured as the difference between the colour of samples and unfortified tea designated as two points in the lab colour space [34]. A blind taste test was conducted on the acceptability of regular and iron-fortified tea. Participants were asked to fill in a survey to rate each sample of tea based on 5 parameters: overall, flavour, colour, mouthfeel, and aroma. There were 8 participants, of whom 2 were not able to sense aroma. The participants rated the parameters using a 5-point scale, which was weighted: really bad ($x1$), bad ($x2$), neutral ($x3$), good ($x4$), and very good ($x5$). The responses for each parameter were multiplied by their respective weight and totaled.

3. Results and Discussion

Spray-drying technology is relatively cheap and is widely used in a single-step large microencapsulation for foods and pharmaceuticals. Depending on the starting feed material and process conditions, a very fine (2–50 μm) to large (2-3 mm) sized particles can be obtained [35, 36]. In the present study, ferrous sulphate, as a core iron source, and chitosan, as a primary coating agent, were used, with a variety of wall materials. All the formulation variables with their codes discussed in this paper are summarized in Table 1.

3.1. Spray-Drying Yield and Iron-Loading Efficiency. Initially, iron-free chitosan solution was spray-dried, and the optimum spray solution concentration was found to be 1% w/v of chitosan with a maximum yield of 62.5% by wt (Figure 1). The 2% w/w solution became highly viscous and resulted in the lowest yield, and therefore, it was impractical for spray drying. Several formulation and process variables were investigated to enhance productivity, loading efficiencies, and improved functional properties of capsules for use in tea fortification. The process yields and loading efficiencies of iron microcapsules are presented in Figure 2. The yield was between 58–78%, consistent with the 72% yield reported earlier, using chitosan as the primary coating [37]. The moderate yield of chitosan (CH15%–40% w/w) can be attributed to the fact that it produces a viscous solution at low concentration, which readily adheres to the spray dryer glass chamber during process optimization and leads to the loss of feed mass. The material sticks to the spray chamber and could not be recovered, thus lowering the overall yield of the dried powder [38].

Maltodextrin has diverse applications as a food additive including bulking and film formation, lowering viscosity, flavour and fat binding, and reducing oxygen permeability in encapsulation matrices [39]. The process yield was expected to increase by reducing solution viscosity by adding a secondary polymer. The incorporation of maltodextrin successfully decreased the viscosity and stickiness of the chitosan solution, leading to fewer instances of nozzle clogging during spraying and minimal deposition on the drying chamber walls. As anticipated, the loading efficiency improved (reaching 90.84%) when 10% w/v maltodextrin was added to chitosan with a 30% w/w iron payload. However, this improvement in loading efficiency could not be maintained when the iron concentration was increased to 40%.

Chitosan and polyphosphate groups can be linked with either ionic interaction or protonation. Both PO_4^{-3} and OH^- are present in sodium tripolyphosphate at higher pH and compete to react with the amino (NH_3^+) group of chitosan [30, 31]. At higher pH, deprotonation is a dominant mechanism of interaction due to the change in pH of chitosan solution [40]. At lower pH, the predominant mechanism is the ionic interaction between the negatively charged phosphate group and the positively charged amino groups. When a cross-linking step was introduced at a higher iron concentration (50% and 60% w/w iron), the iron-loading efficiency significantly improved to 70.92% and 76.12%, respectively (Figure 2). Intermolecular and intramolecular cross-linking of native chitosan likely leads to a stable three-dimensional molecular network, which helped stabilize iron in the microcapsules. Alternately, Fe^{+2} and SO_4^{-2} molecules present in the solution may have interacted with the sites available on the polymer. Further studies should be conducted to understand the mechanism at a molecular level.

3.2. Size and Morphology of Microcapsules. The morphology of a spray-dried particle can be described by its size, shape, internal structure, and surface properties. Many drying process parameters, including feed solution concentration, the solubility of the excipient, drying temperature, and ratio between the drying time and diffusion coefficient, affect the morphology of the particle [41]. During spray drying, a droplet of liquid feed undergoes the constant rate of drying until the polymer concentration at the surface becomes very high, resulting in precipitation of the polymer forming a film around the core [42, 43]. The final evaporation from microcapsules formed largely depends on the physicochemical properties of the polymer or polymer matrix, e.g., solution viscosity, molecular weight, permeability, and elastic modulus. It was found that the molecular weight of the polymer (which affects viscosity) played a significant role in the size and shape of microcapsules [44]. Scanning electron microscopy (SEM) of a representative sample was used to take the images of chitosan-based microcapsules formed with different formulation variables (Figures 3(a)–3(f)). Spray-dried iron-containing particles usually spherical, with low variability, in size and shape, were observed. Slight deformation of particles was observed when iron was added at all concentrations unlike in chitosan particles without iron (Figure 3(a)). Addition of ferrous sulphate slightly altered the viscosity of the 1% chitosan solution at all iron concentrations (Figures 3(b)–3(f)). Incorporating maltodextrin did not reduce the irregularity of particle surfaces; however, a reduced incidence of surface aberrations was observed with a slight increase in particle size (Figures 3(d)–3(e)). The average size of microcapsules calculated by using image analysis software (*ImageJ*) is presented in Table 2 along with

Table 1: Description of formulation variables and codes.

No.	Sample description	Codes
1	Spray-dried medium molecular weight chitosan 1% by wt	CH
2	Chitosan loaded with 15% $FeSO_4$ by wt	CH15Fe
3	Chitosan loaded with 20% $FeSO_4$ by wt	CH20Fe
4	Chitosan loaded with 30% $FeSO_4$ by wt	CH30Fe
5	Chitosan loaded with 40% $FeSO_4$ by wt	CH40Fe
6	Chitosan loaded with 30% $FeSO_4$ and 10% maltodextrin by wt	CH30FeMD
7	Chitosan loaded with 40% $FeSO_4$ and 10% maltodextrin by wt	CH40FeMD
8	Chitosan loaded with 40% $FeSO_4$, 10% maltodextrin surface coated with melted soy stearin in DCM	CH40FeMDST
9	TPP-induced cross-linked chitosan loaded with 50% $FeSO_4$ by wt	CH50FeTPP
10	TPP-induced cross-linked chitosan loaded with 60% $FeSO_4$ by wt	CH60FeTPP

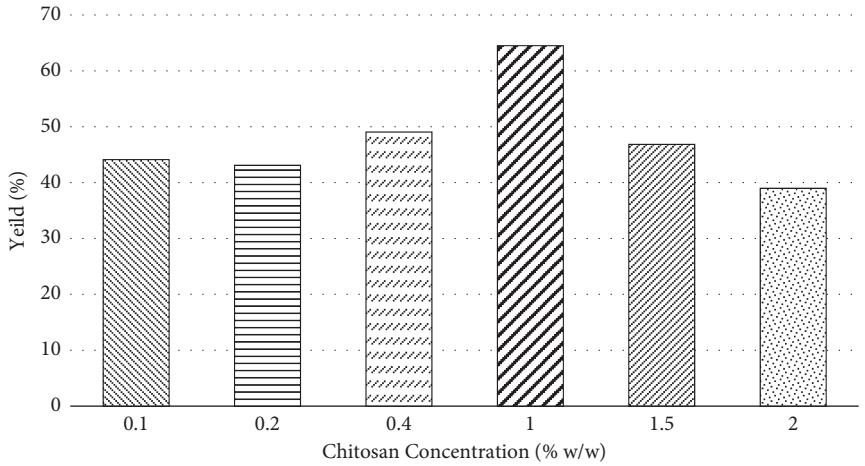

Figure 1: Spray-drying yield (% w/w) of iron-free chitosan microcapsules containing 0.2–2% w/w chitosan in feed solution. Maximum powder recovery was obtained with 1% w/w chitosan in feed solution.

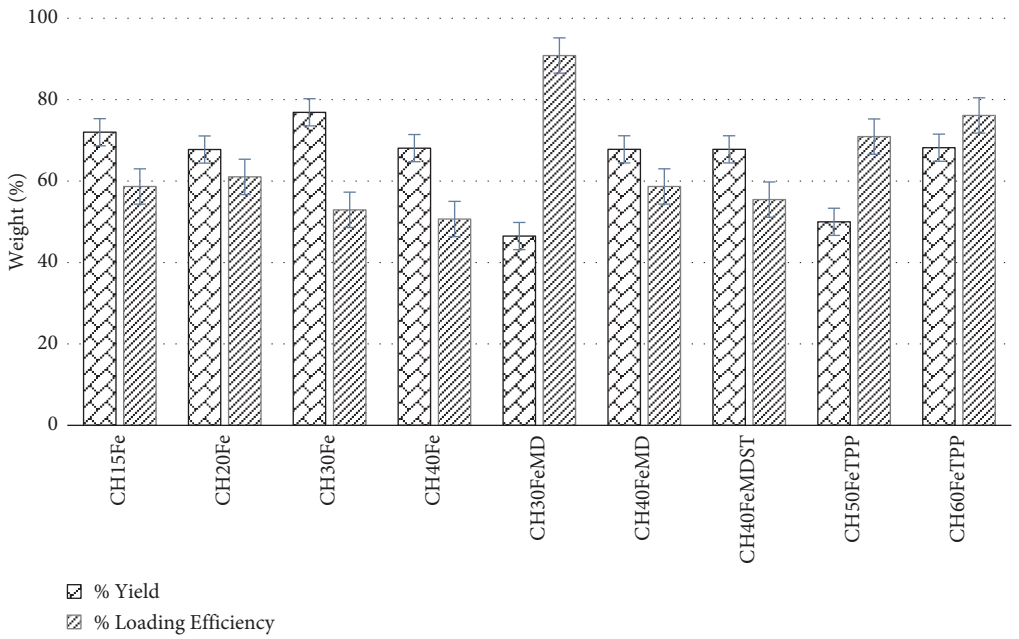

Figure 2: Comparison of spray-drying yield (% w/w powder recovery) and iron-loading efficiencies (% w/w iron in the microcapsules after drying) in various microcapsules (value ± SD, $n = 6$).

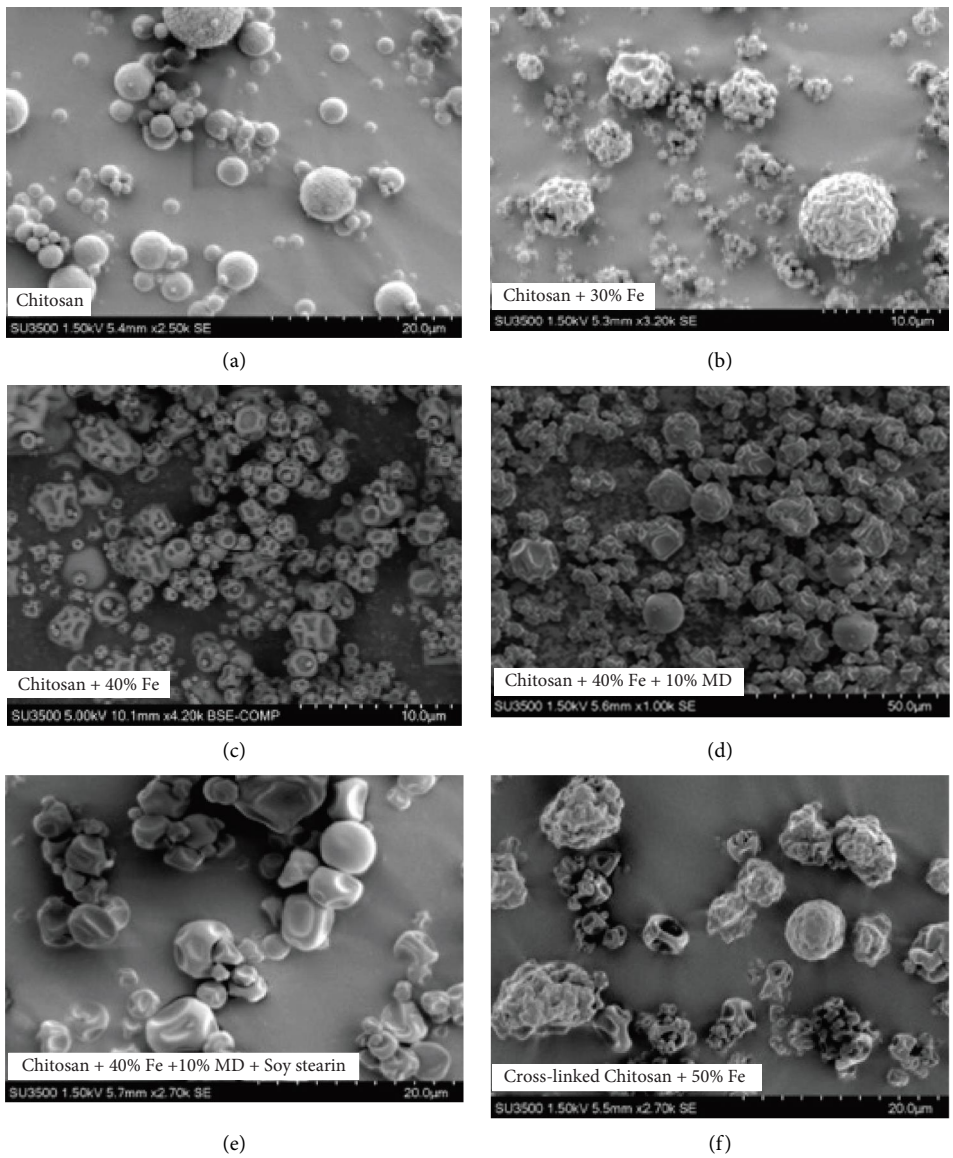

Figure 3: (a–f) SEM images of the selected chitosan microcapsules containing various concentrations of iron and secondary coating, taken at 1000–4000x magnification.

Table 2: Relative atomic abundance of elements at the surface of microcapsules analyzed by XPS at 10 nm depth.

Peaks (P)\peaks	Relative atomic abundance % wt				Av. particle size
Position (pos.)	C1s	O1s	S2p	Fe2p	μm ± SD
Chitosan	62.75	37.16	0.02	0.07	3.55 ± 1.81
CH30Fe	63.49	32.56	1.26	2.68	4.35 ± 1.53
CH40Fe	61.71	33.44	1.33	3.52	2.06 ± 0.68
CH30FeMD	55.48	41.73	1.28	1.52	4.32 ± 1.93
CH40FeMD	55.18	41.86	1.46	1.50	10.98 ± 4.71
CH40FeMDST	65.97	30.28	2.21	1.50	10.27 ± 1.24
CH50FeTPP	53.65	39.79	1.78	4.78	3.75 ± 2.08

Elemental chemical states C1s, O1s, S2p, and Fe2p were chosen as primary XPS regions to quantify elements. Average particle size was determined by SEM.

surface elemental composition. The size of most of the chitosan-based particles was in the range of 2–4 μm, while addition of maltodextrin increased the overall size of particles (~10 μm).

3.3. Surface Composition and Relative Atomic Abundance. Relative atomic abundance at the surface of microcapsules (0–10 nm depth) has been examined using XPS. In this method, X-rays are used to irradiate the sample surface.

Electrons ejected from the sample surface are counted, and their energy spectrum is recorded. A representative spectrum of elements is generated from the sequences of energies from the bound state of electrons at the surface above the background. The peak intensity and peak positions were quantified to determine the elemental and chemical composition of the material at the surface. XPS spectra of chitosan microcapsules with and without loaded iron were recorded for C1s, Fe2p, O1s, and S2p, representing dominant elements in chitosan and ferrous sulphate. Comparing the samples, it was observed that increasing iron with a fixed chitosan coating led to more exposed iron at a depth of 10 nm (Table 2). However, incorporating maltodextrin as a secondary coating material increased the coating to core ratio by 10%, resulting in improved surface protection. Exposed surface iron was reduced from 2.68% and 3.52% to 1.52% and 1.50%, respectively, with the addition of 10% w/w maltodextrin for 30% and 40% w/w. Cross-linking, on the other hand, did not appear to have any major impact on surface iron exposure (Table 2).

3.4. Iron Release. The investigation of iron release served two primary purposes: to assess the in vitro digestibility profile and to examine the effect of boiling temperature, which mimics tea-brewing conditions. The objective was to determine whether particles would retain iron until the tea was consumed, allowing for subsequent release and absorption in the digestive system, thereby ensuring bioavailability. In other words, the coating was expected to completely dissolve in stomach acids while maintaining its integrity at high temperatures for at least 10–15 minutes. The iron release profile of selected microparticles is presented in Figures 4–6. It was observed that release increased with an increase in iron loading in the microparticles in constant boiling water Figure 4. An intriguing phenomenon was observed in the samples with the same iron loading but different surface treatments. In the case of CHFe40MDST, which featured a hydrophobic soy stearin surface overcoat, lower iron release was observed compared to the CH40MD sample with the same iron loading (Figure 5). This difference in iron release was evident between the 10-minute and 25-minute time frame.

Chitosan-based microcapsules exhibited significant iron release in an acidic environment (Figure 5). Sample CH30Fe, with the lowest iron content, released most of it within 30 minutes. In contrast, sample CH40MDST, which had a hydrophobic coating on the outer surface, exhibited distinct release kinetics compared to CHFeMD with the same iron loading and a burst effect. The hydrophobic surface coating of CH40MDST resisted the dissolution and release of iron in the acidic medium for 30–90 minutes. Furthermore, cross-linking at two different iron concentrations also resulted in different release profiles, significantly impacting iron release, even at higher iron concentrations. For example, CH60TPP released 60% of iron within two hours, while CH50TPP released 30% total iron. Except for the soy stearin coating, all of the microcapsules demonstrated a desirable burst effect in the acidic medium, attributed to the solubility of chitosan under acidic conditions.

Iron release at pH 7 using phosphate buffer (Figure 6) was measured. Samples prepared with native non-cross-linked chitosan with 30–40% iron loading released as little as 5-6% of iron after 1.5 hours, indicating reverse-enteric behaviour of chitosan polymers. However, tripolyphosphate-induced cross-linking of chitosan not only modified the viscosity of the solution, which was observed during the sample preparation, but also altered the binding properties and affected the solubility of chitosan at neutral pH. Both CHFe50TPP and CHFe60TPP showed drastic release differences at neutral pH during the same time frame. However, both 30% and 40% iron loading led to similar iron release by the end of two hours.

3.5. Iron-Fortified Indian-Style Milk Tea. The addition of EDTA prevented iron-polyphenol complex formation in iron-fortified tea in the absence of milk [11]. However, in India, tea is generally prepared by boiling tea granules with water and milk. The pH of this tea is higher (~6.0–6.4) (Table 3) than that of tea brewed in water (pH ~4.3–4.5). At higher pH, iron-polyphenol complex formation is more likely to occur. Also, the evaporation of water as a result of boiling increases polyphenol concentration in tea. The complex interactions between milk components, iron, tea polyphenols, and iron absorption enhancers (EDTA or ascorbate) contribute to the darker colour of iron-fortified tea when prepared in milk [20]. For visual evaluation, unencapsulated iron with EDTA as a chelating agent or sodium ascorbate (also at a 1 : 2 molar ratio) as a reducing agent was tested. The colour of the beverage resembles hot chocolate which is expected to be unacceptable to the average consumer (Figure 7). Physical entrapment of iron is required by an inert coating material that can delay iron-polyphenol complex formation until tea is consumed. Unfortunately, most encapsulating agents used in the food and pharmaceutical industry are readily dissolved at high temperatures. It was hoped that the chitosan coating would delay iron release sufficiently for acceptable organoleptic quality in milk tea. Iron capsules with various formulation variables were tested by preparing iron-fortified tea. After analyzing the total iron content of each microcapsule (Table 3), the specified amount of powder and EDTA (1 : 2 Fe: EDTA molar ratio) were added to the tea. As in all previous formulations, the taste of the tea was acceptable, and the colour of the tea is the foremost indicator of the success of a formulation. To compare the difference between regular tea and tea with unencapsulated iron, positive and negative controls were also prepared. Figure 8 shows the visual comparison of colour after five min of cooked milk tea with selected formulation variables 2–5 (listed in Table 3) along with regular tea and unencapsulated ferrous sulphate. After a few minutes, tea prepared with CH30Fe and CH40FeMD started to develop a darker colour, while the colour of cross-linked CH50TPP remained stable for more than 15 min of cooking. Lower delta E values, presented in Table 3 ($\triangle E = 6.60$), and slight visible difference (Figure 8) of colour from regular tea led us to further sensory evaluation.

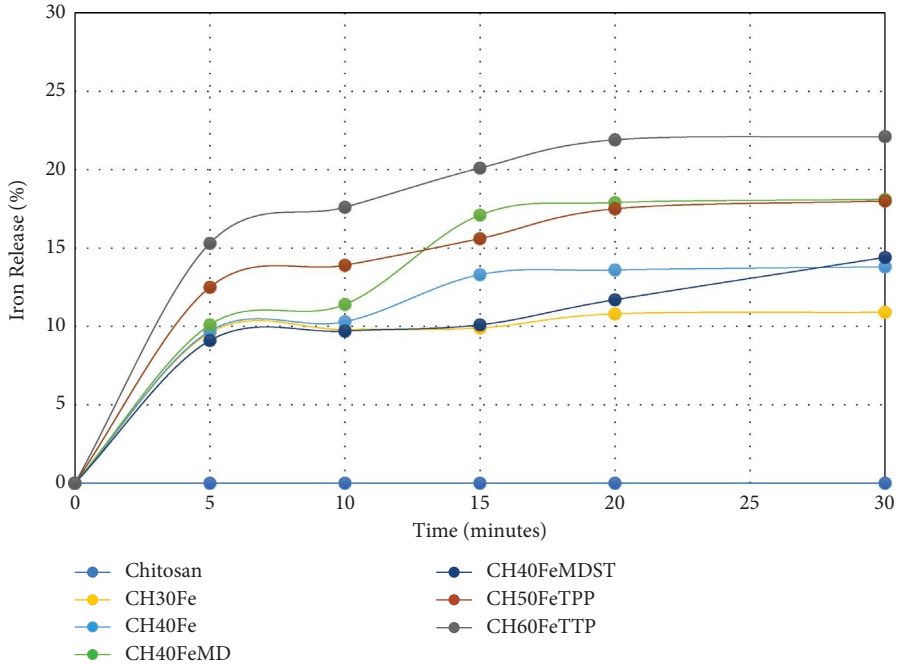

Figure 4: Iron release profile of selected microcapsules in boiling water (95 ± 5°C) for 30 min.

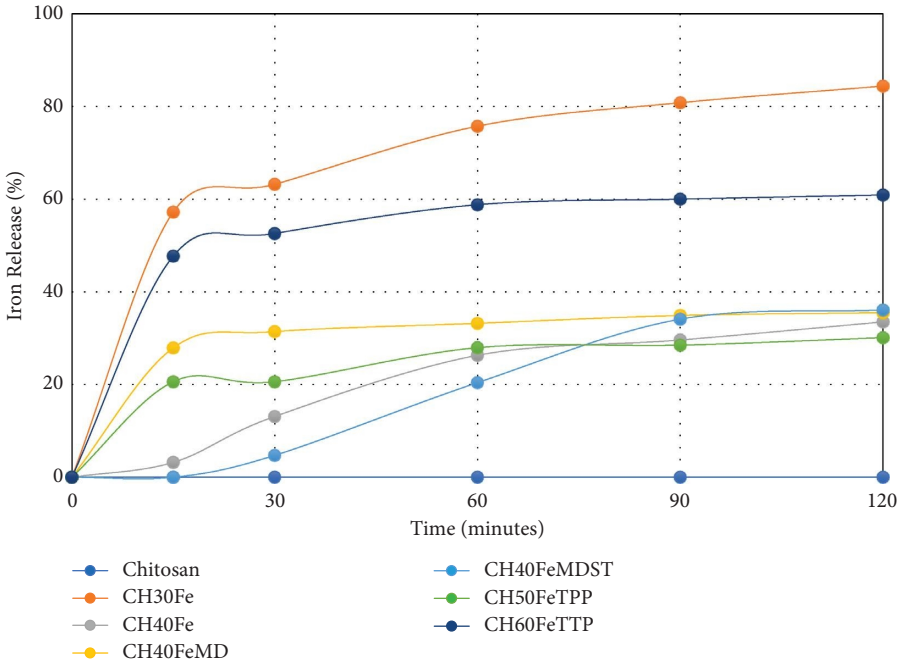

Figure 5: Iron release profile of selected microcapsules in acidic pH 1 in 0.1 N HCl at 37°C for 2 hours.

Cross-linked CH50TPP was selected and assessed for sensory profile analysis as presented in (Table 4). Sensory testing was conducted to score CH50TPP with and without the addition of chelating agents. Two selected agents were disodium EDTA and sodium ascorbate. Also, an additional sample (CH50TPP + EDTA, Cap) was prepared where EDTA was added (1 : 2 molar ratio) in the microcapsules before spray drying as part of coating formulation. It was observed that the most acceptable tea overall was tea-containing iron microcapsules and disodium EDTA added separately as a powder. It was also ranked highest for flavour and colour. The mouthfeel was slightly worse than plain or regular tea, and there is room for improvement in the domain of aroma. Tea with added iron microcapsules in the absence of disodium EDTA was tested to determine if disodium EDTA had a negative effect on acceptability. However, acceptability was higher when disodium EDTA was present. It can be inferred that EDTA has the ability to

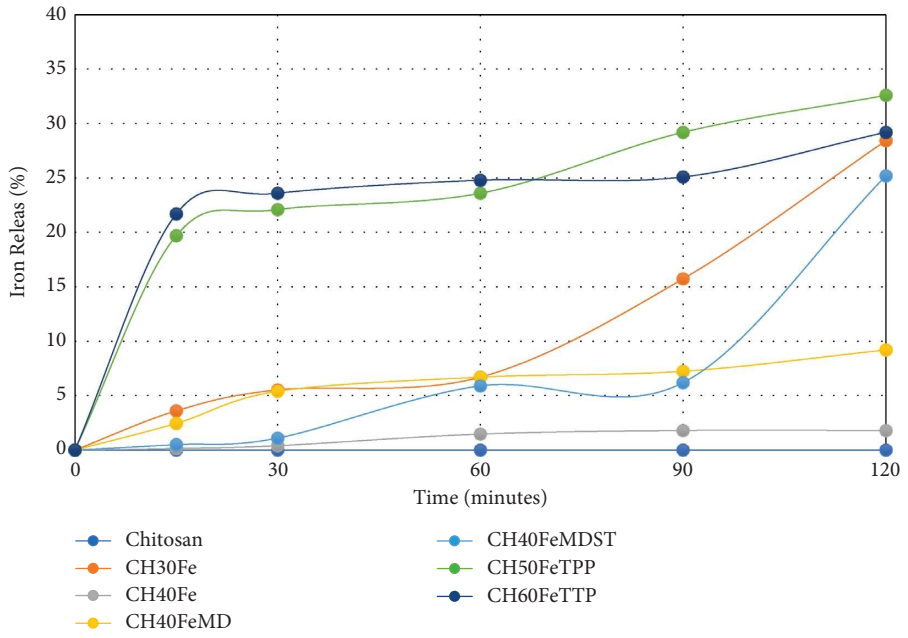

Figure 6: Iron release profile of selected microcapsules at pH 7 in phosphate buffer at 37°C for 2 hours.

Table 3: Colour and pH of milk tea fortified with various iron microcapsules and EDTA (value ± SD, where $n = 3$).

No	Samples	Fe in microcapsules (%)	Water loss on boiling (%)	pH	$\triangle E$	Colour
1	1% w/v tea in milk	0	8.91 ± 0.46	6.35	—	Standard
2	Tea + CH30Fe + EDTA	4.42 ± 0.12	9.68 ± 1.89	6.27	12.46	Unacceptable
3	Tea + CH40MD + EDTA	7.45 ± 0.20	9.51 ± 0.65	6.28	8.86	Acceptable
4	Tea + CH40MDST + EDTA	7.04 ± 0.15	8.99 ± 0.53	6.29	12.33	Unacceptable
5	Tea + CH50TPP + EDTA	9.31 ± 0.31	8.69 ± 0.68	6.33	6.60	Acceptable
6	Tea + $FeSO_4$ $7H_2O$ + EDTA	20.14	8.74 ± 1.15	6.35	19.91	Negative control

The iron concentration in tea was adjusted to 4 mg per cup of tea.

Figure 7: Indian-style milk tea (1) control (no iron), (2) tea with a 1 : 2 molar ratio iron to ascorbate, and (3) tea with a 1 : 2 molar ratio iron to EDTA.

chelate exposed iron from the microcapsules, thus protecting against colour changes. Tea formulations incorporating both iron microcapsules and disodium EDTA could be advantageous particularly in terms of colour acceptability in addition to improving Fe bioavailability. Tea with iron microcapsules and ascorbate powder added separately matched the acceptability of plain tea in every category including the highly ranked mouthfeel and aroma. However, implementing such a strategy would require further testing into the adequate amount of ascorbate to be added to tea to improve iron bioavailability. Therefore, the most promising formulation for iron-fortified tea contains iron microcapsules and disodium EDTA added as a separate powder.

While chitosan was found to be the best coating material, it still has some limitations. The high viscosity at low concentrations limits its use at low solid loading. Addition of maltodextrin increased the solid concentration and improved the spray process and coat to core ratio, but no additional advantage for its use in sensory or iron release profile was observed. Cross-linking, on the other hand, provides an opportunity to utilize the flexibility of chitosan as a coating material with desired and improved functional properties. Further investigation can be conducted to determine the optimal concentration of the cross-linking agent, such as TPP or other suitable cross-linkers. In

Figure 8: Indian-style milk tea brewed with iron microcapsules and EDTA (2–4), visual colour comparison with regular tea as a positive control (1), and unencapsulated iron as a negative control (6) as listed in Table 3.

Table 4: Sensory profile of milk tea fortified with selected iron microcapsules, disodium EDTA, and sodium ascorbate.

Samples	Overall ($n = 8$)	Flavour ($n = 8$)	Colour ($n = 8$)	Mouthfeel ($n = 8$)	Aroma ($n = 6$)*
Tea	27	28	30	28	24
Tea + CH50TPP	26	21	27	26	15
Tea + CH50TPP + EDTA	29.5	29	31	27	17
Tea + (CH50TPP + EDTA) cap	26	28	23.5	24	17
Tea + CH50TPP + ascorbate	27	28	30	28	24
Total	135.5	134	141.5	133	97

Participants rated the parameters using a 5-point scale, which was weighted (really bad (x1); bad (x2); neutral (x3); good (x4); very good (x5)). The responses for each parameter were multiplied by their respective weight and totaled. *There were 8 participants, of whom 2 were not able to sense aroma. The iron concentration in tea was adjusted to 4 mg per cup of tea.

addition, employing different techniques to explore the mechanism and extent of chitosan cross-linking should provide a better understanding of the process and potentially improve the sensory quality of fortified milk tea.

4. Conclusions

We proposed to fortify black tea with iron in a manner that does not impact the sensory properties of Indian-style milk tea upon brewing while maintaining its iron bioaccessibility. In order to prevent the formation of colour due to the complexation of iron with polyphenols in the presence of milk, iron can be encapsulated to inhibit its release and interaction with tea polyphenols prior to tea consumption. Iron complexes dissociate in the stomach, and absorbable FeEDTA complexes re-form as pH is raised in the intestine. Therefore, it is suggested that iron be separated from the tea until it is ingested by using a polymer coating such as chitosan. However, more work will be required to find an encapsulation formulation that can be stabilized in hot tea for the typical duration of consumption, preferably for 30 min or more. Considering that many commercial sources of chitosan are derived from animal-based materials, there may be limitations to its use for populations where animal products are avoided due to vegetarianism or religious reasons. Therefore, it is recommended that plant-based chitosan formulations be explored as an alternative to animal-based chitosan. This could improve the applicability and acceptance of chitosan-based products among diverse populations with varying dietary preferences and cultural considerations.

Data Availability

The data supporting the findings of this study are available within the paper. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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