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Research Article

Iron-Polyphenol Interaction Reduces Iron Bioavailability in Fortified Tea: Competing Complexation to Ensure Iron Bioavailability

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Tea seems to be like a logical substrate for iron fortification; however, its fortification with iron presents technical challenges as tea polyphenols form a blue complex with iron that makes both of them unavailable for absorption. The objective of this work was to develop an effective technology, to prevent the interaction of iron and polyphenols by using EDTA as a competing complexing agent. Fortified tea was prepared from premix, prepared by spraying iron and sodium EDTA into tea leaves. Iron concentration in tea was adjusted to 5 mg/cup. Iron content was measured by AAS and the iron-polyphenol complex by spectrophotometry at 560 nm. Sensory evaluation was carried out in order to determine if fortification affects the properties of tea. A molar ratio of 1 : 2 Fe : EDTA was able to avoid complex formation and provide 4 mg of iron per cup of brewed tea. The fortified tea had a similar colour and flavour as ordinary tea, without the development of off-flavours. However, fortified tea with a ratio lower than 1 : 2 had a darker colour and off-flavours. By the addition of EDTA in a molar ratio ≥1 : 2, it is possible to produce an iron fortified tea without the formation of off-flavours.

1. Introduction

Iron deficiency is the most commonly recognized form of nutritional deficiency in developing countries as well as in affluent societies. Food fortification involves the addition of nutrients to foods irrespective of whether or not the nutrients were originally present in the food. When using appropriate vehicles food fortification can lead to relatively rapid improvements in the micronutrient status of a population at a very reasonable cost, especially if advantage can be taken of existing technology and local distribution networks.

Iron is the most difficult mineral to add to foods as it is difficult to ensure adequate absorption. A main problem is the presence of absorption inhibitors, such as phytic acid or phenolic compounds, in the food vehicle [1], that may reduce the bioavailability and affect the sensory properties of the fortified food. Another critical step in the development of an iron fortified food is the selection of an iron compound that is both unobtrusive and well absorbed. Most soluble and absorbable iron compounds cause unacceptable colour and flavour changes in foods. Although less water-soluble iron compounds typically cause no organoleptic problems, they are poorly absorbed because they do not dissolve completely in the gastrointestinal tract during digestion. A key task in developing a fortification program is choosing a suitable food vehicle.

Tea is the most highly consumed beverage in the world, after water. It is a relatively low cost beverage consumed by all socioeconomic strata in many parts of the developing world. Tea contains substantial amounts of polyphenols that have unique biological activities and may be responsible for many of the health benefits of tea. However, tea polyphenols interfere with iron absorption by complex formation with iron in the gastrointestinal lumen, making iron less available for absorption [2, 3]. The degree of iron absorption inhibition can be related to the amount and type of phenolic compounds, type of iron compound, and pH. Hurrell et al. [4] and Disler et al. [5] found that the consumption of tea

along with iron can reduce iron absorption by 90%, due to the formation of coloured iron-polyphenol complexes. Ferrous sulphate fortified sugar added to beverages containing high concentrations of polyphenols, such as tea or coffee, promotes rapid changes in the colour of the beverages.

The effect of inhibitors of iron absorption can be avoided by using protected iron fortification compounds [6] such as ferric sodium EDTA. Iron in this form is stable, highly bioavailable, and not affected by preparation conditions and has fewer undesirable effects, such as rancidity and organoleptic problems, than other water-soluble fortificants [7]. In the presence of inhibitors, Fe(III)-EDTA is better absorbed, since it prevents iron from binding to these inhibitor compounds [1]. Evidence supports the use of Fe(III)-EDTA to fortify cereal products which contains considerable amounts of phytic acid, increasing iron absorption 2- to 3-fold compared to ferrous sulphate [8, 9]. Fe(III)-EDTA was tested as sugar fortification resulting in a pale yellow fortified sugar. However, the addition of fortified sugar to coffee or tea also resulted in an immediate marked colour change to deep blue, due to its complexation with polyphenols [10, 11]. The effectiveness of EDTA as an iron enhancer depends on its stability constant, which is affected by pH, the molar ratio of the chelator-to-metal ion, and the presence of competing metal ions capable of forming complexes with EDTA. Sodium EDTA is recognized as Generally Recognized as Safe (GRAS) by the Food and Drug Administration (FDA) with a limit of 165 mg/kg weight and unlike ascorbic acid is stable during processing and storage. Sodium EDTA acts as a chelating agent which prevents iron from binding to phytic acid or phenolic compounds that would otherwise inhibit iron absorption. The use of NaFeEDTA as a food additive has recently been reviewed by the International Nutritional Anemia Consultative Group (INACG) and was strongly recommended as the most suitable iron fortificant for use in developing countries.

Consequently, the objective of this study was to study the effect of EDTA on iron-polyphenol complex formation in iron fortified tea and the bioavailability of added iron. The sensory properties of iron fortified tea, prepared using different iron : EDTA ratios, were compared with natural brewed tea (no fortification), in order to assess the acceptability of iron fortified tea.

2. Materials and Methods

2.1. Materials. Behora (Assam, Golaghat, India) black tea leaves were used for all the experiments. HPC used as adhesive was kindly donated by Ashland Aqualon Functional Ingredients (Wilmington, USA). Ferric EDTA was purchased from Bio Basic (Ontario, Canada), EDTA disodium salt was obtained from BioShop (Ontario, Canada), and ferrous sulphate was obtained from Fisher Scientific (Ontario, Canada).

2.2. Methodology

2.2.1. Fortified Tea Preparation. The target iron content for fortified tea was 5 mg iron/cup, which can supply the 30% of the RDI when consuming 2 cups of fortified tea daily. The fortification procedure was carried out by preparing a premix containing 10 mg iron per gram of tea leaves, using ferric EDTA or ferrous sulphate as iron source and with the addition of different molar ratios Fe : EDTA (1 : 1, 1 : 1.5, 1 : 1.75 and 1 : 2) to determine the minimum ratio able to avoid the reaction. Iron compounds and EDTA were attached to tea leaves by spraying a solution containing pharmaceutical grade HPC (10% of total solids). Tea leaves were dried in a freeze dryer for 24 h. Finally, the premix was sieved (<350 μm) and nonadhering added powder was collected.

Fortified tea was prepared by mixing 10 g of premix with 40 g of tea leaves, which gives a final iron concentration of 2 mg/g of fortified tea. Each cup of brewed tea was prepared from 2.5 g of tea leaves in 250 g of boiling water.

2.2.2. Iron Analysis. In order to evaluate if the iron compounds can be attached to tea leaves, the amount of iron in the premix and fortified tea was determined using AAS. Briefly, samples were digested with 25 mL of sulfuric acid and 2.5 mL of nitric acid at ~250°C for 90 minutes. Then, it was cooled at room temperature and diluted to 250 mL with distilled water. The extracts were analysed for iron content using AAS. A calibration curve from 0 to 20 ppm was prepared in a 2 M aqueous solution of sulfuric acid. Iron content in brewed tea was measured directly using a calibration curve prepared in water.

2.2.3. Effect of EDTA on Iron-Polyphenol Complex Formation. Iron-polyphenol complex was measured using spectrophotometry. 2 L of tea was freshly prepared and brewed for 5 minutes. Tea leaves were removed and the liquid was distributed into 6 beakers (250 mL each). A stock solution of ferric EDTA was prepared and increasing volumes were added to each beaker. The maximum UV and visible light absorbance wavelength for the iron-tea polyphenol complex was 560 nm. Maximum absorbance at this wavelength was measured after 5, 20, and 60 minutes.

2.2.4. Sensory Analysis

(1) Tea Leaves. The visual impact of iron fortification on the colour and appearance of tea leaves was evaluated by 8 panelists. They received a set of 3 samples and they were asked to evaluate differences between the samples.

(2) Brewed Tea. A Flash Profile test was used for evaluation of the tea as it allows a rapid positioning of products according to their major sensory differences [12]. Iron fortified tea samples, prepared using different iron : EDTA ratios, were compared to natural brewed tea (no fortification). Samples were prepared by brewing 2.5 g of tea leaves in 250 g of boiling water and evaluated during the next 5 minutes. Selected discriminating attributes were natural colour, clear appearance, presence of off-flavours, and natural flavour. Each attribute was evaluated on a 0 to 9 scale, where 0 means no perception of the attribute and 9 intense perception of the attribute. This test can give us the sensory map of each sample so that it can be compared with the corresponding to natural tea.

Table 1: Iron content in premix (target 10 mg/g), fortified tea (target 2 mg/g), and brewed tea (5 mg/cup).

Fe : EDTA ratio	Iron content in premix (mg/g)	Iron content in fortified tea (mg/g)	Iron content in brewed tea (mg/cup)
1 : 0 (FeSO4)	$9.1^{a} \pm 0.2$	$1.9^{a} \pm 0.2$	$4.5^{a} \pm 0.2$
1 : 1	$9.1^{a} \pm 0.2$	$1.8^{a} \pm 0.1$	$4.5^{a} \pm 0.1$
1 : 1.5	$8.9^{a} \pm 0.3$	$1.8^{a} \pm 0.2$	$4.4^{a} \pm 0.1$
1 : 1.75	$8.9^{a} \pm 0.2$	$1.8^{a} \pm 0.2$	$4.4^{a,b} \pm 0.2$
1 : 2	$8.3^{b} \pm 0.1$	$1.7^{a} \pm 0.1$	$4.2^{b} \pm 0.1$

Note. Different letters in the same column indicate significant differences ($P \leq 0{,}05$).

Table 2: Iron (mg/cup) forming complex at different iron : EDTA ratios at tea pH.

Time (min)	Complexed iron (mg/cup) at different Iron : EDTA ratios				
	1 : 0	1 : 1	1 : 1.5	1 : 1.75	1 : 2
5	$3.8^{a} \pm 0.3$	$3.1^{b} \pm 0.1$	$2.1^{c} \pm 0.2$	$2.1^{c} \pm 0.2$	$0.1^{d} \pm 0.0$
20	$4.0^{a} \pm 0.2$	$3.2^{b} \pm 0.2$	$2.2^{c} \pm 0.3$	$2.3^{c} \pm 0.1$	$0.1^{d} \pm 0.1$
60	$4.1^{a} \pm 0.1$	$3.2^{b} \pm 0.1$	$2.3^{c} \pm 0.2$	$2.3^{c} \pm 0.3$	$0.1^{d} \pm 0.0$

Note. Different letters indicate significant differences between groups ($P < 0.05$). Values represent means ± SD ($n = 3$).

2.2.5. In Vitro Bioaccessibility. Tea was prepared by brewing 2.5 g of fortified tea leaves in 250 g of boiling water. The bioaccessibility was estimated by digesting 100 mL of brewed tea at pH 1 and 4 mL of 4% pepsin solution for 2 h at 37°C. In order to stop the reaction, the sample was cooled down in an ice bath for 10 minutes. To simulate gut digestion, the pH was increased to 6.5 and pancreatin/bile salt solution was added and agitated for 2 h at 37°C. The liquid was centrifuged and the supernatant was filtered using a 0.45 μm syringe filter. The final iron content was measured using AAS and the bioaccessible portion was reported as the amount of added iron remaining in the liquid after the digestion process. The resulting liquid was used to assess the transport through Caco-2 cells using a Caco-2 Assay kit (ReadyCell, Spain) in a ready-to-use cell-based assay format. After 2 h of incubation at 37°C, the decrease in the concentration of iron in both sides of the monolayer was measured, in order to determine the bioavailability of iron in fortified tea.

2.3. Statistical Analysis. The reported results correspond to the arithmetic mean of three batches ± standard deviation. One-way variance analyses were carried out to establish significant differences between values obtained for the same sample. The differences of means between samples were resolved by confidence intervals using a Bonferroni test in the Statgraphics 5.0 program (Manugistics Inc., Rockville, MA). The level of significance was set for $P \leq 0.05$.

3. Results

3.1. Iron Content in Premix, Fortified Tea, and Brewed Tea. The amount of iron that can be attached to tea leaves for premix preparation determines the iron content in fortified tea leaves and brewed tea, and consequently the amount of iron that would be ingested through normal tea consumption. Table 1 shows the iron content in premix, fortified tea, and brewed teas prepared by adding different ratios Fe : EDTA. Our technology for fortifying tea with iron allows us to attach more than 80% of the added iron and EDTA to tea leaves, depending on the Fe : EDTA ratio. As can be observed, iron content decreased when increasing the Fe : EDTA ratio. This might be due to the higher amount of solids that needs to be dissolved in the same amount of water. All samples have good attachment results as HPC is a good adhesive and film-forming polymer [13] that keeps iron and EDTA attached to the tea leaves. When tea leaves are brewed in hot water, HPC will dissolve and release the iron compound and EDTA into de brew.

3.2. Iron-Polyphenol Complex Formation. Iron-polyphenol complex cannot be transported through the intestinal cells, so it cannot be absorbed by the organism, and instead, it is excreted in the feces. The reaction of polyphenols and iron is pH dependant as catechol and gallol groups need to be deprotonated for metal binding. Polyphenols are easily deprotonated at physiological pH in the presence of iron and form very stable complexes [14]. While decreasing the pH, H+ ions affect the protonation state of the polyphenol ligands decreasing their ability to chelate iron [15]. At tea pH (around 5) the stoichiometry of the reaction between iron : gallic acid is 1 : 2. At pH 7, the stoichiometry iron : gallic acid is 1 : 3, while at pH 1 (stomach) the reaction does not occur. A cup of 250 mL of black tea, prepared by brewing 2.5 g of tea leaves in 250 mL of water, used for these experiments has 187 mg of polyphenols measured as gallic acid equivalents, which is in accordance with the values reported by Pérez-Jiménez et al. [16]. In our fortification experiments, 10 mg (0.18 mmol) of iron was added per 2.5 g of tea leaves (187 mg of gallic acid; 1.1 mmol) and the stoichiometry at tea pH (1 : 2); the amount of polyphenols in a cup of tea is more than enough to complex the added iron; so iron is the limiting reactant and the complex will be expressed as moles of iron forming the complex. Iron-polyphenol complex formation, measured as mmol of iron, when adding different ratios of Fe : EDTA, is shown in Table 2. Complex formation decreases when increasing the Fe : EDTA ratio. Complex formation increased

Table 3: Bioaccessibility of iron in fortified water and tea when added in different Fe : EDTA ratios.

Fe : EDTA ratio	Bioaccessibility (% of added iron)	Complex formation (mg iron)
1 : 0 (FeSO4)	$8^{c} \pm 1$	$4.1^{a} \pm 0.1$
1 : 1	$54^{b} \pm 2$	$3.2^{b} \pm 0.1$
1 : 2	$97^{a} \pm 2$	$0.1^{c} \pm 0.0$
1 : 2 (in water)	$99^{a} \pm 1$	$0.0^{c} \pm 0.0$

Note. Different letters in the same column indicate significant differences ($P \leq 0.05$).

with time for the first hour of reaction; however, there was no further increase after 60 minutes.

When ferrous sulphate was added to tea more than 90% of the iron was complexed with polyphenols. The utilization of EDTA for protecting added iron resulted in reduced complexation of the added iron. Increasing the Fe : EDTA ratio decreased the amount of iron forming the complex from 71% at molar ratio of 1 : 1 to 2% of added iron forming complex when the molar ratio was 1 : 2. Further increase in Fe : EDTA molar ratio did not provide a significant further reduction in complex formation.

EDTA's effectiveness for iron protection depends on the pH, the molar ratio of the chelator to the ferric ion, and the presence of competing metal ions capable of forming complexes with EDTA. Of the nutritionally important metals, ferric ion (Fe^{+3}) has the highest stability constant ($\log k$ = 25.1), giving good iron protection even in the presence of other minerals from the diet (European Food Safety Authority (EFSA), 2010). Sodium EDTA acts as chelating agent and as such prevents iron from binding to phytic acid or phenolic compounds and the effect is dependant on the molar ratio.

3.3. Sensory Evaluation of Fortified Tea Leaves and Brewed Tea

3.3.1. Tea Leaves. The judges received a set of 3 samples of tea leaves (fortified tea at ratios 1 : 1; 1 : 2 and unfortified natural tea) and they were asked to evaluate differences between the samples. Fortified tea leaves using a ratio 1 : 1 were similar to natural tea leaves. The judges detected the presence of gray leaves in fortified tea leaves prepared using a Fe : EDTA ratio 1 : 2. Gray leaves were the result of the addition of higher amounts of EDTA as it is white. However, it was not considered detrimental since natural tea leaves are not uniform in colour. The consumption of instant tea (soluble tea powder) is increasing, which would make its fortification easier and less noticeable.

3.3.2. Brewed Tea. Tea quality judgment is a very challenging task due to multidimensional attributes of tea; however, the most important quality descriptors for black tea are aroma, astringency (bitterness), cleanliness (presence of off-flavours), and finish (lasting of flavour in mouth). It has been developed several methodologies for the evaluation of the sensory properties of tea such as electronic nose and electronic tongue and they describe aroma and smell, and astringency, briskness, and taste, respectively. In this study, judges were asked to evaluate three brewed teas, prepared with Fe : EDTA ratios 1 : 1; 1 : 2 and natural tea, in terms of natural colour, appearance, natural flavour, and presence of off-flavours, using a 0–9 hedonic scale. Figure 1 shows the sensory maps of fortified tea prepared with different Fe : EDTA ratios. As it can be observed the sensory map of samples 1 : 1 is very different from natural tea as it reveals the presence of off-flavours, absence of natural flavour, and colour. Fortified tea using a ratio 1 : 2 showed a very similar sensory map to natural tea, showing that the fortification procedure was able to avoid colour development and off-flavours due to the formation of iron-polyphenol complex.

In India, one of the principal problems encountered for evaluating black tea is that as tea industries are spread over dispersed locations and quality of tea varies considerably on agroclimatic condition, type of plantation, season of flush, and method of manufacturing [17]. To avoid differences, in this study we used for the entire project the same tea.

3.4. In Vitro Bioaccessibility of Iron Fortified Tea. Bioavailability is a key factor when developing a fortified food, as the micronutrient has to be delivered and released at the proper time and location in the body [18]. Bioavailability of many minerals can be readily assessed by in vitro methods. Gangloff et al. [19] evaluated the in vitro bioavailability of iron in meat using a simulated digestion system; while pepsin was used for the gastric phase, the intestinal phase was simulated by adding pancreatin and bile extract before the uptake of iron by Caco-2 cell monolayers was measured. Zhu et al. [20] used this approach to evaluate the iron uptake by Caco-2 cells when examining the influence of other food components, such as an iron absorption inhibitor, of different iron compounds (NaFeEDTA, $FeCl_3$, and $FeSO_4$). The authors observed that when the inhibitor was added, the absorption of each of the three iron compounds decreased to a similar extent.

The bioavailability depends on the bioaccessibility, defined as the amount of total micronutrient that is released after the digestion process to the gastrointestinal tract and is available for being transported and absorbed across de intestine. Bioavailability is the actual amount of the nutrient transported across the intestine into the bloodstream and it can be simulated using caco-2 cells. Table 3 shows the bioaccessible portion of added iron after simulated digestion of fortified tea prepared with different Fe : EDTA ratios. Three ratios were chosen (1 : 0; 1 : 1; 1 : 2). As it can be observed, there is a relationship between complex formation and bioaccessibility of iron from fortified tea: increased complex formation clearly decreases bioaccessibility. There

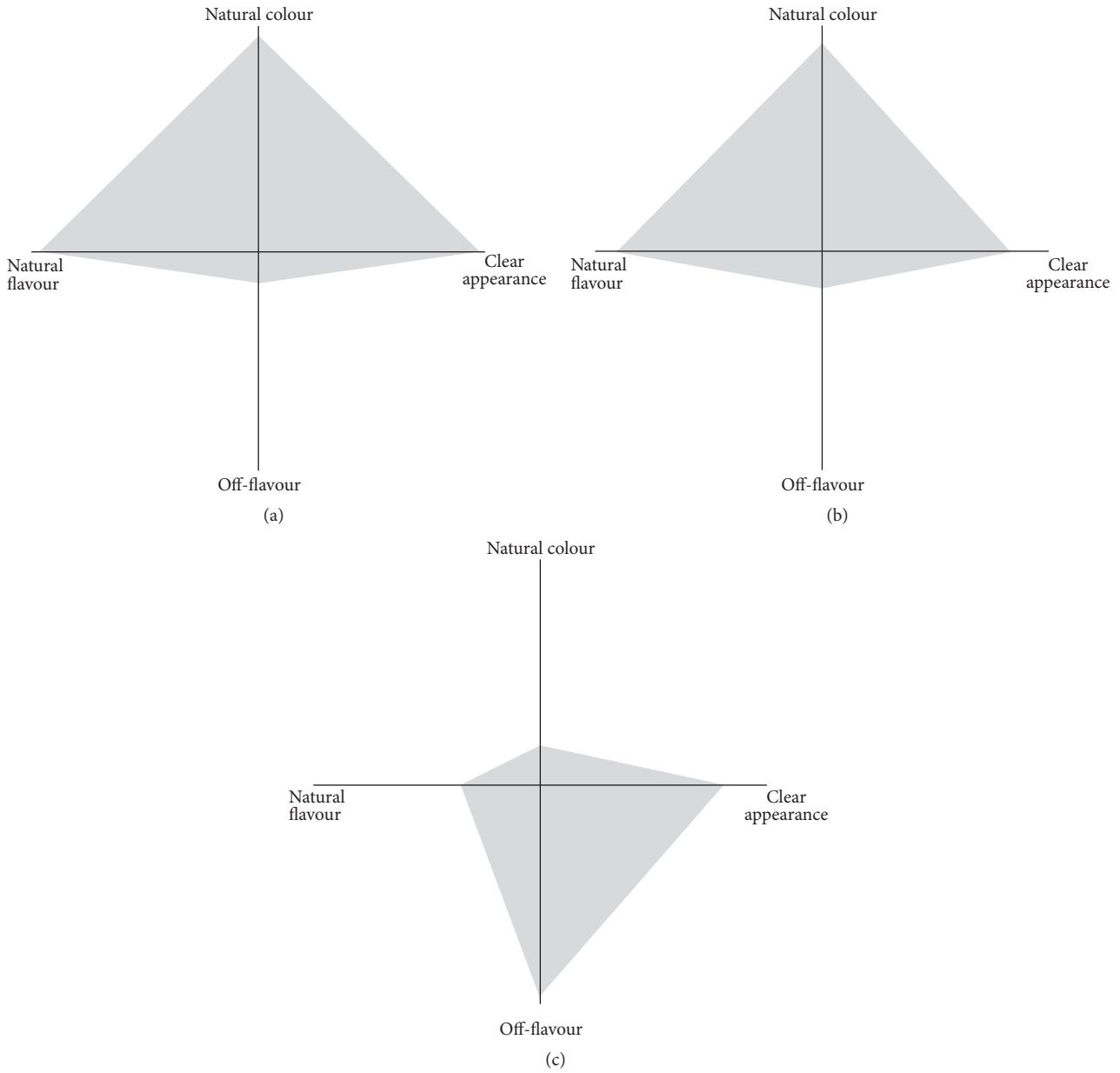

Figure 1: Sensory map of tea and fortified tea prepared using different Fe : EDTA ratios. (a) Natural tea, (b) 1 : 2 fortified tea, and (c) 1 : 1 fortified tea.

was a strong inverse linear relationship between complex formation and bioaccessibility. It seems that the iron-tea polyphenols complex cannot be digested by enzymes in the stomach and gut, making iron inaccessible for the next step. Fortified tea using a Fe : EDTA ratio 1 : 2 was as bioaccessible as Fe : EDTA ratio 1 : 2 in water, with almost 100% bioaccessibility.

From the three chosen ratios, 1 : 2 ratio was selected for transport and absorption experiments as it seems to be a promising formulation for obtaining a high iron bioavailability in fortified tea. A control using water instead of tea was carried out in order to evaluate if the tea matrix has an effect in iron bioavailability. Bioavailability of iron in 1 : 2 fortified tea was 62%, which means that from total added iron the 62% was transported through Caco-2 cells and becomes available for being used by the organism. Bioavailability of iron in water was 64%, which suggests that the addition of Na_2EDTA blocks the effect of iron absorption inhibitors present in black tea. These results suggest that when adding iron into tea in a molar ratio 1 : 2 it is possible to develop a fortified tea with the same bioavailability as it is when added to water. Zhu et al. [20] suggested that uptake of iron from NaFeEDTA by intestinal enterocytes is regulated by the dissociation of iron from EDTA and its reduction, just as simple inorganic iron sources do at the brush border membrane of the enterocyte in order to be absorbed. Even though, iron added along

with EDTA in fortified tea was significantly more bioavailable than when added with no protection (ratio 1 : 0), suggesting that the major effect of EDTA in iron bioavailability is the capacity of protecting it from complexation. Our results are consistent with work reported by MacPhail and Bothwell [21] who studied the iron absorption from a series of rice meals containing Na_2EDTA. The authors observed a significant improvement of absorption, over three times, at high EDTA concentrations.

4. Conclusions

Iron fortification of tea was successfully achieved by adding iron with Na_2EDTA to avoid complex formation with tea polyphenols. EDTA was added to tea leaves at Fe : EDTA ratios of 1 : 0; 1 : 1; 1 : 1.5; 1 : 1.75; 1 : 2. The different formulations were sprayed and attached to tea leaves using an adhesive to achieve a final iron concentration of 10 mg/g of premix. In the laboratory 83% of the added iron was attached to the tea leaves, forming a premix which was used to prepare fortified tea. Brewed tea achieved an iron concentration > 4 mg/cup. Complex formation was inhibited by adding EDTA in a Fe : EDTA ratio 1 : 2. In vitro bioavailability of added iron in the fortified tea was 62%, while in water it was 64% suggesting that the inhibitory effect of tea in iron absorption can be blocked by using EDTA at this molar ratio of 1 : 2. Initial sensory evaluation suggested that fortified tea was similar to unfortified tea in terms of flavour, and no noticeable flavours or colour changes were observed. Our approach was effective in preventing iron-polyphenol complex formation, providing a fortified tea with 4 mg iron per cup in a bioaccessible form.

Additional Points

Practical Application. Fortified tea is a feasible alternative for improving the iron status of iron deficient people. Tea is a cheap, consumed worldwide, rarely home-grown beverage and its fortification can be achieved without changing the production lines. However, iron fortification of tea has technical challenges due to complex formation between iron and tea polyphenols. The authors have developed a feasible technology able to provide bioavailable iron in tea.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] R. F. Hurrell, "Fortification: Overcoming technical and practical barriers," *Journal of Nutrition*, vol. 132, no. 4, pp. 806S–812S, 2002.

[2] M. Brune, L. Rossander, and L. Hallberg, "Iron absorption and phenolic compounds: importance of different phenolic structures," *European Journal of Clinical Nutrition*, vol. 43, no. 8, pp. 547–557, 1989.

[3] M. Brune, L. Hallberg, and A. N. N. B. Skanberg, "Determination of Iron-Binding Phenolic Groups in Foods," *Journal of Food Science*, vol. 56, pp. 128–131, 1991.

[4] R. F. Hurrell, M. Reddy, and J. D. Cook, "Inhibition of non-haem iron absorption in man by polyphenolic-containing beverages," *British Journal of Nutrition*, vol. 81, no. 4, pp. 289–295, 1999.

[5] P. B. Disler, S. R. Lynch, R. W. Charlton et al., "The effect of tea on iron absorption," *Gut*, vol. 16, no. 3, pp. 193–200, 1975.

[6] R. Gibson, "Technological approaches to combatting iron deficiency," *European Journal of Clinical Nutrition*, vol. 51, p. S25, 1997.

[7] R. Hurrell and J. Cook, "Strategies for iron fortification of foods," *Trends in Food Science Technology*, vol. 1, pp. 56–61, 1990.

[8] L. Davidsson, T. Dimitriou, E. Boy, T. Walczyk, and R. F. Hurrell, "Iron bioavailability from iron-fortified guatemalan meals based on corn tortillas and black bean paste," *American Journal of Clinical Nutrition*, vol. 75, no. 3, pp. 535–539, 2002.

[9] T. H. Bothwell and A. P. MacPhail, "The potential role of NaFeEDTA as an iron fortificant," *International Journal for Vitamin and Nutrition Research*, vol. 74, no. 6, pp. 421–434, 2004.

[10] J. D. Cook and M. E. Reusser, "Iron fortification: an update," *American Journal of Clinical Nutrition*, vol. 38, no. 4, pp. 648–659, 1983.

[11] F. E. Viteri, E. Alvarez, R. Batres et al., "Fortification of sugar with iron sodium ethylenediaminotetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations," *American Journal of Clinical Nutrition*, vol. 61, no. 5, pp. 1153–1163, 1995.

[12] J. Delarue and J.-M. Sieffermann, "Sensory mapping using Flash profile. Comparison with a conventional descriptive method for the evaluation of the flavour of fruit dairy products," *Food Quality and Preference*, vol. 15, no. 4, pp. 383–392, 2004.

[13] S. Karki, H. Kim, S. Na, D. Shin, K. Jo, and J. Lee, "Thin films as an emerging platform for drug delivery," *Asian Journal of Pharmaceutical Sciences*, vol. 11, pp. 559–574, 2016.

[14] N. R. Perron and J. L. Brumaghim, "A review of the antioxidant mechanisms of polyphenol compounds related to iron binding," *Cell Biochemistry and Biophysics*, vol. 53, no. 2, pp. 75–100, 2009.

[15] N. R. Perron, H. C. Wang, S. N. Deguire, M. Jenkins, M. Lawson, and J. L. Brumaghim, "Kinetics of iron oxidation upon polyphenol binding," *Dalton Transactions*, vol. 39, no. 41, pp. 9982–9987, 2010.

[16] J. Pérez-Jiménez, V. Neveu, F. Vos, and A. Scalbert, "Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database," *European Journal of Clinical Nutrition*, vol. 64, supplement 3, pp. S112–S120, 2010.

[17] B. Tudu, A. Jana, A. Metla, D. Ghosh, N. Bhattacharyya, and R. Bandyopadhyay, "Electronic nose for black tea quality evaluation by an incremental RBF network," *Sensors and Actuators, B: Chemical*, vol. 138, no. 1, pp. 90–95, 2009.

[18] O. Li, V. Dueik, and L. Diosady, "Microencapsulation of vitamins, minerals, and nutraceuticals for food applications," in *The Art and Science of Microencapsulation: An application Handbook for Food Industry*, A. Gaonkar, N. Vasisht, A. Khare, and R. Sobel, Eds., 2014.

[19] M. B. Gangloff, R. P. Glahn, D. D. Miller, and D. R. Van Campen, "Assessment of iron availability using combined in vitro digestion and Caco-2 cell culture," *Nutrition Research*, vol. 16, no. 3, pp. 479–487, 1996.

[20] L. Zhu, R. P. Glahn, K. Y. Chi, and D. D. Miller, "Iron uptake by Caco-2 cells from NaFeEDTA and FeSO4: Effects of ascorbic acid, pH, and a Fe(II) chelating agent," *Journal of Agricultural and Food Chemistry*, vol. 54, no. 20, pp. 7924–7928, 2006.

[21] P. MacPhail and T. H. Bothwell, "The prevalence and causes of nutritional iron deficiency anemia," in *Nutritional anemias*, S. J. Fomon and S. Zlotkin, Eds., vol. 30 of *Nestle Nutrition Workshop Series*, pp. 1–12, Raven Press, Vevey, Switzerland, 1992.