

Iron Fortified Sugar: Evaluation of Four Fortificants

Honors Thesis

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by

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ABSTRACT

Acronyms and abbreviations: NaFe-EDTA: iron (III) ethylene diaminetetraacetic acid; RDA: Recommended Dietary Allowance; BPDS: bathophenanthroline disulfonic acid; PVC: polyvinyl chloride

Key words: Sugar, Candy, Green Tea, Iron, Fortification

The objective of this study was to compare the impact of selected iron fortificants on the color of granulated sugar and two products sweetened with the sugar.

Granulated white sugar was fortified with NaFe-EDTA, iron bis-glycinate, elemental iron powder, or ferrous sulfate to yield an iron concentration of 0.12 mg/g sugar, with plain sugar as a control. Color change was monitored over three months by dissolving samples in water, and measuring the absorbance at 350 nm. A 12.5 g sugar sample was added to 250 mL of brewed green tea after each month and was monitored at 560 nm. Hard candy was made with each fortified sugar and the control (unfortified sugar), stored for three months, and monitored for color changes at 350 nm.

Color intensities of the fortified sugar relative to the unfortified control were as follows: iron bis-glycinate, NaFe-EDTA, ferrous sulfate, and elemental iron. These rankings changed little over time. Tea sweetened with fortified sugars ranked (most to least different from the control) as ferrous sulfate, iron bis-glycinate, NaFe-EDTA, and elemental iron. The candy didn't visually change over time, although there were initial differences from the control candy. The color of the samples ranked (most to least different from the control candy) as iron bis-glycinate, ferrous sulfate, elemental iron, and NaFe-EDTA.

The best fortificant for sugar depended on the application. Elemental iron was the best for granulated sugar, but NaFe-EDTA was most suitable for the candy, and perhaps the tea, since the elemental iron is insoluble in water.

INTRODUCTION

Over two billion people worldwide are iron deficient (Veneman 2006). According to the World Health Organization, 15-20% of the US population under 18 years of age suffers from iron deficiency (Rastogi 2002). Iron deficiency can cause several health disorders, loss of productivity, and economic loss (Cardoso de Paula R A and M Fisberg 2001). The best way to solve this issue, according to Cook and Reusser (1983), is food fortification because individuals that use a fortified product will then have access to more iron. The food selected for fortification should be affordable and compatible with the cultural practices in the target population. Refined table sugar (sucrose) is an acceptable vehicle because consumer intake is high in the US, 62.5 lbs. per capita in 2006 (Anonymous 2007). It is also a stable ingredient due to its dry, uniform, crystalline nature (Anonymous 2004). However, a form of iron that doesn't produce off colors or flavors, but is still adequately absorbed is required. Off colors might develop over time in the sugar as the iron reacts with the small amount (0.2%) of impurities found in refined sugar (Beckett and others 2006). Some of these impurities might be phenolic compounds, left over from the plant matter from which the sugar was extracted. Phenolic compounds are also found in drinks such as tea and if iron isn't strongly bound to chelating agents it will readily react with the tea tannins to form a violet complex (South P.K. and D.D. Miller 1998). Green tea's pale color, vs. black tea's dark brown color, makes the violet color more obvious in green tea. Very rarely is sugar consumed in its refined granulated state without being combined with other foods or transformed. The fortificants, for use in some confectionery items, should be able to withstand the melting and recrystallization of the sugar without reacting. Heme iron is more bioavailable than non-heme, but its dark color would be an issue. Also, sugar

fortified with heme iron would not be acceptable to many vegetarians. Therefore, non-heme iron was chosen as the focus of this experiment.

A study by Sari and others (2001) examined the effects of an iron-fortified chewy candy on the iron status of elementary school children in Indonesia. This study used 1mg of elemental iron/gram of candy. Their results were encouraging, showing a decrease in anemia and an increase of serum ferritin in the experimental group after the twelve-week study. The children seemed to have very little complaints about the organoleptic qualities of the fortified sugar. Sugar was fortified, in an Indian study, with 500ppm or 1000ppm iron from ferrous sulfate heptahydrate, ferrous glycine sulfate, ferrous sulfate, or ferrous fumarate and the shelf life of the fortified sugar was examined. In this study, the sugar was stored in clear containers; however, sugar is typically sold in paper bags (Anonymous 2004). There was no change in iron content during the six-month study, but an unacceptable discoloration was observed in all mixtures but the ferrous glycine sulfate. Panelists accepted the sugar in foods but due to the reaction with tannins, not in tea (Anonymous 2004). Based on the literature, an experiment that tests color differences and iron content of some of these fortificants would be a useful approach for determining an ideal iron fortificant for sugar.

Therefore, the objective of this study was to compare the impact of selected iron fortificants on the color of granulated sugar and on products made with the fortified sugar. It also aimed to verify that each serving (one tablespoon or 12.5g of sugar) contains 10% of the RDA, thus ensuring the targeted amount will be delivered to the consumer. It was hypothesized that the fortified granulated sugar wouldn't show any

color change over the three-month trial, but that there would be differences in color when the fortified sugar is mixed with green tea (South and Miller 1998) or made into candy.

MATERIALS & METHODS

Part I: Creating the Fortified Sugar Samples

Preparation of the Granulated Sugar Samples

Dry mixing and storage in plastic containers was recommended for sugar fortification with iron (Anonymous 2004). The variation used in this experiment was a spectrophotometric assay which analyzed color changes in candy, tea, and sugar that was fortified with sodium iron (III) EDTA (Sigma Chemicals, St. Louis, Mo.), Ferrochel (iron bis-glycinate chelate, Albion), hydrogen reduced elemental iron (Nutrafine RS, Hogan), and granular ferrous sulfate (7-hydrate, Mallinckrodt) after 0, 1, 2, and 3 months [see experimental design, Tables 1-4].

Eight, 200g aliquots of NaFe-EDTA fortified sugar were prepared by mixing thoroughly 200g of sugar and 0.158g of NaFe-EDTA in a clear plastic zip bag. Then each 200g aliquot was split into two 100g aliquots and one 100g aliquot was placed in a paper bag. The other was left in the plastic zip bag. The treatments were labeled according to the sample design in Appendix Table 1. The above procedure was then replicated using 0.088g iron bis-glycinate chelate, 0.024g elemental iron, and 0.120g ferrous sulfate. For the control, 100 g of sugar was placed into eight paper bags. Also, 100g of sugar was put into eight plastic bags and labeled according to the design in Appendix Table 1. After all the sugar was placed in bags, they were placed in a lab cabinet at room temperature.

The Hard Candy Preparation

In a non-reactive saucepan, 200g of sugar was mixed with 0.158g of iron (III) EDTA. To the sugar in the saucepan, 10mL of light corn syrup and 83mL of distilled

water was added. This was then heated over medium high heat and stirred until the sugar was dissolved. After the sugar was dissolved, stirring ceased and the temperature was raised to 149°C. The saucepan was then removed from the heat and the mixture was dropped by tablespoon (each approximately 12.5g), to yield 12 candies, on parchment paper. All candies were wrapped in plastic PVC film and two of the candies were labeled 0 Mo. with two labeled batch “A”. Two more were labeled 1 Mo. “A”, two with 2 Mo. “A”, and two with 3 Mo. “A”. The remaining candies were then saved and all candies were then labeled “NaFe-EDTA”. The above candy procedure was replicated one more time for NaFe-EDTA, except the samples were labeled batch “B” instead of “A”. Then the entire above candy procedure was repeated using 0.088g iron bis-glycinate chelate, 0.024g elemental iron, 0.120g ferrous sulfate, and just 200g of sugar (for the control).

Part II: Measuring the Color Change

To prepare samples for measuring the color of the sugar, a 12.5g sample of each sugar labeled 0 mo. was dissolved in 250mL of distilled water. To prepare to measure the color of the candy, each candy labeled 0 mo. was crushed with a hammer and each sample dissolved in 250mL of distilled water. Then a 1mL sample of each sugar solution was transferred into a cuvette. The cuvette was then read on a Beckman Coulter DU 520 general purpose UV/Vis spectrophotometer set to scan between 350-650nm. Since, the highest absorbance in the scans occurred at the 350nm, for the samples, they were read at 350nm and the absorbance of the samples was compared. This procedure was repeated again after 1, 2, and 3 months. Absorbances for each treatment at each time point were subtracted from the control values with results presented as this difference. The color change was also noted visually each month by examining the color of the sugar in

weighing boats and rating it on a scale from 0-5, with zero equaling the control sugar's color and five indicating a definitive change in color.

Part III: Measuring the Iron Content

An assay by Zhu (2007) that analyzes total non-heme iron was modified and used. This assay measures solutions at 535nm against a standard curve for iron (Zhu L 2007). Iron standard solutions were prepared by mixing 20 μ L of iron atomic absorption standard solution and 1980 μ L of water in a 2mL Eppendorf tube. Then 25 μ L, 50 μ L, 100 μ L, 200 μ L, 300 μ L, 400 μ L of iron stock solution were put in 5mL test tubes. 975 μ L, 950 μ L, 900 μ L, 800 μ L, 700 μ L, 600 μ L of distilled water was placed into the corresponding 5mL test tubes to yield solutions containing 0.25 μ g/mL, 0.5 μ g/mL, 1 μ g/mL, 2 μ g/mL, 3 μ g/mL, and 4 μ g/mL iron. To each tube, 1mL hydroxylamine (3% w/v water) was added. The tubes were then mixed and 1.5mL BPDS (0.3mg/mL water) was added to each tube. After 15min, 1mL of the solution was transferred into a cuvette. The cuvette was read on a spectrophotometer set to 535nm against a blank of water and a standard curve was constructed. For each of the dissolved sugar samples a 0.1mL aliquot and a 0.9mL aliquot of water was mixed in a 5mL test tube to create a 1/10 dilution. For each of the dissolved candy samples a 0.1mL aliquot of each and a 0.9mL of water were mixed in a 5mL test tube. Then, in a different 5mL test tube, 1mL of the 1/10 diluted sugar solution and 1mL hydroxylamine was mixed and 1.5mL BPDS was then added to each tube. After 15min, 1mL of solution was transferred into a cuvette and read on a spectrophotometer set to 535nm against a blank of water. After one month when the data were compared against the standard curve (Figure 1) and the expected amount of iron was not recovered, this procedure was stopped. Figure 2 shows that there was less than

the desirable amount recovered from the strongly chelated fortificants and more than the desirable amount recovered for ferrous sulfate. Therefore, this method was abandoned and it was assumed that the amount of iron added didn't change over time.

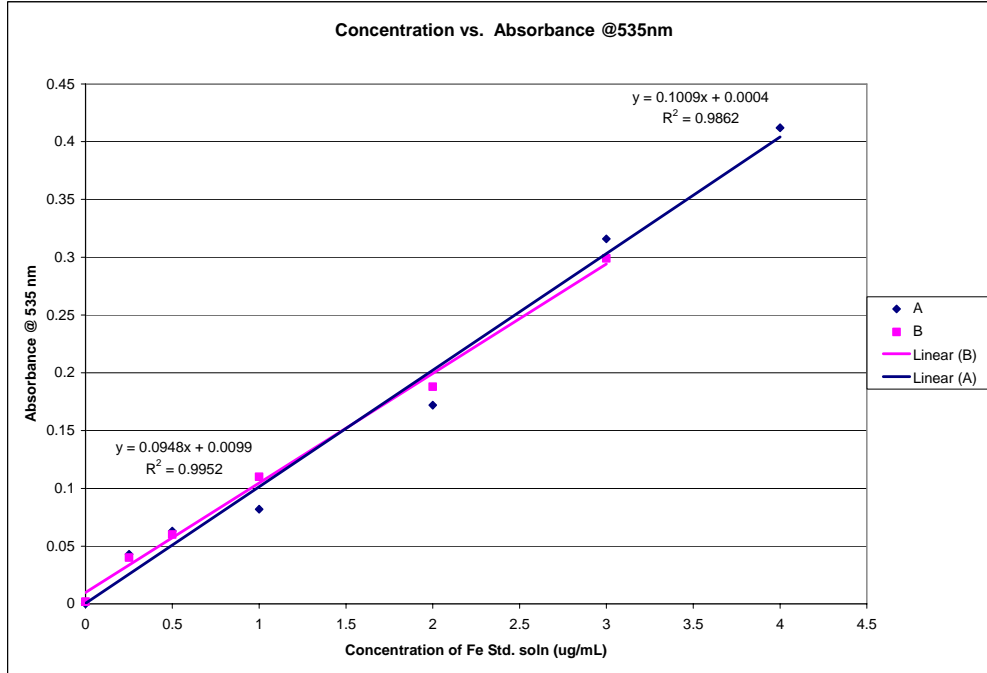


Figure 1. Standard Curve for Iron Recovery, Absorbance at 535 nm

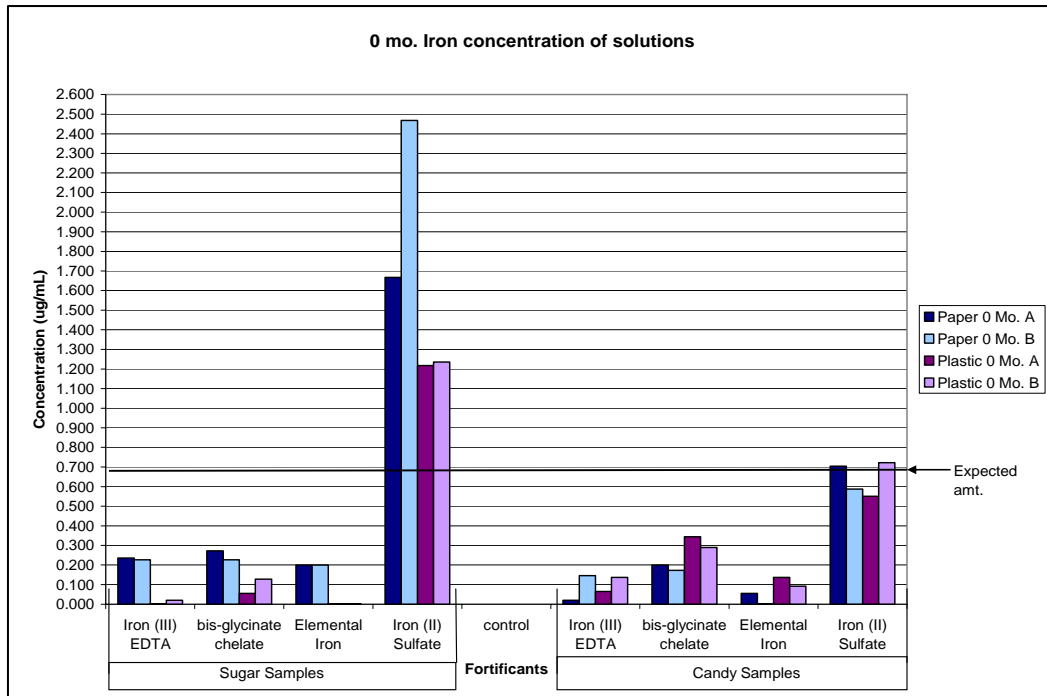


Figure 2. Iron Concentration of Fortified Granulated Sugar and Candy Samples at 0 Mo. (For the Candy samples: Paper = Batch A and Plastic = Batch B, A = Sample 1 and B = Sample 2)

Part IV: The Green Tea Reaction

Ten cups of Bromley green tea, purchased from a local grocery store, were prepared according to the box directions by putting 250mL of distilled water at 65.5°C in 10 300mL beakers and adding one green teabag to each. A 12.5g sample of each “0 Mo.” sugar was added to 250mL of green tea, which was brewed for 2 minutes, and observed for a change in color at 560nm. The tea with unfortified sugar was the control. A positive linear relationship between absorbance at 560nm and the concentration of an iron-galloyl complex has been reported (South and Miller 1998). This procedure was repeated at 1, 2, and 3 Mo. increments. The results from the green tea experiment were compared with the results from the candy and granulated sugar assays. The results were analyzed with line graphs and bar graphs. In addition to this analysis, paired t-tests were performed with H_0 : mean of the differences = 0 and H_A : mean of the differences \neq 0. From this analysis, it was determined which fortified sugar had the least color change over the three-month period.

RESULTS & DISCUSSION

Results from this study show that, of the fortificants used in this study, there isn't one fortificant that can be recommended for all sugar fortification applications. The visual appearance (see Figure 3) of the granulated sugar shows that all but the elemental iron differed in visual appearance from the unfortified sugar. Some of the fortificants (iron bis-glycinate chelate and NaFe-EDTA) colored the granulated sugar when they were first mixed, due to the larger particle size and dark color of the fortificant itself. This issue should be addressed in any future experiments and care should be used to select finely ground and pale fortificants.

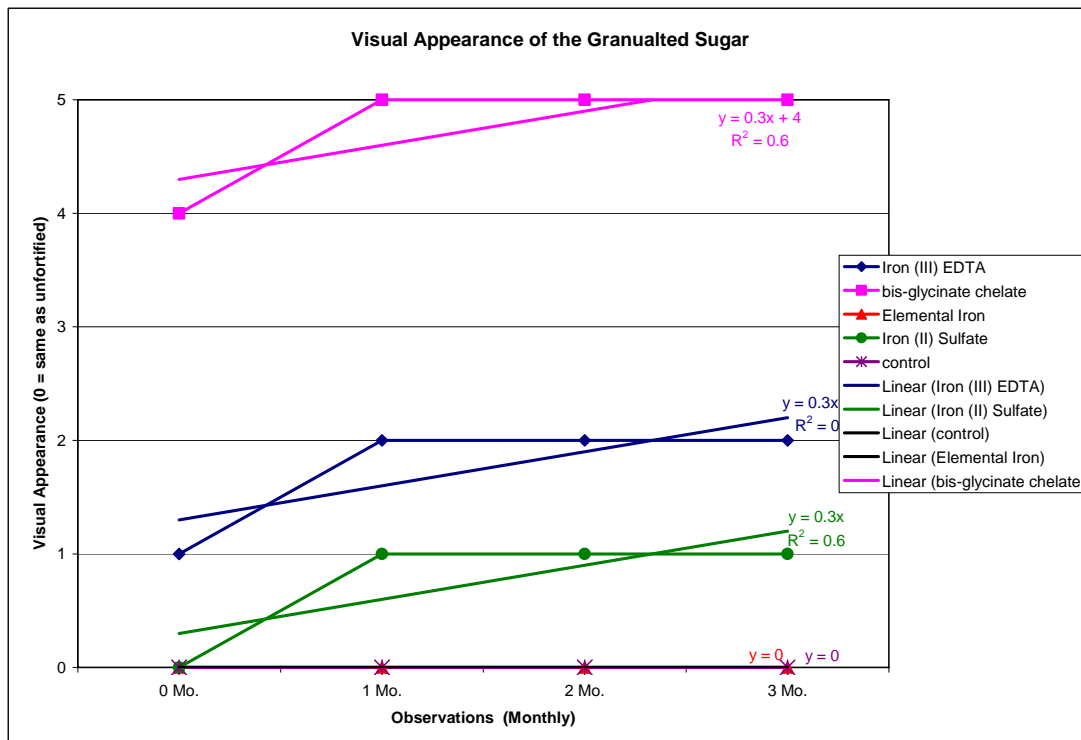


Figure 3. Visual Appearance of the Granulated Sugar Samples Over Time

Paired t-tests performed on the duplicate samples showed that only the batch B candy samples for month 2 ($p = 0.001$) and the paper samples for 1 mo. ($p = 0.043$) were statistically significantly different. Since these were the only statistically different samples, an average of samples A and B for each month's treatment, excluding these statistically different sample values, could be used to analyze the results.

Figure 4 shows some of the general trends that occurred in the average granulated sugar samples over the three-month period. According to Figure 4, the NaFe-EDTA and the iron bis-glycinate chelate were the most different from the control samples at 350nm, with elemental iron the closest to the control. Ferrous sulfate, in Figure 4 appears to differ slightly more over the three-month period than the sugar fortified with elemental iron. The difference in the ferrous sulfate fortified sugar's color from the control sample is consistent with the results from the study from India (Anonymous 2004). For many of

the samples it appears that the 0 Mo. reading was much higher than the remaining readings. This higher reading might be attributed to a slight difference in the procedure. A scanning spectrophotometer scanned the 0 Mo. samples and a graph was printed that showed the highest absorbance was 350nm. In order to increase precision and streamline the procedure, 350nm was used as the sole wavelength for the remainder of the experiment. According to Figure 4, during the three months there doesn't appear to be a dramatic change in the samples.

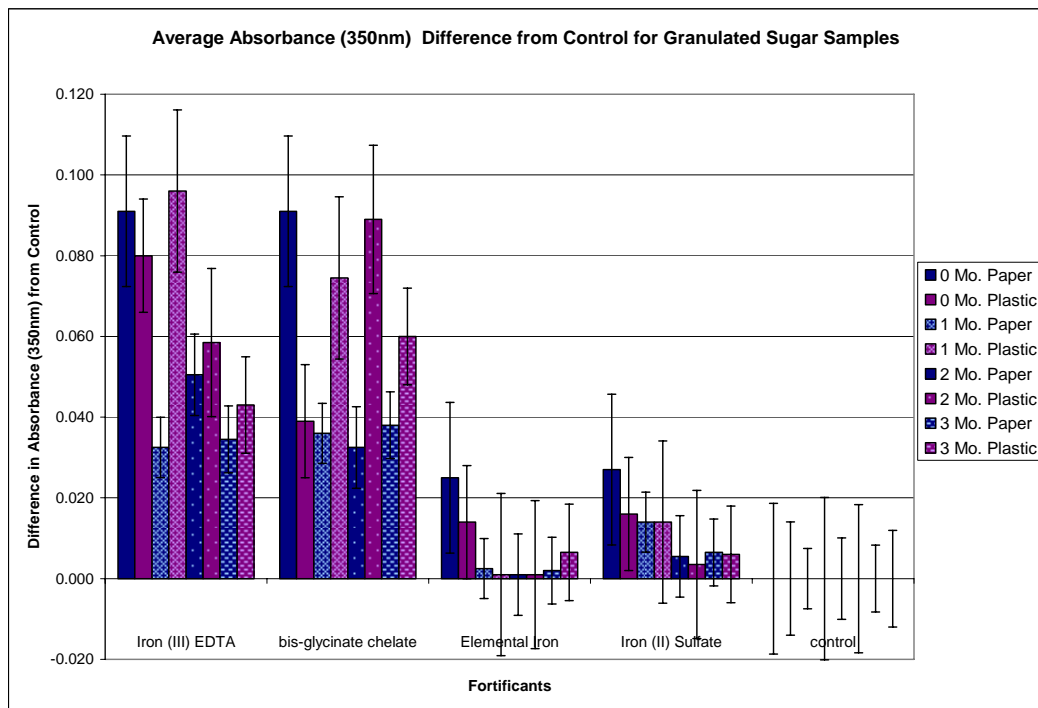


Figure 4. Monthly Comparison (at 350nm) over Three Months for Granulated Sugar Samples, means± SE, n = 2

Figures 5 and 6 do not suggest much of a difference in color from the samples stored in plastic vs. the samples stored in paper bags because all the slopes are about zero.

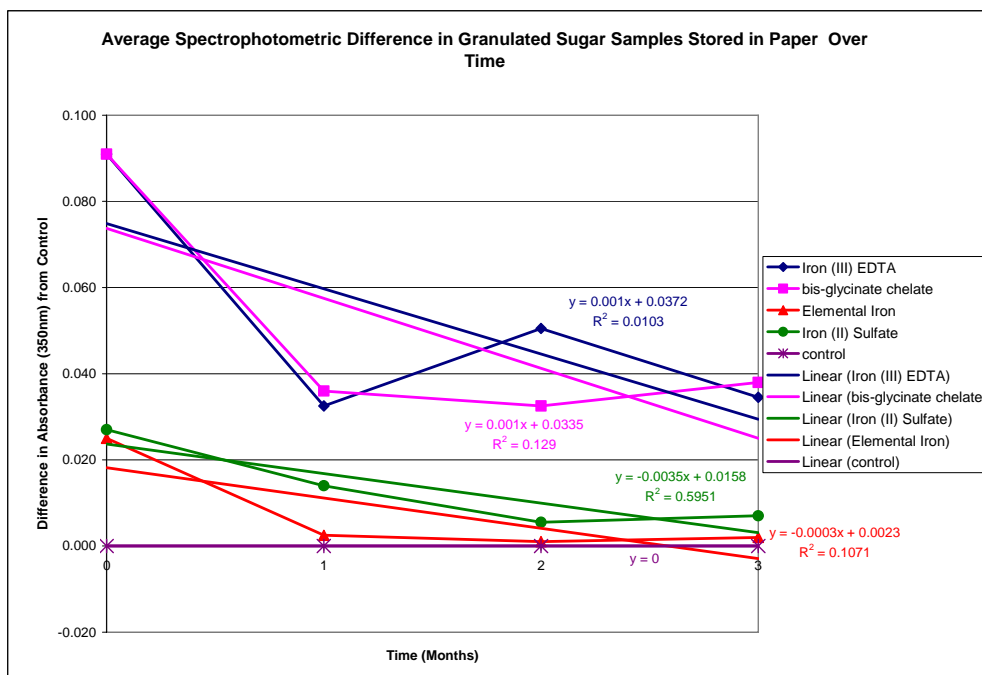


Figure 5. Average Spectrophotometric Difference in Granulated Sugar Samples Stored in Paper Over Time

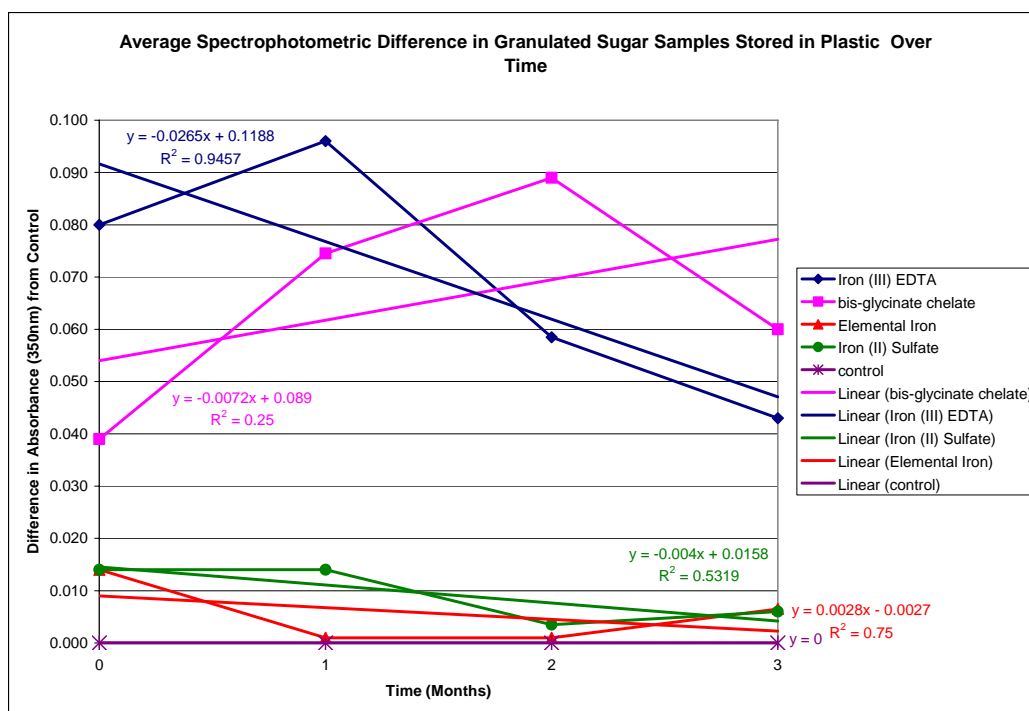


Figure 6. Average Spectrophotometric Difference in Granulated Sugar Samples Stored in Plastic Over Time

The results, from the granulated sugar samples that were dissolved in the green tea, varied in reactivity compared to the control (unfortified) sample. As shown in Figure 7,

the iron sulfate and the iron bis-glycinate chelate have a much higher absorbance, and therefore a large difference from the control sample. These two samples were visibly brownish-purple when the fortified sugar samples were added. The NaFe-EDTA became slightly darker in color when the sugar sample was added, but it was much less noticeable, which is consistent with the results in Figures 7 and 8. The study by South and Miller (1998), also found that strong chelating agents, such as EDTA, worked well in preventing a violet color from forming. Elemental iron, yet again, had the least amount of change from the control sample. This is most likely because the elemental iron was unable to ionize and react with the tea tannins.

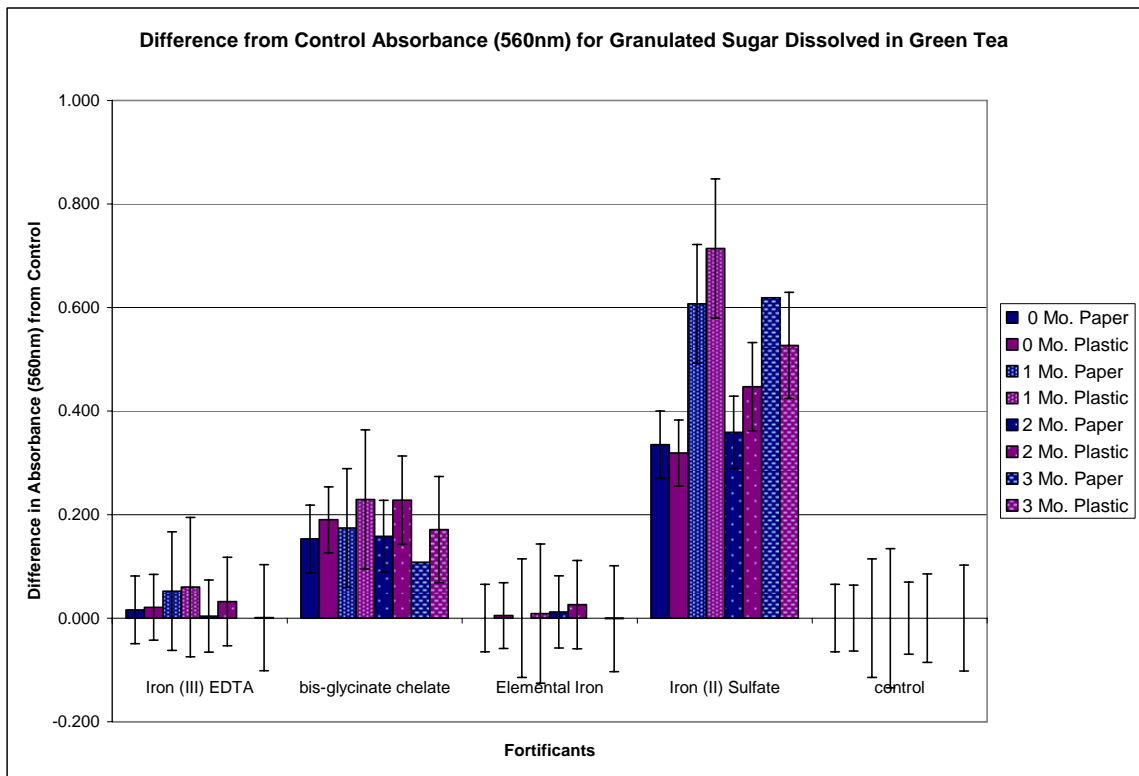


Figure 7. Monthly Comparison (at 560nm) over Three Months for Granulated Sugar Samples Dissolved in Green Tea, means \pm SE, n = 2



Figure 8. Visual Appearance of Fortified Green Tea Samples, from the left: NaFe-EDTA, Ferrous bis-glycinate, Elemental Iron, Ferrous sulfate, Control

There doesn't appear to be much of a difference in absorbance in the fortified sugar samples from the control over the three-month period.

The other application in which fortified sugar was used was in the making of hard candies. When the candies were made, it was apparent visually, see Figure 9, that there were some differences between some of the fortified sugar candy samples and the unfortified candy samples.

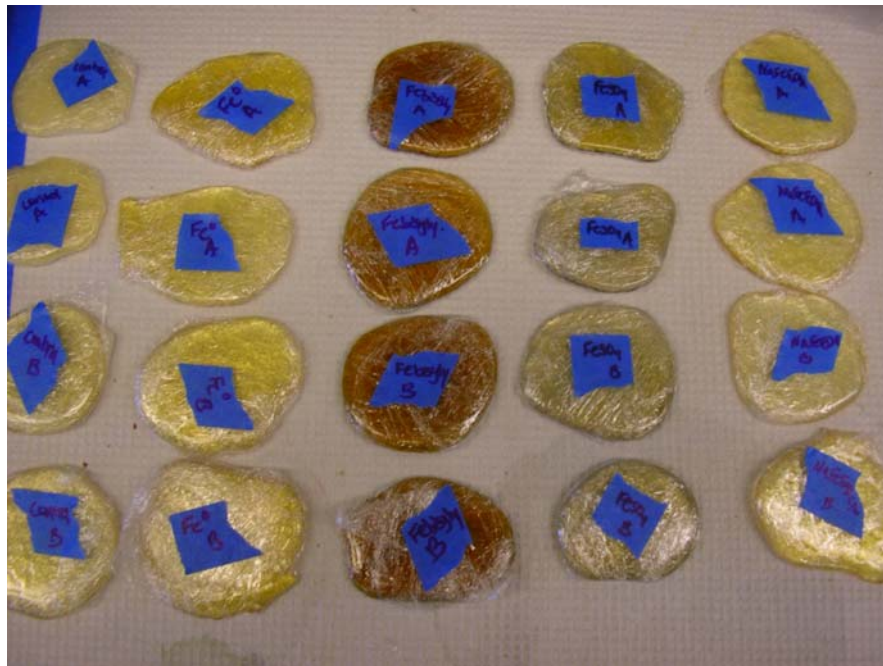


Figure 9. Visual Appearance of Candy Samples, from the left: Control, Elemental Iron, Ferrous bis-glycinate, Ferrous sulfate, NaFe-EDTA

The candy recipe included the use of corn syrup. Corn syrup contains the reducing sugar glucose and this when mixed with the iron bis-glycinate (a source of an amino acid) likely underwent Maillard browning, turning the candies brown. The other fortified candy sample that visually appeared drastically different from the unfortified candy was the ferrous sulfate candy. This candy had a greenish tinge that might have come about because the ferrous sulfate used was green in color. Although the goal was to achieve strict uniformity in the batches, there was some error introduced by the need to individually mix and mould each batch of candies. In future experiments, this could be eliminated by using larger volume equipment and hard candy moulds. This process yielded slightly differing times and temperatures for each batch. However, after performing t-tests between sample batches A and B there weren't statistically significant differences ($p > 0.05$) between batches. Figure 10 indicates that the iron bis-glycinate was the most different from the unfortified candy. Elemental iron and ferrous sulfate seemed to have similar differences in absorbance at the 350nm wavelength from the control candy samples. However, the greenish color of the ferrous sulfate candies might not have been as highly absorbed at the 350nm wavelength, since absorbance is the highest for violet at that wavelength (Hagar and Bullerwell 2003). The NaFe-EDTA candy samples were the most similar to the control sample. The candy samples visually did not appear to change in color over the three month experimental period.

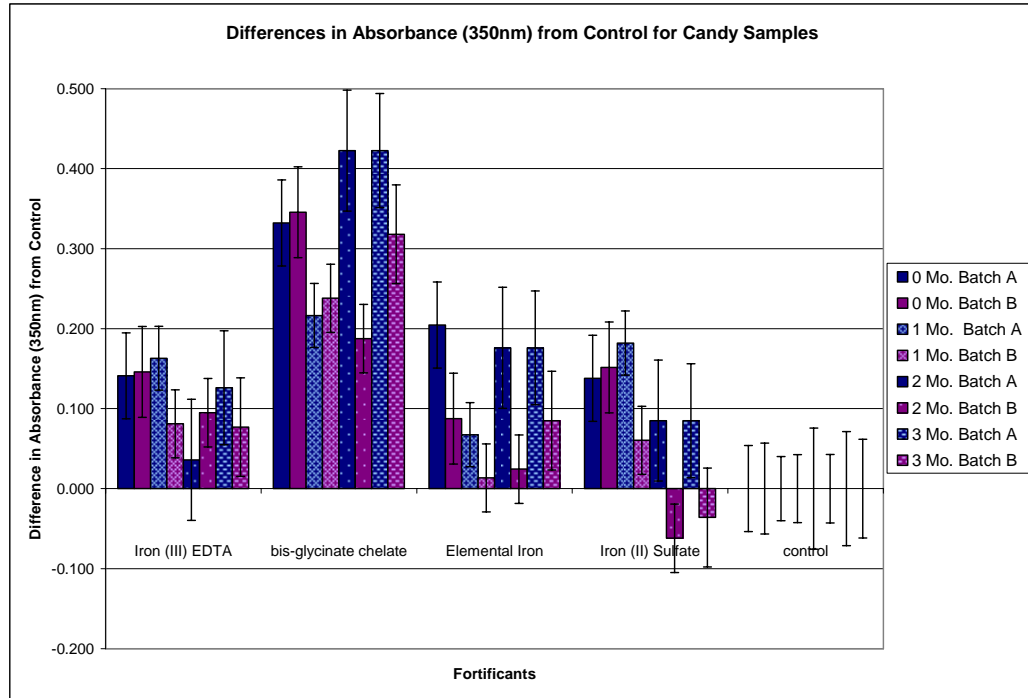


Figure 10. Monthly Comparison (at 350nm) over Three Months for Candy Samples, means± SE, n = 2

After examining all of the results, the best fortificant, in terms of limiting color change in granulated sugar, was elemental iron. NaFe-EDTA would be a good choice for fortifying sugar candies or perhaps tea because it is soluble in water and elemental iron is not. However, according to Allen and others, elemental iron is not necessarily the best fortificant for use in the fortification of granulated sugar because it can have a very low bioavailability (2006). This would mean that, since the body can't absorb this form as readily as other forms, for example ferrous sulfate or NaFe-EDTA, consumers would need to consume more of the fortified sugar to absorb the same amount of iron. However, the study by Sari and others (2001) helps to verify that elemental iron, presented in a candy form does help to reduce the prevalence of anemia, which is encouraging for the continued exploration of using elemental iron as a sugar fortificant. Relative cost might also be a factor in picking a fortificant for sugar. Hydrogen reduced

elemental iron is only half the cost (per mg iron) of ferrous sulfate 7H₂O, but ferrous bis-glycinate and NaFe-EDTA are almost 17 times more expensive than ferrous sulfate 7H₂O (Allen and others 2006). By examining how different fortificants would change the color of hard candies, in addition to verifying that a change in color occurs when certain fortificants are added to granulated sugar and the sugar is added to green tea, this study helps to add to the iron fortification literature base.

CONCLUSION

These findings suggest elemental iron fortified granulated sugar had the least amount of change from unfortified granulated sugar and NaFe-EDTA was the best fortificant for fortification of hard candy and possibly green tea. Therefore, of the fortificants used, there isn't one that clearly can be recommended for all types of sugar fortification. The implications on the feasibility of elemental iron fortified sugar are also dependant on sensory testing, which should be incorporated in future experimentation to examine if differences are detected at this usage level. More research is also needed to examine other possible fortificants.

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APPENDIX: Experimental Design

Table 1. Data Table for Recording Absorbance of Granulated Sugar and Candy at 350nm

Scanning Spectrophotometer Peaks & Absorbance at 350nm																
sugar																
Treatments	0 Mo. paper		1 Mo. paper		2 Mo. Paper		3 Mo. Paper		0 Mo. plastic		1 Mo. Plastic		2 Mo. Plastic		3 Mo. Plastic	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Iron (III) EDTA																
bis-glycinate chelate																
Elemental Iron																
Iron (II) Sulfate																
control																
candy																
Treatments	0 Mo. paper		1 Mo. paper		2 Mo. Paper		3 Mo. Paper		0 Mo. plastic		1 Mo. Plastic		2 Mo. Plastic		3 Mo. Plastic	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Iron (III) EDTA																
bis-glycinate chelate																
Elemental Iron																
Iron (II) Sulfate																
control																

Table 2. Data Table for Recording the Absorbance of Granulated Sugar Samples Dissolved in Green Tea

Green Tea Absorbance @ 560nm									
Treatments	0 Mo.		1 Mo.		2 Mo.		3 Mo.		
	paper	plastic	paper	plastic	paper	plastic	paper	plastic	
Iron (III) EDTA									
bis-glycinate chelate									
Elemental Iron									
Iron (II) Sulfate									
control									

Table 3. Data Table for Recording the Absorbance of the Granulated Sugar and Candy Samples at 535nm for Iron Content Calculation

Absorbance @ 535nm																
sugar																
Treatments	0 Mo. paper		1 Mo. paper		2 Mo. Paper		3 Mo. Paper		0 Mo. plastic		1 Mo. Plastic		2 Mo. Plastic		3 Mo. Plastic	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Iron (III) EDTA																
bis-glycinate chelate																
Elemental Iron																
Iron (II) Sulfate																
control																
candy																
Treatments	0 Mo. paper		1 Mo. paper		2 Mo. Paper		3 Mo. Paper		0 Mo. plastic		1 Mo. Plastic		2 Mo. Plastic		3 Mo. Plastic	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Iron (III) EDTA																
bis-glycinate chelate																
Elemental Iron																
Iron (II) Sulfate																
control																

Table 4. Data Table for Recording the Visual Appearance of the Granulated Sugar Samples

Granulated Sugar: Visual Appearance			
Treatments	scale of 0-5, 0 = same as unfortified sugar		
	0 Mo.	1 Mo.	3 Mo.
Iron (III) EDTA			
bis-glycinate chelate			
Elemental Iron			
Iron (II) Sulfate			
control			