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Investigation of the effect of black tea acidulation with citric acid or its natural source on dietary iron bioaccessibility

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ABSTRACT

Despite the established inhibitory action of tea polyphenols on dietary iron absorption, the consumption of tea at mealtimes remains common in many regions of the globe where anaemia is still a public health concern. The objective of this study was to investigate the effect of acidulating black tea with citric acid or lime juice on dietary iron bioaccessibility. Firstly, the effect of citric acid on iron-polyphenol complex formation and iron speciation was studied using molecular spectrophotometry. Then, the bioaccessibility of dietary iron from pearl millet ball in the presence of black tea acidulated with citric acid or lime juice was assessed through a simulated *in vitro* digestion. Finally, the different species of bioaccessible iron were quantified. Citric acid reduced the absorbance of iron-polyphenol complex by 58.06% and 59.52% at pH 5 and 7, respectively, while promoting the formation of organic iron at pH 5 and ferrous iron at pH 5 and 7. Dietary iron bioaccessibility increased in the presence of acidulated black teas, with the greatest variation (28.57%) being observed in the digest of pearl millet ball mixed with lime juice-acidulated black tea. The more abundant bioacessible iron species was organic iron, and ferrous iron was more concentrated in digests of pearl millet ball mixed with lime juice-acidulated black tea. Acidulation of tea beverage with lime juice is a culinary technique that could mitigate the inhibitory action of tea polyphenols on dietary iron absorption and alleviate iron deficiency in populations consuming tea at mealtimes.

Keywords: Iron, Bioaccessibility, Speciation, Citric Acid, Iron-Polyphenol Complex.

INTRODUCTION

Iron deficiency is the most common form of malnutrition in the world affecting more than two billion people. Children and women of childbearing age are the most vulnerable groups of the population (Camaschella, 2019). The harmful consequences of iron deficiency on growth, immune system, cognitive development, physical performances are well documented (Mantadakis et al., 2020; Georgieff, 2020; Snook et al., 2021; Ouedraogo et al., 2023). One of the major causes of iron deficiency is the presence of high amount of anti-nutritional factors such as polyphenols in plant foods (Piskin et al., 2022). Although having demonstrated bioactive properties, polyphenols inhibit the bioaccessibility of iron by forming with this trace element stable chelation complexes, reducing its intestinal

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absorption (Piskin et al., 2022; Naco et al., 2023).

Tea consumption at mealtimes has been identified as a risk factor of iron deficiency (Temme and Van Hoydonck, 2002; Hayat et al., 2015). Tea is the most consumed beverage in the world after water and is characterised by high polyphenol content (Khan and Mukhtar, 2018). Furthermore, 83.1% of total tea consumption occurs in developing countries where iron deficiency remains a public health concern (FAO, 2014).

The preparation of tea is subjected to different culinary techniques, including acidulation with lime juice just before consumption. This is done to improve the organoleptic quality of acidulated tea. Also, acidulated tea granules are a common form of selling this beverage across the globe. Lime juice is characterised by its high content in citric acid, an organic acid with concentration reaching 50 g/L (Smati et al., 2017). Previous studies have demonstrated the positive effect of organic acids in enhancing dietary non-haem iron solubility and intestinal absorption (Teucher and Cori, 2004; Perera et al., 2023).

In a study conducted by Mellican et al. (2003), ferric citrate, a chelated iron compound, strongly prevented the formation of coloured iron-polyphenol complex. Also, a more recent study revealed sodium citrate to be effective in preventing the formation of iron-polyphenol complex in iron-fortified black tea; this competing chelating agent produced about 60% less iron-polyphenol complex than the control (McGee and Diosady, 2018a). It can be noticed that these previous studies were conducted in a context of food fortification with synthetic iron, and do not match with situations where natural dietary iron is targeted. Moreover, it seems costly and difficult to safely use synthetic chelating agents at a household level, especially in low-income countries. A food-to-food fortification approach to explore implies incorporating a natural source of citric acid to tea, in other to counteract the inhibitory effect of polyphenols on dietary iron absorption. Food-to-food fortification is an emerging food-based strategy used to fight micronutrients deficiencies, and which is easily applicable at household level.

The objective of this study was to investigate the effect of acidulating a polyphenols-rich beverage (black tea) with citric acid or its natural source on dietary iron bioaccessibility.

MATERIALS AND METHODS

Study of the effect of citric acid on iron-polyphenol complex formation and iron speciation

Experimental solutions

Stock solutions of gallic acid (10 g/L), iron chloride $FeCl_3$ (50 mmol/L), and citric acid (50 g/L), were prepared. Deionised water was used as solvent in this study.

Control solutions

Two citric acid-free solutions with final concentrations of 1 g/L of gallic acid and 1 mmol/L of ferric iron (Fe^{3+}) were prepared. Before reaching the final volume of 11 mL, solutions of HCl 1 mol/L or NaOH 1 mol/L were used to adjust the pH to 5 for one solution and 7 for another one.

Tests solutions

Two solutions with final concentrations of 5 g/L of citric acid, 1 g/L of gallic acid and 1 mmol/L of ferric iron (Fe^{3+}) were prepared. As mentioned before, solutions of HCl 1 mol/L or NaOH 1 mol/L were used to adjust the pH to 5 or 7 before reaching the final volume of 11 mL.

The pH levels used are relevant to brewed tea and sites of iron absorption into the body (gut). The gallic acid/iron/citric acid proportions were chosen on the basis of tea consumption during a cereal-based meal as revealed by a field survey (not published).

Measurement of the absorbance of iron-polyphenol complex in experimental solutions

The measurement of the absorbance related to iron-polyphenol complex was carried out according to the method proposed by McGee and Diosady (2018b). This simple method specifies the maximum absorption wavelengths of iron-polyphenol complex at different pHs. Two millilitres of each experimental solution were introduced into the UV-Vis spectrophotometer cuvette, and the absorbance was read at 555 nm and 565 nm for pH 5 and 7, respectively.

Study of iron speciation in experimental solutions

The study of iron speciation was carried out according to the method described by da Silva et al. (2017). This method is based on the

principle that ferric iron (Fe^{3+}), in the presence of hydroxylamine hydrochloride, is reduced to ferrous iron (Fe^{2+}). The Fe^{2+} ions form a stable coloured (red orange) complex with 1-10-phenanthroline which absorbs at the maximal wavelength of 510 nm.

Quantification of inorganic iron

Inorganic iron refers to both ferrous iron (Fe^{2+}) and ferric iron (Fe^{3+}). To 2 mL of experimental solution, 1 mL of hydroxylamine hydrochloride 1% and 1 mL of 1,10-phenanthroline 0.25% were added and the whole was homogenised. Then, the mixture was allowed to stand for 15 minutes, and the absorbance was read at 510 nm. A standard curve ($R^2 = 0.986$) obtained from 0 – 5 mg/L ferrous sulphate ($FeSO_4$) solutions was used to quantify Fe^{2+} ions, corresponding to total inorganic iron initially found in experimental solutions.

Quantification of organic iron

Organic iron is iron chelated by organic molecules. Its concentration was determined by deductive calculation using the following formula:

Organic iron = Soluble iron - Inorganic iron

Quantification of ferrous iron

To 2 mL of experimental solution, 1 mL of 1,10-phenanthroline (0.25%) was added and the whole was homogenised. Then, the mixture was allowed to stand for 15 minutes, and the absorbance was read at 510 nm. The same standard curve mentioned for inorganic iron was used to quantify ferrous iron (Fe^{2+}) in experimental solutions.

Quantification of ferric iron

Ferric iron (Fe^{3+}) concentrations were determined by deductive calculation using the following formula:

Ferric iron = Inorganic iron - Ferrous iron

Study of the bioaccessibility of dietary iron in the presence of black tea acidulated with citric acid or lime juice

Biological material

Black tea leaves (*Camelia sinensis*), lime fruits (*Citrus limon*) and pearl millet grains (*Pennisetum glaucum*) used in this study were all purchased at a local market in the city of Maroua (Far-north region of Cameroon).

Preparation of acidulated brewed black tea

In a clean cup of polystyrene, one black tea bag (2.5 g) was steeped in 100 mL of hot deionised water. The whole was stirred and cooled for 3 minutes. Then, 20 mL of citric acid solution (50 g/L) was added and the whole was homogenised. A second preparation was made in the same conditions, but citric acid solution was replaced by 20 mL of lime juice, its natural source. A third preparation without acidulant served as control.

The tea/acidulant ratio was chosen according to local habits of preparing acidulated tea (half a lime in a cup of tea). The concentration of citric acid solution was the same as in lime juice (Smati et al. 2017).

Preparation of pearl millet ball

Pearl millet grains were sorted and cleaned before milling (Saint-Donkey mill). Twenty grams (20 g) of pearl millet flour was dissolved in 250 mL of water and added to 500 mL of boiling water while stirring. Then, 180 g of pearl millet flour was added, and the mixture was stirred with a wooden spatula until a consistent dough was obtained. After 2 minutes of additional cooking, the dough was used to make balls.

Preparation of test meals

Three test meals were prepared:

- 200 g of pearl millet ball + 100 mL of non-acidulated black tea
- 200 g of pearl millet ball + 100 mL of black tea acidulated with citric acid solution
- 200 g of pearl millet ball + 100 mL of black tea acidulated with lime juice

Each meal was homogenised in a home blender (Model tyb-351). An aliquot of the homogenate was dried at 105°C for 12 hours for the quantification of total iron, and the other aliquot was kept below 0°C for the simulated *in vitro* digestion.

Quantification of total iron in test meals

Dried samples (0.5 g) were incinerated at 500°C for 10 hours. The ashes were then dissolved in 5 mL of deionised water and 15 mL of Aqua regia (mixture of nitric acid HNO_3 and hydrochloric acid HCl, in a molar ratio of 1:3). One millilitre of the obtained solution was diluted in 50 mL of deionised water. Iron was quantified by atomic absorption spectrophotometry (Jones Jr and Case, 1990).

Determination of iron bioaccessibility using simulated in vitro digestion

The simulated *in vitro* digestion method described by Minekus et al. (2014) was used. Simulated digestive fluids (SSF: Simulated

Salivary Fluid, SGF: Simulated Gastric Fluid, and SIF: Simulated Intestinal Fluid) were prepared from stock solutions summarised in Table 1. Using solutions of HCl 1 mol/L and NaOH 1 mol/L, the pHs of SSF, SGF and SIF were adjusted to 7, 2 and 7, respectively.

The simulated digestive fluids served as solvent for the preparation of enzymes solutions. The pH of these solutions was adjusted to 2 and 7 using diluted solutions of HCl and NaOH 0.1 mol/L.

Oral digestion

Five grams (5 g) of each test meal was mixed with 5 mL of 150 U/mL α-amylase solution in a test tube. After homogenisation, the whole was incubated for 2 minutes at 37°C.

Gastric digestion

Ten millilitres (10 mL) of the oral bolus was mixed with 7.5 mL of SGF solution and 1.6 mL of porcine pepsin solution of 25000 U/mL. The pH was adjusted to 2 with HCl 1 mol/L. The mixture was incubated for 2 hours at 37°C while shaking gently.

Intestinal digestion

Twenty millilitres (20 mL) of gastric chyme was mixed with 11 mL of SIF solution and 5 mL of pancreatic-bile extract solution 800 U/mL. The pH was then adjusted to 7 with NaOH 1 mol/L. The mixture was incubated for 2 hours at 37°C while shaking gently.

The obtained digests were then filtered on a Whatman No. 42 filter-paper. The filtrates were mineralised and used to quantify soluble iron by atomic absorption spectrophotometry, as described for total iron.

Determination of iron bioaccessibility

The percentage of bioaccessible iron was determined according to the formula below:

$$\text{Bioaccessibility (\%)} = \frac{\text{Quantity of iron in the filtrate}}{\text{Quantity of iron in the sample}} \times 100$$

Study of the speciation of bioaccessible iron

Two millilitres (2 mL) of filtrate from *in vitro* digestion was mixed with 0.16 mL of sodium nitrite 0.39%, 1 mL of protein precipitating solution (5 g TCA + 5 mL of concentrated HCl for 50 mL solution) and 6.84 mL of deionised water. The mixture was incubated at 100°C for 10 minutes, then centrifuged at 3500 rpm for 15 minutes.

The different species of soluble iron in the supernatants (bioacessible iron) were quantified according to the methodology previously described.

Statistical analyses

Results were reported as mean ± standard deviation. The data were subjected to analysis of variance (ANOVA) and the differences between means were analysed by Duncan's multiple comparison with a significance level of $p < 0.05$.

Table 1: Composition of simulated digestive fluids.

Stock solutions	SSF	SGF	SIF
	pH 7	pH 2	pH 7
KCl 68 g/L (mL)	15.1	6.9	6.8
KH_2PO_4 68 g/L (mL)	3.7	0.9	0.8
$NaHCO_3$ 84 g/L (mL)	6.8	12.5	42.5
NaCl 117 g/L (mL)	-	11.8	9.6
$MgCl_2 \cdot 6H_2O$ 30.5 g/L (mL)	0.5	0.4	1.1
$(NH_4)_2CO_3$ 48 g/L (mL)	0.06	0.5	-

SSF: Simulated Salivary Fluid; SGF: Simulated Gastric Fluid ; SIF: Simulated Intestinal Fluid.

RESULTS

Effect of citric acid on iron-polyphenol complex formation

Table 2 shows the absorbance of the experimental solutions in the absence or presence of citric acid at different pHs. It can be observed that in citric acid-free solutions (controls), the absorbance was lower ($p < 0.05$) at pH 5 compared to pH 7, indicating a more important formation of iron-polyphenol complex at neutral pH. In the presence of citric acid, the absorbance was reduced ($p < 0.05$) irrespective of the pH. This result demonstrates the preventing action of citric acid on the chelation of iron by gallic acid.

Effect of citric acid on iron speciation in experimental solutions

The concentrations of different iron species in the experimental solutions are presented in Table 3. It appears that iron was mainly found in the organic form at all the studied pHs. In control solutions, the concentration of organic iron was higher ($p < 0.05$) at pH 7, confirming the favourable effect of neutral pH on the formation of iron-polyphenol complex. The presence of citric acid increased ($p < 0.05$) the concentration of organic iron at pH 5, but reduced it at pH 7. The logical contrary was observed with inorganic iron, since iron is found either be in the organic, or in the inorganic form.

As far as each inorganic species is concerned, there was a reduction ($p < 0.05$) in Fe^{3+} concentration at pH 5, with a concomitant increase in Fe^{2+} concentration. At pH 7, both Fe^{2+} and Fe^{3+} concentrations increased in the presence of citric acid. The reducing effect of citric acid on ferric iron at pH 5 was not observed at pH 7.

Bioaccessibility of dietary iron in the presence of black tea acidulated with citric acid or lime juice

Table 4 presents the contents of total iron, soluble iron and bioaccessible iron in the digests of test meals. The total iron content of the test meal containing black tea acidulated with lime juice was lower, compare to other samples, indicating a diluting effect of other minerals found in lime juice on the iron content.

In the presence of acidulated black tea, the soluble and bioaccessible iron levels were higher ($p < 0.05$) than those observed in the control. The test meal containing lime juice-acidulated tea exhibited the highest increase in iron bioaccessibility (28.57%). This result revealed the superiority of lime juice in promoting the solubility of iron in the studied digests, compared to the pure citric acid solution.

Speciation of bioaccessible dietary iron in the presence of black tea acidulated with citric acid or lime juice

The concentrations of the various species of bioaccessible iron are presented in Table 5. Bioaccessible iron is mainly found in the form of organic iron which was more concentrated ($p < 0.05$) in the digest of the test meal containing citric acid-acidulated black tea. This suggests a complexation of citric acid with iron. The higher concentrations of inorganic and ferrous iron in the digest of the test meal containing lime juice-acidulated black tea indicates the reducing action of lime juice on iron, although a paradoxal absence of a significant variation of ferric iron (Fe^{3+}) was noticed.

Table 2 : Absorbance of iron-polyphenol complex in experimental solutions.

Solutions	Absorbance	Reduction of Absorbance (%)
pH 5		
Control	$0,155^{b} \pm 0,009$	-
Test	$0,065^{a} \pm 0,000$	58.06
pH 7		
Control	$0,914^{d} \pm 0,010$	-
Test	$0,370^{c} \pm 0,001$	59.52

Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

Table 3 : Iron speciation in experimental solutions.

Solutions	Inorganic iron (mg/mL)	Organic iron (mg/mL)	Ferrous iron (Fe^{2+}) (mg/mL)	Ferric iron (Fe^{3+}) (mg/mL)
pH 5				
Control	$0,130^{c} \pm 0,005$	$0,897^{b} \pm 0,002$	$0,009^{a} \pm 0,003$	$0,121^{c} \pm 0,008$
Test	$0,048^{a} \pm 0,002$	$0,952^{c} \pm 0,002$	$0,047^{c} \pm 0,001$	$0,009^{a} \pm 0,003$
pH 7				
Control	$0,085^{b} \pm 0,001$	$0,943^{c} \pm 0,04$	$0,012^{a} \pm 0,003$	$0,048^{b} \pm 0,04$
Test	$0,203^{d} \pm 0,004$	$0,797^{a} \pm 0,06$	$0,037^{b} \pm 0,003$	$0,166^{d} \pm 0,001$

Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

Table 4: Bioaccessibility of dietary iron in the presence of acidulated black tea.

Meals	Total iron (mg/100gMS)	Soluble iron (mg/L)	Bioaccessible iron (%)	Increase of Bioaccessibility (%)
M0	$21,59^{b} \pm 0,04$	$0,42^{a} \pm 0,00$	$6,16^{a} \pm 0,01$	-
M1	$21,65^{b} \pm 0,18$	$0,48^{b} \pm 0,01$	$6,97^{b} \pm 0,04$	13.15
M2	$20,51^{a} \pm 0,09$	$0,47^{b} \pm 0,02$	$7,92^{c} \pm 0,21$	28.57

M0: pearl millet ball + non acidulated black tea
M1: pearl millet ball + black tea acidulated with citric acid solution
M2: pearl millet ball + black tea acidulated with lime juice
Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

Table 5 : Speciation of bioaccessible dietary iron in the presence of black tea acidulated with citric acid or lime juice.

Meals	Inorganic iron (mg/L)	Organic iron (mg/L)	Ferrous iron (Fe^{2+}) (mg/L)	Ferric iron (Fe^{3+}) (mg/L)
M0	$0.169^{a} \pm 0.009$	$0.251^{a} \pm 0.009$	$0.035^{a} \pm 0.000$	$0.133^{a} \pm 0.009$
M1	$0.174^{a} \pm 0.00$	$0.314^{b} \pm 0.009$	$0.047^{a} \pm 0.021$	$0.126^{a} \pm 0.020$
M2	$0.226^{b} \pm 0.02$	$0.239^{a} \pm 0.024$	$0.118^{b} \pm 0.027$	$0.108^{a} \pm 0.026$

M0: pearl millet ball + non acidulated black tea
M1: pearl millet ball + black tea acidulated with pure citric acid
M2: pearl millet ball + black tea acidulated with lime juice
Mean values in the same column with different superscript letters are significantly different ($p < 0.05$).

DISCUSSION

Polyphenols are among dietary factors that inhibit the intestinal absorption of iron (Piskin et al., 2022). These compounds can chelate iron and form a non-absorbable coloured complex. The absorbance of iron-polyphenol complex.is proportional to its concentration, as indicated by the Beer-Lambert law. Our study revealed that the formation of iron-polyphenol complex was sensitive to pH. In 2018, Diosady and Elisa demonstrated that black tea polyphenols precipitate in very acid conditions. Polyphenols available to chelate iron in the studied experimental solutions would have been reduced in acidic conditions. As it was the case in our study, these authors also demonstrated that a neutral pH favoured the formation of iron-polyphenol complex. The reduction of absorbance in the presence of citric acid corroborates the findings of McGee and Diosady (2018a) who observed 60% less iron-polyphenol complex in an iron-fortified black tea containing sodium citrate. A competition between citric acid and polyphenols for chelating iron could explain this result. The negatively charged carboxyl groups of citric acid could compete with deprotonated hydroxyl groups of polyphenols in chelating iron cations (Fe^{2+} or Fe^{3+}), hence reducing the concentration of iron-polyphenol complex.

Studying speciation is identifying and quantifying the different species or chemical forms of metals in a sample (Bou Khouzam et al., 2012). In food and nutritional biochemistry, the sample can be foods, digests, body fluids or tissues (Bou Khouzam et al., 2012; Wan et al., 2019). Speciation provides information on the availability for absorption of certain dietary trace elements important in human nutrition (da Silva et al., 2017). In the context of our study, investigating iron speciation was carried out to get precise insights on the relationship between citric acid and this trace element. The increased organic iron concentration in the presence of citric acid at pH 5 could be attributed to the chelating phenomenon mentioned before. Previous studies have shown that organic acids can chelates iron to form soluble complexes (Salovaara et al., 2003; Teucher and Cori, 2004; Perera et al., 2023). However, the reduction of organic iron concentration in the presence of citric acid at pH 7 suggests that citric acid disfavoured the formation of iron-polyphenol complexes, but not through a competitive complexation mechanism. The reduction of Fe^{3+} concentration at pH 5 suggest that citric acid had preferably chelated this ionic form of iron, while promoting at the same time its reduction to Fe^{2+}.

Bioaccessibility refers to the fraction of a nutrient released from a food matrix in the

gastrointestinal tract and available for absorption (Heaney, 2001). The improvement of bioaccessible dietary iron in the presence of acidulated black teas could be due to the enhancing effect of organic acids on iron solubility, by chelation or chemical reduction (Teucher and Cori, 2004). The presence of acidulant could have prevented the formation of iron-polyphenol complex, hence the inhibitory effect of black tea polyphenols. This result corroborates with what was observed in experimental solutions. The bioaccessibility of iron was more improved in the presence of lime juice-acidulated black tea. This could be due to the additional contribution of ascorbic acid present in lime juice (Smati et al., 2017). Indeed, ascorbic acid is known as one of the most powerful enhancers of the solubility of non-haem iron (Reddy et al., 2022).

Contrary to what was observed with experimental solutions, the presence of citric acid promoted the formation of organic iron at intestinal pH which is around 7. This observation would be due to the chemical complexity of the digests, favourable to the complexation of iron by citric acid. The increased concentration of Fe^{2+} in the digest of the test meal containing lime juice-acidulated black tea could be due to the action of ascorbic acid which, although in relatively small quantity in lime juice, would have reduced ferric iron to ferrous iron through the oxidation-reduction reaction (Allen, 1998). Ferrous iron (Fe^{2+}) and iron complexed by soluble organic molecules such as organic acids are better assimilated in the gastrointestinal tract (Bou Khouzam et al., 2012; Wan et al., 2019).

Conclusion

The general objective of this study was to investigate the effect of acidulation of black tea with citric acid or lime juice on the bioaccessibility of dietary iron. It appeared from the preliminary experimental study that citric acid inhibited the formation of iron-polyphenol complexes at pHs corresponding to the sites of absorption of iron in the digestive tract. Dietary iron was more bioaccessible in the presence of black tea acidulated with citric acid or its natural source (lime juice). Bioaccessible iron was more found complexed to organic molecules and the the presence of acidulated black tea promoted the formation of ferrous iron which is its better absorbed inorganic species. Acidulation of tea beverage with lime juice is a culinary technique that could mitigate the inhibitory action of tea polyphenols on dietary iron absorption and alleviate iron deficiency in populations consuming tea at mealtimes.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualisation: SM, MM; Methodology: SM, MM, NN, PHB, CND; Formal analysis and investigation: SM, MM, NN, PHB, CND; Writing - original draft preparation: MM; Writing - review and editing: SM; Resources: SM, MM; Supervision: SM.

REFERENCES

Allen LH. 1998. Iron-ascorbic acid and iron-calcium interactions and their relevance in complementary feeding. In *Micronutrient Interactions: Impact on Child Health and Nutrition.* International Life Sciences Institute: Washington DC; 11-20.

Jones Jr JB, Case VW. 1990. Sampling, handling, and analyzing plant tissue samples. In *Soil Testing and Plant Analysis*, Westerman RL (Ed.). Book Series 3, Soil Science Society of America: Madison; 389-427.

Bou Khouzam R, Szpunar J, Holeman M, Lobinski R. 2012. Trace element speciation in food: State of the art of analytical techniques and methods. *Pure and Applied Chemistry,* **84** (2012): 169-179. DOI: http://dx.doi.org/10.1351/PAC-CON-11-08-14

Camaschella C. 2019. Iron deficiency. *Blood, The Journal of the American Society of Hematology,* **133**(1): 30-39. DOI:

https://doi.org/10.1182/blood-2018-05-815944.

da Silva I J G, Fonseca L, Costa ADS, Moita GC. 2017. Bioaccessibility and Speciation Study of Iron in Bovine Tissue. *Revista Virtual de Química*, **9**(2): 476-491. DOI: https://doi.org/10.21577/1984-6835.20170027

FAO 2014. Current market situation and medium term outlook. Committee on Commodity Problems Intergovernmental Group on Tea (21st Session). Bandung.

Georgieff MK. 2020. Iron deficiency in pregnancy. *American Journal of Obstetrics and Gynecology*, **223**(4): 516-524. DOI: https://doi.org/10.1016/j.ajog.2020.03.006

Hayat K, Iqbal H, Malik U, Bilal U, Mushtaq S. 2015. Tea and its consumption: benefits and risks. *Critical Reviews in Food Science and Nutrition*, **55**(7): 939-954. DOI: https://doi.org/10.1080/10408398.2012.678949

Heaney RP. 2001. Factors influencing the measurement of bioavailability, taking calcium as a model. *The Journal of Nutrition*, **131**(4): 1344-1348. DOI: https://doi.org/10.1093/jn/131.4.1344S

Khan N, Mukhtar H. 2018. Tea polyphenols in promotion of human health. *Nutrients*, **11**(1): 39. DOI: https://doi.org/10.3390/nu11010039

Mantadakis E, Chatzimichael E, Zikidou P. 2020. Iron deficiency anemia in children residing in high and low-income countries: risk factors, prevention, diagnosis and therapy. *Mediterranean Journal of Hematology and Infectious Diseases*, **12**(1): 1-12. DOI: https://doi.org/10.4084/MJHID.2020.041

McGee EJT, Diosady LL. 2018. Development of spectrophotometric quantification method of iron-polyphenol complex in iron-fortified black tea at relevant ph levels. *Food Analytical Methods*, **11**: 1645-1655. DOI: https://doi.org/10.1007/s12161-018-1147-8

McGee EJT, Diosady LL. 2018. Prevention of iron-polyphenol complex formation by chelation in black tea. *LWT*, **89**: 756-762. DOI: https://doi.org/10.1016/j.lwt.2017.11.041

Mellican RI, Li J, Mehansho H, Nielsen SS. 2003. The role of iron and the factors affecting off-color development of polyphenols. *Journal of Agricultural and Food Chemistry*, **51**(8): 2304-2316. DOI: https://doi.org/10.1021/jf020681c CCC: $25.00

Minekus M, Alminger M, Alvito P, Ballance S, Bohn N, Bourlieu C, Brodkorb A. 2014. A standardised static in vitro digestion method suitable for food–an international consensus. *Food & Function*, **5**(6): 1113-1124. DOI: https://doi.org/10.1021/jf020681

Naco MEB, Tirera H, Tangara BY, Sarr SO, Fall D, Diop YM, Diop A. 2023. Potentiel thérapeutique et nutritionnel de quelques échantillons de thé vert consommé dans la région de Dakar au Sénégal. *International Journal of Biological and Chemical Sciences*, **17**(4): 1357-1370. DOI: https://dx.doi.org/10.4314/ijbcs.v17i4.6

Ouedraogo O, Kambire D, Zida S, Savadogo B, Soulama I, Zoure AA, Nebie I. 2023. Anémie et infection palustre chez des enfants de moins de 15 ans vivant en zone endémique du paludisme au Burkina Faso. *International Journal of Biological and Chemical Sciences*, **17**(7): 2653-2662. DOI: https://dx.doi.org/10.4314/ijbcs.v17i7.5

Perera DN, Palliyaguruge C L, Eapasinghe DD, Liyanage DM, Seneviratne RH, Demini SMD, Samarakoon SR. 2023. Factors affecting iron absorption and the role of fortification in enhancing iron levels. *Nutrition Bulletin*, **48**(4): 442-457. DOI: https://doi.org/10.1111/nbu.12643

Piskin E, Cianciosi D, Gulec S, Tomas M, Capanoglu E. 2022. Iron absorption: factors, limitations, and improvement methods. *ACS Omega*, **7**(24): 20441-20456. DOI:

https://doi.org/10.1021/acsomega.2c01833

Reddy MB, Agbemafle I, Armah S. 2022. Iron bioavailability: enhancers and inhibitors. In *Nutritional anemia*. Springer International Publishing: Cham; 141-149.

Salovaara S, Sandberg AS, Andlid T. 2003. Combined impact of pH and organic acids on iron uptake by Caco-2 cells. *Journal of Agricultural and Food Chemistry*, **51**(26): 7820-7824. DOI: https://doi.org/10.1021/jf030177

Smati I, Rebey IB, Hammami M, Hamdaoui G, Tounsi MS. 2017. Variation of the quality of lemon (Citrus limon L.) juice during stage of fruit maturity. *Journal of New Sciences, Agriculture and Biotechnology*, **43**(1): 2334-2343.

Snook J, Bhala N, Beales IL, Cannings D, Kightley C, Logan RP, Goddard AF. 2021. British Society of Gastroenterology guidelines for the management of iron deficiency anaemia in adults. *Gut*, **70**(11): 2030-2051. DOI: https://doi.org/10.1136/gutjnl-2021-325210

Temme EHM, Van Hoydonck PGA. 2002. Tea consumption and iron status. *European Journal of Clinical Nutrition*, **56**(5): 379-386. DOI: https://doi.org/10.1038/sj.ejcn.1601309

Teucher O, Cori. 2004. Enhancers of iron absorption: ascorbic acid and other organic acids. *International Journal for Vitamin and Nutrition Research*, **74**(6): 403-419. DOI: https://doi.org/10.1024/0300-9831.74.6.403

Wan D, Wu Q, Ni H, Liu G, Ruan Z, Yin Y. 2019. Treatments for iron deficiency (ID): prospective organic iron fortification. *Current Pharmaceutical Design*, **25**(3): 325-332. DOI: https://doi.org/10.2174/1381612825666190319111437