Research on the Iron Content in Plant-based Beverages

D A Volkova¹, I V Smotraeva¹ and P E Balanov¹
¹ITMO University, Faculty of Food Biotechnologies and Engineering, Lomonosova Street 9, St. Petersburg, 191002, Russian Federation

E-mail: irinasmotraeva@yandex.ru

Abstract. The current study considers tackling iron deficiency and nutritional anaemia through consumption of functional iron-rich foods, which are produced from plant-based ingredients. The study proposes the approach of developing functional beverages recipes. It includes ingredients selection, formula calculation, drink sample evaluation and iron measurement. Manufactured drinks are aimed at vulnerable groups and cover certain amount of iron daily dosage. The approach may act as the way of producing safer food options to avoid immoderate iron intake and prevent chronic iron overload. As the result of the study, the iron content of the best-evaluated sample was measured, and it was estimated that produced Hibiscus-based beverage may be utilized as the tool to prevent deficiency and anaemia in specific groups, without creating the threat for non-targeted individuals.

1. Anaemia as the public health problem
During several decades, anaemia is described as one of the main global public health issues. It is defined as low blood haemoglobin concentration, which negatively influences the well-being of specific age and gender groups [1]. The most vulnerable groups are represented by women of reproductive age, pregnant women and children. According to WHO statistic data, it affects over 800 million of children and women [2]. Anaemia is associated with poor cognitive and motor development among children, adverse consequences of childbirth, maternal and perinatal mortality for women.

There are several mechanisms of anaemia development, including impairment in blood cells production, excessive blood loss and through specific diseases, such as malaria and HIV. However, nearly 50% of the cases are considered to be the implications of iron deficiency, which are described as nutritional anaemias [2]. Iron deficiency is mainly the result of insufficient iron intake through food products. Certain life stages require even more iron – early childhood, female reproductive period, which makes people, experiencing these stages, target groups to obtain potential iron deficiency and anaemia as the following consequence.

2. Iron metabolism and Recommended Daily Intake (RDI)
Two types of iron are identified in food: haem and non-haem iron [3]. Meat sources provide haem-iron, which bioavailability and absorption are not influenced by other food factors, since iron is protected by haem molecule. Non-haem iron is a component of both meat and plant-based sources, and its absorption, on the contrary, is largely influenced by nutritional compounds. The effective non-haem iron absorption is reached by improved bioavailability, which depends on the content of synergists and antagonists of iron digestion. Synergists, such as vitamin C (ascorbic acid) and lemon acid, help increasing iron absorption, whereas antagonists as phytates, polyphenols and calcium lead to
highly reduced absorption [4; 5]. Vitamin C, for instance, has a greater impact on digestion process than the negative effect of the mentioned inhibitors, which are widely found in wholegrains, tea, wines and dairy products [3].

Daily intake of iron depends on the age and gender, but the average number for men and postmenopausal women is 10 mg per day, for children – 11 mg per day, for women of reproductive age is 18 mg/day, and for pregnant women it could be increased up to 27-30 mg/day [6].

3. Deficiency treatment and possible adverse effects
Iron deficiency treated with consumption of iron-rich products, iron-fortified products, additional supplementation and injection of iron medications in severe cases [7]. The research undertaken among primary school children in Vietnam has showed fortified food demonstrating a better effect in decreasing anaemia presence than iron additives [8]. Examples of fortified foods are flour, rice, dry powders for child nutrition, spices and sauces, cacao powder [9].

However, fortified products, such as flour, rice or sauces, used for larger social segments, might pose a threat of iron overload for groups that are not in risk of developing iron deficiency and anaemia, such as men, postmenopausal women and elderly people. This becomes significantly dangerous due to absence of iron excretion mechanisms, therefore, all the iron amount that has accessed the body will be used or stored [3]. Furthermore, gene mutations, prevalent in north-western European population, particularly among people of Denmark and Iceland, lead to excessive iron accumulation in specific body organs [3]. Iron overload damages the function of heart, liver and pancreas, which, henceforth, leads to liver cirrhosis, liver cancer, heart failure, arthritis, and endocrine disease.

To avoid negative iron fortification circumstances of mass-used products, the fortification should be applied to certain products categories. Another perspective direction is the functional foods, which contain natural iron, for instance, in soluble forms. Functional foods are produced to fulfil selected nutritional needs, such as mineral deficiencies, therefore, they do not create the overload risk for unrelated to deficiencies groups.

4. Selection of iron rich plant sources for beverage production
Variety of plant raw materials contain iron: fruits, vegetables, berries, herbaceous plants. To identify the best-matching functional drink components, the amount of iron should be considered together with the presence of synergists and inhibitors of absorption. The essential synergist is ascorbic acid, substances like polyphenols and calcium should be avoided.

As the result of the detailed literature review, Hibiscus sabdariffa, also known as roselle, was chosen as the drink main compound. The flowers and leaves contain significant amount of ascorbic acid and iron. According to USDA (United States Department of Agriculture) data, 100 gr of dry roselle provides 18,4 mg of vitamin C and 1 mg of calcium, which is a positive relation of catalyst against inhibitor [10].

Polyphenols represent a large class of multiple chemicals. Therefore, it is rather hard to estimate the exact amount of those in Hibiscus. Some of the components, such as hibiscitrin and sabdaritrin, are present in the relatively low concentrations, while quercetin content is 3,2 mg/gr and chlorogenic acid – 2,7 mg/gr [11]. Despite the polyphenols demonstrating positive effects (reducing body weight [12; 13], risk of type 2 diabetes [14], blood pressure [15; 16], low-dense cholesterol level [17], supressing melanoma cells growth [18]), they are rather strong inhibitors of iron bioavailability, consequently, their presence should be minimized.

Polyphenols are mainly responsible for antioxidant activity, which is classified as a safe body mechanism. The higher polyphenols content corresponds to the elevated antioxidant properties. Comparative study of antioxidant activity among soy, yellow tea, red wine and Hibiscus sabdariffa has revealed the minimum activity of Hibiscus, comparing to the other analysed samples [19]. The outcome allows to assume that the present polyphenols levels in Hibiscus are relatively low to interfere with iron absorption, especially in the presence of strong synergist as ascorbic acid.
Fruits and berries were reviewed as the potential non-haem iron sources [10]. To make them the components of the beverage, the juice was produced out of the selected raw materials.

Considering synergist against inhibitors ratio, the following fruits and berries have been chosen:

| Raw materials | Fe, mg/100 gr | Ascorbic acid, mg/100 gr | Ca, mg/100 gr | Polyphenols, mg/100 gr |
|---------------|--------------|---------------------------|--------------|-----------------------|
| Lemon         | 0,6          | 53,0                      | 26,0         | 42,0                  |
| Cherry        | 0,3          | 10,0                      | 16,0         | 274,0                 |
| Raspberry     | 0,7          | 26,2                      | 25,0         | 215,0                 |
| Strawberry    | 0,4          | 58,8                      | 16,0         | 235,0                 |

5. Evaluation of physicochemical properties of the ingredients

The chosen ingredients have been analysed to review their physicochemical qualities:

| Commodity         | Juice extraction, % | DM content, % | TA, mmol/l | RP, mV |
|-------------------|----------------------|---------------|------------|--------|
| Hibiscus infusion | -                    | 0,10          | 0,15       | 165,00 |
| Lemon juice       | 34,80                | 7,50          | 1174,00    | 124,00 |
| Cherry juice      | 47,30                | 19,50         | 2,85       | 152,00 |
| Strawberry juice  | 51,50                | 5,50          | 1,00       | 111,00 |
| Raspberry juice   | 54,40                | 7,10          | 2,00       | 95,00  |

Juice extraction barely exceeds 50% with the hand extraction method. However, this technique is still preferable, since much less berries sediment enters the juices in comparison to mechanized extraction. The sediment can negatively alter the beverage consistency and shelf-life.

Dry matter (DM) content is comparatively high to avoid adding sugars to the final drink sample. The taste profile may be modified by including greater amount of the cherry juice, that has the highest DM percentage. This inclusion will elevate the sweetness and decrease the acidity.

The highest titratable acidity (TA) expectedly belongs to the lemon juice. Titratability is one of the crucial iron-enriched drink indicators, since the higher titratability corresponds to better digestion of metal forms found in fruits. Lemon juice will ensure the essential vitamin C content and certain acidity of the product itself, which will have an impact on the better iron absorption.

Redox potential (RP) measurement has been conducted to define antioxidant activity of the ingredients. The low potentials are correlated with high antioxidant activity. This effect has not been identified among analysed commodities; all the RP numbers are relatively high. Nevertheless, redox potentials of the strawberry and raspberry juices are rather decreased, therefore, it can be suggested that the reasonably lowered potential can be achieved with the certain ingredients’ combination.

6. Calculation the iron-enriched beverage recipe №1 and №2 and assessment implementation

Information for the recipe calculation was utilized from USDA database [10]. The portion amount has been estimated as 500 gr. Recommended daily intake was chosen as the average amount for reproductive age women – 18 mg/day. The ingredients ratio was selected experimentally, by enhancing the amount of some ingredients, while reducing the quantity of the others.

| RDI (women), mg/day | Ingredients of the product, 100 gr |
|---------------------|-----------------------------------|
| Fe, mg              | Hibiscus | Lemon | Cherry | Raspberry | Strawberry |
| 18,00               | 1.29     | 0.6   | 0.53   | 0.69      | 0.75       |
According to the recipe №1, the beverage has been produced and evaluated by physicochemical and organoleptic parameters. Sample №1 has demonstrated successful results of the instrumental assessment, however, organoleptic evaluation has revealed the elevated acidity, which forms the uncommon and rather unpleasant taste for an average consumer. It was decided to modify the initial formula by decreasing the volume of lemon juice and adding the berry juices instead. The formula has been modified to recipe №2, using the same data and calculation process.

The beverage sample №2 was produced, according to the modified recipe, and analysed using the same evaluation criteria. It has demonstrated significantly good results in both physicochemical and sensory assessments, the latter of which showing the improved and balanced flavour profile.
7. Measurement of the iron content in beverage portion

7.1. Method description
Photocolorimetry is used as a method of iron content evaluation in food products [20]. It is based on the chemical reaction between phenanthroline and iron (II) at pH 3-9 with the formation of orange-red coloured complex compound.

The methodology includes drink filtration, preparation of control solution, analysed solution, measurement of optical density, construction of calibration curve and iron content calculation.

Optical density of analysed solution is measured relative to control solution at wavelength of 490 nm. Calibration curve is constructed with the usage of Mohr’s salt (ammonium iron (II) sulphate) solution with concentration of 20 μg/ml.

Formula for iron in portion calculation:

\[ m_{Fe} = \frac{C(Fe)_{st} \cdot V(Fe)_{cur} \cdot V_{al}}{V_{tot}}. \]  

where \( C(Fe)_{st} \) – iron concentration in standard solution, μg/ml
\( V(Fe)_{cur} \) – iron volume according to calibration curve, ml
\( V_{al} \) – drink aliquot used for analysis, ml
\( V_{tot} \) – total drink volume, ml

7.2. Results of assessment
The calibration curve was prepared using standard iron solutions with different volumes.

Figure 2. Standard curve for identifying substance concentration.

The drink volume prepared for the analysis was 50 ml. Measured optical density of the analysed solution is \( A_r = 0.232 \). According to the curve, \( A_r = 0.232 \) corresponds to the following volume of the standard iron solution: \( V_{x(Fe)} = 0.57 \) ml. Mass of iron, corresponding to this volume, is:

\[ m_{x(Fe)} = C(Fe) \times V_{x(Fe)} = 20 \mu g/ml \times 0.57ml = 11.48 \mu g \]  

This iron content is present in the aliquot used for conducting the analysis, which is 2 ml. Total produced beverage is 50 ml, hence, iron mass \( m(Fe) \) in the whole volume is:

\[ m(Fe) = m_{x(Fe)} / 2 \times 50 = 11.48 / 2 \times 50 = 287 \mu g \text{ in } 50 \text{ ml of drink} \]  

The amount was recalculated for 100 ml. Calculated during statistical processing, observational error of the three parallel measurements (0.56; 0.58; 0.57) is 0.01 mg/100 ml, consequently:

\[ C_{Fe} = 0.57 \pm 0.01 \text{ mg/100 ml} \]  

The final iron per drink portion content is:
\[ C_{Fe} = 2.85 \pm 0.01 \text{mg/500 ml} \] (5)

RDI was taken as an average of 18 mg/day for reproductive women. The total iron amount in portion, which is 2.85 mg, covers 15.83% of the daily intake. Theoretical calculation of the expected daily intake coverage is 25.73%, according to the second formula calculation. Therefore, it is 9.9% higher than the intake coverage, calculated based on the measured iron amount of the drink.

The produced beverage can be classified as functional, since it covers certain percentage of the recommended daily intake for women of reproductive age. The functionality has been discussed, including possible application to prevent the development of iron deficiency and anaemia. The ingredients of the beverage have been identified. All the components are plant-based, and iron is derived from natural substances, the beverage includes no added sugars or preservatives. The formula has been calculated and modified through instrumental and sensory evaluation of the drink samples. Iron content in the best evaluated sample has been measured and compared to the recommended daily intake for women of reproductive age. The functionality has been discussed, including possible application to prevent the development of iron deficiency and anaemia. This option can be defined as relatively safe due to moderate iron content, which will not lead to iron chronic overload.

8. Results

To create an effective and safe tool to prevent iron deficiency and anaemia, this study has proposed the approach of creating functional drink recipes. As the result of the literature review, the ingredients of the iron-rich beverage have been identified. All the components are plant-based, and iron is derived from natural substances, the beverage includes no added sugars or preservatives. The formula has been calculated and modified through instrumental and sensory evaluation of the drink samples. Iron content in the best evaluated sample has been measured and compared to the recommended daily intake for women of reproductive age. The functionality has been discussed, including possible application to prevent the development of iron deficiency and anaemia. This option can be defined as relatively safe due to moderate iron content, which will not lead to iron chronic overload.

9. Conclusion

The study has described the approach of developing recipes for iron-rich plant-based beverages. Those food options can be described as functional products, which represent potential solutions to tackle iron deficiency without causing a danger of excessive iron consumption.

10. References

[1] WHO 2015 The Global Prevalence of Anaemia in 2011 (Geneva–WHO) p 48
[2] WHO 2017 Nutritional Anaemias: Tools for Eff. Prevention and Control (Geneva–WHO) p 96
[3] Truswell S and Mann J 2012 Essentials of Human Nutrition (Oxford–OUP) pp 162–70
[4] Lönnerdal B 2010 Calcium and iron absorption mechanisms and public health relevance Int. J. Vitam. and Nutr. Res. 80 pp 293–99
[5] Zijp I, Korver O and Tijburg L 2000 Effect of tea and other dietary factors on iron absorption Food Sci Nutr. 40 pp 371–98
[6] Institute of Medicine 2001 Food and Nutrition Board. Dietary Reference Intakes for Vitamin A, Vitamin K, Copper, Iodine, Iron et al (Washington, DC–National Academy Press) p 800
[7] WHO 2016 Strategies to Prevent Anaemia: Recommendations from an Expert Group Consultation (New Delhi–WHO) p 35
[8] Thi Le H, Brouwer I, Burema J, Nguyen K C and Kok F J 2006 Efficacy of iron fortification compared to iron supplementation among Vietnamese schoolchildren Nutr. J. 5 pp 1–8
[9] WHO and FAO 2006 Guidelines on Food Fortification with Micronutrients (France–WHO) chapter 5 pp 97–110
[10] USDA Food Data Central: https://fdc.nal.usda.gov/index.html
[11] Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I and Heinrich M 2014 Hibiscus sabdariffa L. – a phytochemical and pharmacological review Food Chem. 165 pp 424–43
[12] Chang HC, Peng CH, Yeh DM, Kao ES and Wang CJ 2014 Hibiscus sabdariffa extract inhibits obesity and fat accumulation, and improves liver steatosis in humans Food Funct. 5 pp 734–9
[13] Heranz-Lopez M, Olivares-Vicente M, Encinar J A, Barrajón-Catalán E, Segura-Carretero A, Joven J and Micol V 2017 Multi-targeted molecular effects of Hibiscus sabdariffa polyphenols: an opportunity for a global approach to obesity Nutrients 9 pp 1–26
[14] Williamson G 2017 The role of polyphenols in modern nutrition Nutr. Bull. 42 pp 226–35
[15] Hopkins A L, Lamm M G, Funk J and Ritenbaugh C 2013 Hibiscus sabdariffa L. in the treatment of hypertension and hyperlipidemia Fitoterapia 85 pp 84–94
[16] Beltrán-Debón R, Rodríguez-Gallego E, Fernández-Arroyo S, Senan-Campos O, Massucci F A, Hernández-Aguilera A, Sales-Pardo M, Guimerà R, Camps J, Menendez J A et al 2015 The acute impact of polyphenols from Hibiscus sabdariffa in metabolic homeostasis: an approach combining metabolomics and gene-expression analyses Food Funct. 6 pp 57–66
[17] Mohagheghi A, Maghsoud S and Ghazi-Khansari M 2011 The effect of Hibiscus sabdariffa on lipid profile, creatinine, and serum electrolytes: a randomized clinical trial Hindawi 3 pp 1–4
[18] Goldberg K H, Yin A C, Mupparapu A, Retzbach E P, Goldberg G S and Yang C F 2017 Components in aqueous Hibiscus rosa-sinensis flower extract inhibit in vitro melanoma cell growth Trad.i Complement. Med. 7 pp 45–9
[19] Andzi Barhe T and Feuya Tchouya G R 2016 Comparative study of the anti-oxidant activity of the total polyphenols extracted from Hibiscus Sabdariffa L, Glycine max L Merr, yellow tea and red wine through reaction with DPPH free radicals Arabian J. of Chem. 9 pp 1-8
[20] Intergovernmental Standard GOST 26928 – 86 “Food-stuffs Method for determination of iron”