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Sorbet Made from Moringa Leaves and Red Guava as an Alternative for the Management of Iron Deficiency Anemia in Adolescent Girls

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Abstract

Moringa leaves contain high iron (Fe) but low bioavailability of Fe because it contains calcium and tannins. Vitamin C in red guava can increase Fe absorption. Sorbet made from red guava and Moringa leaves can be an alternative to overcome iron deficiency anemia in adolescent girls. This study aims to analyze the level of acceptance, levels of Fe, Ca, vitamin C, and tannins, as well as the bioavailability of Fe sorbet made from red guava and Moringa leaves. Sorbet consists of 5 formulas including, F1 (strawberry sorbet), F2 (red guava sorbet), F3 (Moringa leaf sorbet), F4 (red guava sorbet + 18% Moringa leaves), and F5 (red guava sorbet + 22% Moringa leaves). Acceptance level was tested with the hedonic method (appearance, smell, taste, and texture) and statistically analyzed using Friedman and Wilcoxon test. The Fe and Ca were analyzed using X-Ray Fluorescence method, vitamin C and tannins were analyzed using High-Performance Liquid Chromatography and spectrophotometry methods. Fe bioavailability test was performed using the inverted bowel bag method. The level of preference for each attribute was significantly different ($p < 0.05$). The level of preference chosen was F2. The mean levels of Fe, Ca, vitamin C, and tannins in F5 were 0.21 ± 0.29, 6.03 ± 0.67, 46.72 ± 0.69, and 87.76 ± 0.40 mg/100 g. The highest Fe bioavailability was F5 while the lowest was F3. The best formula based on the parameters of preference, Fe, Ca, vitamin C, and tannin levels, as well as Fe bioavailability was sorbet F5.

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Introduction

Anemia is a condition in which the body has a low red blood cell count (Balarajan *et al*. 2011). Based on DALY (Disability-Adjusted Life Years), the prevalence of iron deficiency anemia at the age of 15–44 years is ranked third as a global health problem (Departemen Gizi dan Kesehatan Masyarakat, 2007). On one hand, the prevalence of anemia in adolescent girls aged 13–18 years and women of childbearing age in 2013 in Indonesia reached 22.7%, respectively (Badan Penelitian Kesehatan Kementrian Kesehatan RI., 2013). This prevalence increased in 2018 by 32% in adolescent girls, which means 3–4 out of 10 adolescents suffer from anemia (Badan Penelitian Kesehatan Kementrian Kesehatan RI., 2018).

Iron deficiency anemia in adolescents especially in young women has an impact on decrease concentration in learning, drowsiness, frequent fatigue, lower body resistance (Damayanti *et al*. 2021;Soliman *et al.* 2014; Hassan *et al.* 2016). Menstrual patterns, knowledge of anemia, dietary regulation, and nutritional status are risk factors for iron deficiency anemia in adolescent girls (Kounnavong *et al*. 2020; Chandra *et al.* 2016; Cairo *et al.* 2014; Shekhawat *et al.* 2014). To overcome anemia, we can modify food, diversify food, and fortify iron in food. A sustainable food-based approach using sufficient food sources of iron can control iron deficiency anemia effectively (Saini *et al.* 2014).

One of the potential food-based approaches is the development of food products. Product development by combining food sources of iron, namely Moringa leaves. Moringa leaves as alternatives food source to solve malnutrition problem cause contains iron (Fe) therefore quantitative analysis result is 61.006mg/kg (Nurhaini *et* *al.* 2018). Red guava fruit contains vitamin C level is 87mg/100gr (Khairiyah *et al.* 2022). Moringa leaves and food sources of vitamin C as a source of nutrients that increase iron absorption, namely red guava, was carried out in this study (Saunders *et al*. 2012). Sorbet is a frozen dessert made of water and sugar with a fresh fruit content of at least 25%. Sorbet is not added with fat (e.g., milk) in the manufacturing process. The product in this study was a sorbet made from Moringa leaves and red guava. This research may provide information about the level of acceptance, levels of Fe, Ca, vitamin C, and tannins, as well as the bioavailability of Fe sorbet made from red guava and Moringa leaves.

Materials and methods

Sorbet Production

The sorbet was made from fresh red guava (*Psidium guajava*) and Moringa leaves (*Moringa oleifera*), sugar, water, and CMC (Carboxy Methyl Cellulose) stabilizer. Meanwhile, strawberry sorbet (control) was made from raw materials of strawberry (*Fragaria x ananassa*), granulated sugar, water, and CMC stabilizer.

This study was conducted from October 2020 to April 2021, and it was an experimental study with a one-factorial completely randomized design, namely a sorbet formulation made from Moringa leave and red guava. Sorbet formulations were divided into five groups, including strawberry sorbet (F1/control), 100% Moringa leaf sorbet (F2), 100% red guava sorbet (F4) (F5), red guava sorbet + 18% Moringa leaves (F4), and red guava sorbet + 22% Moringa leaves (F5).

Organoleptic Test Procedures

The organoleptic test of sorbet by hedonic method. The data analysis technique used this study is organoleptic test conducted by panelists and hedonic method (Sudiarta, 2022). The analysis involved 34 moderately trained panelists consisting of undergraduate and postgraduate students, as well as the academic staff of the Department of Nutrition, Faculty of Medicine, Diponegoro University (UNDIP). Each sample was randomly coded. The hedonic test shows appearance, smell, taste, and texture.

Level of Iron and Calcium

The level of iron and calcium of sorbet was analyzed using the X-Ray Fluorescence (XRF) method in Integrated Laboratory of UNDIP. The element like iron (Fe) and calcium was performed by XRF analysis (Bora *et al.,* 2014)

Level of Vitamin C and Tannins

The level of Vitamin C and Tannins of sorbet was analyzed using High-Performance Liquid Chromatography (HPLC) and spectrophotometry methods in Food Technology Laboratory Soegijapranata Catholic University. HPLC method to quantify the content of vitamin C (Chen *et al.*, 2022)

Iron Bioavailability and Iron Absorption Levels

The analysis of iron bioavailability and iron absorption levels, it was carried out on samples of male Wistar rats (*Rattus Norvegicus Strain Wistar*) at the Laboratory of the Center for Food Nutrition Studies (PSPG) UGM. The method using the inverted bowel bag and spectrophotometry methods. In this study, the best formula was determined using the effectiveness index method.

Data Analysis

Data were statistically analyzed with SPSS software version 22 and presented by describing the results of the test of hedonic method level of preference, levels of iron, calcium, vitamin C, tannins, and iron bioavailability in the form of mean ± SD (univariate analysis). Bivariate analysis was carried out by analysing the differences in the level of preference for the five sorbet groups using the Friedman test (data not normally distributed) and Wilcoxon test (data not normally distributed) to determine the differences in each formula and each hedonic attribute. This study has been approved by the Ethics Commission of the Faculty of Medicine, Diponegoro University, number 16/EC/H/FK-UNDIP/II/2021.

Results and Discussion

Level of Preference of Sorbet

Table 1. The result of the level of preference test of sorbet

Category	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5	*p-value*
Appearance	7.24 ± 1.33	7.44 ± 1.08	5.09 ± 1.76	3.94 ± 1.43	4.41 ± 1.52	0.000*
Smell	6.47 ± 1.52	6.71 ± 1.27	3.56 ± 1.56	4.18 ± 1.38	5.00 ± 1.21	0.000*
Taste	5.15 ± 1.91	6.06 ± 1.70	3.09 ± 1.64	4.91 ± 1.38	4.91 ± 1.29	0.000*
Texture	6.21 ± 1.27	6.41 ± 1.71	5.06 ± 1.72	5.15 ± 1.28	5.38 ± 1.37	0.000*

*: *p value* <0,005 (significantly different)

The level of preference was analyzed by the hedonic method to analyze the level of preferences of the panelists on the appearance, smell, taste, and texture attributes of sorbet with five different formulas. The results of the analysis using the Friedman test (table 1) showed that there were differences in the appearance, smell, taste, and texture of the sorbet ($p < 0.05$).

Based on Wilcoxon's post hoc test, the panelists preference for appearance attributes of sorbet F1 was different from that of sorbets F2, F4, and F5. Their preference for the appearance of sorbet F2 was also different from that of sorbets F3 and F4. Their preference for the appearance of sorbet F3 was different from that of sorbets F4 and F5 while their preference for the appearance of sorbet F4 was different from that of sorbet F5.

Appearance/color is an important attribute of food quality because color is the first attribute seen by consumers, so it determines the level of preference for a food product (Hardjanti, 2008). Sorbet F2 has a pink color, which comes from the main ingredient, red guava. The natural color of food products will change, which is influenced by the level of the composition of the ingredients (Winarno, 2006). Sorbet F4 has a brownish-green color, which is a mixture of the green color from Moringa leaves and the pink color from red guava. The Moringa leaf color of F4 was pale green because Moringa leaves undergo a rapid blanching process before being blended. This process can inhibit the browning process, so the resulting color is not too thick (Muchlisun *et al*., 2015; Afrianti, 2013).

Smell/aroma is also a quality specification of organoleptic assessment which is very subjective and difficult to measure because each person has a different level of smell. The smell of food determines its taste/delicacy. A food product will be more easily accepted by consumers if it has a distinctive and attractive odor (Winarno, 2006). The smell of sorbet F2 is caused by the ingredients used, such as the distinctive smell of red guava. Moringa leaves have a distinctive unpleasant smell (Miftahur & Rita, 2014), which is caused by the Moringa leaf containing the lipoxidase enzyme (an enzyme found in green vegetables). This enzyme hydrolyzes or decomposes fat into compounds that cause unpleasant smells (including hexanal 7 and hexanol groups) (Ilona & Ismawati, 2015).

Based on the hedonic test on taste, sorbet with the main raw material from Moringa leaves has a slightly bitter taste. The Moringa leaves have a bitter taste because they contain tannin compounds. Tannins are astringent compounds that have a bitter taste from their polyphenol groups, so they can bind and precipitate proteins (Ismarani, 2012). Tannins can also cause an astringent taste because, when they are consumed, cross-links are formed between tannins and proteins/glycoproteins in the oral cavity (Rosyidah, 2016). The bitter taste of Moringa leaves can be lost if the Moringa tree is harvested regularly for consumption (Aminah *et al*., 2015).

Meanwhile, the textures produced from the five sorbet formulas have almost the same texture because they are made with the same procedure, namely in a blender with the addition of water, sugar, and stabilizer/CMC (Carboxyl Methyl Cellulose). CMC was added to refine the texture of the sorbet because, in general sorbet has a coarse texture due to the presence of ice crystals. The stabilizer used in making ice cream/sorbet was CMC. This material can change the texture and ice crystals of the sorbet to be more homogeneous, smooth, and not easy to melt. This means that as more water is bound, there is no formation of coarse ice crystals, resulting in a soft texture (Rini & Ishartani, 2012).

Iron, Calcium, Vitamin C, and Tannins

Table 2. The Fe, Ca, vitamin C and tannin content of sorbet

Formula	Fe	Ca	Vitamin C	Tannin
F1	0.20 ± 0.12	2.50 ± 0.45	24.94 ± 0.53	189.50 ± 0.00
F2	0.12 ± 0.08	1.36 ± 0.26	29.74 ± 0.09	129.50 ± 0.08
F3	0.16 ± 0.04	29.14 ± 1.99	29.83 ± 1.42	123.77 ± 1.09
F4	0.95 ± 0.14	5.91 ± 0.73	26.29 ± 0.13	135.65 ± 0.91
F5	0.21 ± 0.29	6.03 ± 0.67	46.72 ± 0.69	87.76 ± 0.40

Based on the results of the analysis of iron, calcium, vitamin C, and tannins (table 2) was found that the sorbet with the highest iron content was F4 (red guava sorbet + 18% Moringa leaves) with an average iron content of 0.95 mg/100 g. The iron content of sorbet F3 (100% Moringa leaf sorbet) was lower than that of sorbets F4 and F5 sorbet (red guava sorbet + 22%). This is because the iron found in red guava is higher than in Moringa leaves (1.10 mg/100 g in guava and 0.85 mg/100 g in Moringa leaves) (BPPHP., 2002; Gopalakrishnan, Doriya, & Kumar, 2016; Olagbemide & Alikwe, 2015).

The calcium content of sorbet F3 was higher than the others and sorbet with the lowest calcium content was sorbet F2 (red guava sorbet) with an average calcium content of 1.36 mg/100 g. Because fresh Moringa leaves have a fairly high calcium content of 1,077 mg/100 g (Direktrorat Gizi Masyarakat, 2018). Previous research on ice cream products added with Moringa leaf flour showed that the more Moringa leaf flour was added to the ice cream, the higher calcium it contains (Iskandar AB, Ningtyias FW, 2019). Meanwhile, fresh red guava contains 14 mg/100 g of calcium (BPPHP., 2002), lower than the calcium of fresh Moringa leaves.

Sorbet F5 had the highest levels of vitamin C (46.72 mg/100 g). The vitamin C content of 100% Moringa leaf sorbet was higher than that of 100% red guava sorbet because fresh Moringa leaves have higher levels of vitamin C than red guava. The vitamin C content of fresh Moringa leaves was 220mg/100g (Gopalakrishnan et al., 2016; Mahattanatawee *et al*., 2006) while red guava contains 87 mg/100g of vitamin C (BPPHP., 2002).

Sorbet that uses redder guava mixture (F5) has the lowest tannin content (87.76 mg/100 g) because the tannin content of Moringa leaves is higher than the tannin content of red guava. The research of moringa leaves has results of total tannin content (total phenol minus non-tannin phenolic phenol) 35-44g/kg of dry weight (du Toit *et al*. 2020). Meanwhile, another study revealed that the total soluble phenolic (TSP) content of red guava was 231.67mg gallic acid/100g puree. Gallic acid is a subclass of hydrolyzed tannins commonly found in plants (Mahattanatawee *et al*., 2006).

Iron Bioavailability

The results of the bioavailability test showed that the greatest bioavailability of iron in all formulas was found at 120 minutes. The bioavailability of iron in all formulas increased from 0 minutes to 30, 60, 90, up to 120 minutes. Sorbet F5 had the highest mean bioavailability of iron at 120 minutes while sorbet F3 had the lowest mean (Figure 1).

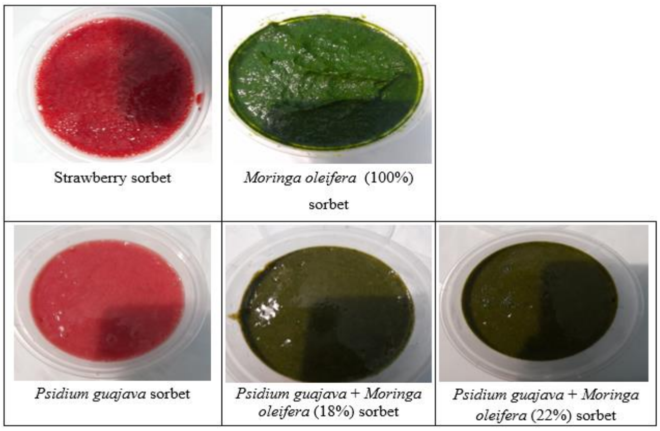

Figure 1. Sorbet with five different formula

The bioavailability at 120 minutes means that the amount of iron in the sorbet made from red guava and Moringa leaves is absorbed by the small intestine (duodenum) within 2 hours. Digestion in the small intestine (duodenum) is divided into 3 phases, including bolus transit in the small intestine, degradation of macromolecules (carbohydrates, proteins, or lipids) into smaller ones, and absorption through the intestinal wall. A study on the digestive model in the small intestine (influence of dietary fibre) stated that the transit time of insoluble dietary fiber bolus in the small intestine ranged from 3.9 to 5 hours while the soluble dietary fibre ranged from 5 to 6.7 hours (Taghipoor *et al*., 2014).

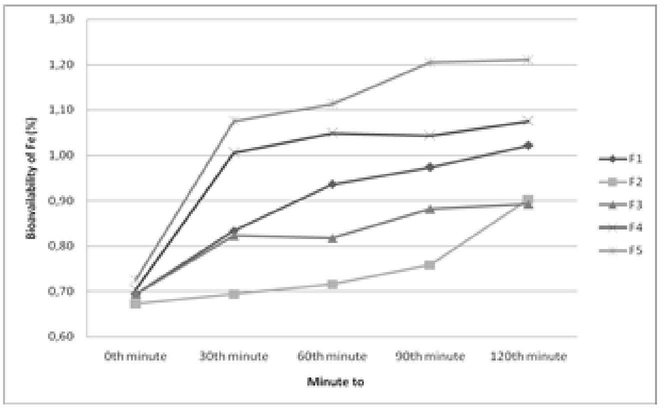

Figure 2. Bioavailability of Fe (%)

This study used Moringa leaves (vegetable) and red guava (fruit), so it is classified as soluble dietary fibre. The transit time of a bolus of sorbet made from red guava and Moringa leaves in the small intestine is estimated to be around 5 to 6.7 hours. The refined form of the sorbet and the iron content, which is a micromolecule, may speed up the transit time for further absorption by the intestinal wall. Two hours has described the absorption of iron in the small intestine.

Absorption is the process of taking nutrients from the body's surface into the circulation/bloodstream. Bioavailability is defined as how well a nutrient can be absorbed by the body and used to reduce micronutrient deficiencies (Tessera *et al*., 2015). The bioavailability of iron from plant-based foods is lower than iron from animal-based foods. In addition, the absorption from plant-based foods is strongly influenced by interactions with triggering and inhibiting nutrients for iron bioavailability. Calcium can inhibit iron absorption when given in the form of inorganic calcium compounds or consumed from dairy products, such as milk or cheese (the degree of inhibition depends on the amount of calcium consumed) (Yang *et al*., 2006). The presence of anti-nutritional factors in food, such as phytates, tannins, and polyphenols can also inhibit the bioavailability of iron (Tessera *et al.*, 2015).

The bioavailability of iron in fresh dried and boiled Moringa leaves increased by 3 and 3.5 times, respectively (Yang *et al*., 2006). Cooking Moringa leaves also increases the total available iron. The making of sorbet in this study did not involve a cooking process with the main ingredients (Moringa leaves, red guava, and strawberries) as well as other additional ingredients (sugar, CMC, and water). Moringa leaves were blanched (a quick dip for 1–2 minutes in boiling water) while red guava and strawberries were processed fresh. Therefore, the iron bioavailability of the sorbets with these five different formulas only ranged from 0.6% to 1.2%.

Research on the bioavailability of iron in Moringa leaves in vitro was carried out by adding ascorbic acid to samples during the digestion to increase iron extraction during peptic-pancreatic digestion of plant-based food products. Ascorbic acid further increased the bioavailability of iron from plant-based food products when added to the digestive process exposed to Caco-2 cells (iron uptake process), compared to the addition of ascorbic acid during the medium digestion process (Khoja *et al*., 2020).

This study used guava (a fruit rich in vitamin C) in making sorbets F4 and F5. Vitamin C can increase the absorption of iron when taken at the same time because vitamin C changes the form of iron from the ferricyanide form to ferrocyanide (the more easily absorbed form). Vitamin C forms an iron-ascorbate group which remains soluble at higher pH in the small intestine (duodenum) (Almatsier, 2009). The previous study had shown that vitamin C played a role in increasing iron absorption when the body was in a state of iron deficiency (Garcia *et al*., 2003).

Conclusion

The highest Fe bioavailability was F5 while the lowest was F3. The best formula based on the parameters of preference, Fe, Ca, vitamin C, and tannin levels, as well as Fe bioavailability, was sorbet F5.

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