Iron supplement for anemia developed from home industry waste of snake fruit Pondoh (Snake fruit *edulis* Reinw.)

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Abstract. Anemia due to iron deficiency in young girls and pregnant women remains a nutritional health problem in Indonesia. They take iron tablet/day to improve their iron status but it sometimes has some side effects. In Sleman Regency, snake fruits Pondoh are used to make various processed foods but some home food industries leave snake fruit seeds as industrial waste. Therefore, the aim of this study was to extract gradually seeds of snake fruits Pondoh for development of anemia iron supplement. In this explorative study, we collected seeds of snake fruits Pondoh from a home food industry in Turi district, Sleman Regency, Yogyakarta. In brief, we made simplicia of snake fruit seeds in several steps from washing, selecting, drying, cutting in small pieces, grinding to separating with 100 mesh sieve. After that, the simplicia was extracted using tetrahydrofuran, methanol, acetic acid, hexane and or ethyl acetate reagents to separate lipid, water and organic fractions, which were then dried using a vacuum evaporator. Dried fractions of snake fruit seeds (SFS) were finally measured using the inductively coupled plasma or atomic absorption spectroscopy method for iron levels and using ferric chloride for tannin content. From two different methods of making simplicial, we got 26.88 and 22.82% (weigh/weight) iron levels while ethyl acetate SFS fraction had higher iron levels (2.65% w/v) than lipid and water fractions (1.62 and 1.88% respectively). In addition, there was only the water fraction that contained tannin. In conclusion, the ethyl acetate fraction of SFS Pondoh may be useful for development of anemia iron supplement.

1. Introduction

Anemia which is commonly caused by iron deficiency, remains a nutritional problem in the global community including in Indonesia because the prevalence of anemia in the world is almost stable from 29 to 43%. Children, pregnant women and young girls are more susceptible to get anemia than other age groups [1,2]. The Indonesian government has already implemented iron supplementation, which provides iron tablets containing 60 mg Fe and 400 µg folic acid for pregnant women and young girls but daily administration of iron tablet sometime causes some side effects [3].

Snake fruit (*Salacca edulis* Reinw.) is a typical Indonesian plant which has some varieties based on its growing areas [4]. For example, snake fruit Pondoh is cultivated in Turi, Tempel and Pakem districts, Sleman Regency and has the sweetest flavor among Indonesian snake fruits [5]. From nutrition viewpoints, snake fruits contain complete macro and micronutrients such as carbohydrate, calcium, phosphorus, vitamin C and iron but only about 56 - 65% portion of snake fruit can be consumed and the remaining portion becomes waste like peel and seeds [5,6]. In addition, harvested snake fruits easily decay in room temperature and some home industries have processed flash of snake fruits to become high economic products [7]. However, they leave snake fruit seeds (SFS) as industrial waste and there is limited products from which have been made [8].
In recent years, natural compounds have attracted public attention and some of them have been used as alternative therapies for overcoming health problems. From our research group, Susanti has successfully made SFS powder from which contains 19.9% (w/w) Fe and 5.8% (w/w) mg Zn [9]. Furthermore, SFS ethanol extract contain some secondary metabolites like phenols, flavonoids and tannins, which have $229.27 \pm \mu$g / mL IC50 for antioxidant activity [10]. However, the latter secondary metabolite belongs to phenolic compounds acting as anti-nutritional properties by making macromolecules-binding complexes to reduce 3 - 15% chemically digestion in the small intestine. Tannins also have an astringent effect with bitter flavor, which can bind and precipitate proteins [11].

Another negative effect of tannins is to chelate ferric ion in plant foods so that iron availability is reduced and to decrease protein digestibility and other nutrients bioavailability [12]. A recent study showed that 100g SFS flour consists of 290.88 mg tannins [13]. Therefore, the aim of this study was to improve the SFS nutrients content and to extract gradually SFS Pondoh for development of anemia iron supplement.

2. Methodology
2.1 Making SFS Simplicia
In brief, to produce SFS simplicia, we collected the same size of SFS from one home food industry where was located at Donokerto village, Turi District, Sleman Regency, Yogyakarta. All collected seeds were washed with tap water and allowed to dry at room temperature. To ensure completely dry, cleaned SFS were air-dried under direct exposure of sunlight and continued to be dried in a cabinet dyer for 8 hours and in oven for 90 min. Before grounding, dried SFS were divided into two parts: cutting in small pieces and whole SFS. Subsequently, SFS simplicia was sieved with a 100 mesh sieve and keep it in a tight bottle before further analysis.

2.2 Fractionation of SFS simplicia
All chemical reagents used in this study were purchased from Merck KGaA, Darmstadt, Germany unless otherwise stated. For separating lipid, organic and water soluble fractions of SFS simplicia, we adopted from a study conducted by Tang and co-workers (2018) with a slight modification [14]. At first, SFS simplicia was dissolved in tetrahydrofuran solution at 37°C for 30 minutes in a water bath and was then centrifuged at 4,200 g for 10 min. The extraction was repeated one more with the same reagent to collect supernatant as a lipid-soluble fraction. Collected residues were dissolved in 50: 3.7: 46.3 volume / volume, methanol-acetic acid-water mixtures at 37°C for 30 minutes in a water bath. After centrifugation, the supernatant was collected and the residue was re-extracted to get water-soluble fraction.

The remaining residue was resuspended in a buffer solution containing 2 M NaOH, 10 mM EDTA and 1% ascorbic acid at 37°C for 30 min in a water bath, and then acidified to pH 2 with 6 M HCl solution. The mixture was extracted twice with 5 mL n-hexane to remove fatty acids and then was extracted twice with 5 mL diethyl ether and 5 mL ethyl acetate mixtures. The supernatant was collected as the organic-soluble fraction after 10 min centrifugation. All pooled fractions were dried using a vacuum evaporator. Finally, dried SFS fractions were then put in a closed container and stored in a refrigerator before use.

2.3 Measurement of Protein, Micronutrients, and Tannin
Iron and zinc concentrations in SFS simplicia were measured using a spectroscopy with the inductively coupled plasma (ICP) method at the Central Research and Testing Laboratory, Gadjah Mada University, Yogyakarta while protein and vitamin C concentration was measured using the Kjeldahl method and liquid chromatography-mass spectrometry respectively. Iron concentration and tannin content in three different SFS fractions were tested using atomic absorption spectroscopy and ferric chloride methods respectively and it was performed at the Chemistry Laboratory, Faculty of Life Sciences, Semarang State University, Central Java.
3. Result and Discussion

In this study, we added oven or cabinet drying processes to make SFS simplicia in order to improve its nutrients content. Table 1 indicated that oven-dried whole seeds (OWS) of snake fruit had the highest nutrients contents, compared to other methods (OCS, CWS and CCS). Iron concentration in OWS (26.88%) and OCS (22.82%) was higher than iron concentration in CWS (16.94%) and CCS (14.58%). For zinc, protein and vitamin C concentrations, it had the same pattern that OWS had the highest nutrient contents.

Table 1. Comparisons of Different Drying Methods and SFS Before Grounding on Iron, Zinc, Protein and Vitamin C Concentrations

|            | OWS* | OCS | CWS | CCS |
|------------|------|-----|-----|-----|
| Iron (mg/100g) | 26.88| 22.82| 16.94| 14.58|
| Zinc (mg/100g) | 4.65 | 2.82 | 2.23 | 2.62 |
| Protein (g/100g) | 3.71 | 0.21 | 1.21 | 2.29 |
| Vitamin C (mg/100g) | 0.88 | ND  | 0.21 | ND   |

This method was used by Melati and Ristanti thesis [15,16]. OWS: oven-dried whole seeds; OCS: oven-dried chopped seeds; CWS: cabinet-dried whole seeds; CCS: cabinet-dried chopped seeds; ND: not determined

In our study, we have demonstrated the first time that additional drying processes for whole or chopped SFS influence the nutrients content. In comparison to Karta and Susanti studies, this OWS method is superior in terms of iron concentration but the zinc and protein concentrations are lower than that of those studies [8,9]. Additionally, SFS simplicia has higher contents of Fe, protein and Zn compared to contents of Fe, protein and Zn in fresh flesh of snake fruit [6]. Therefore, the SFS simplicia potentials for iron fortification and iron supplementation for iron deficiency anemia therapy. Although the Fe and Zn concentrations in SFS simplicia is high, the concentration of anti-nutritive tannin is also high [13]. We then extracted SFS simplicia using the Tang and his colleague method to remove the SFS tannin.

Table 2. Iron concentration and tannin content in different SSF fractions

|            | Lipid Fraction | Ethyl Acetate Fraction | Water Fraction |
|------------|----------------|------------------------|----------------|
| Iron (mg/L) | 16.17 ± 0.67   | 26.55 ± 0.54           | 18.82 ± 0.4   |
| Tannin     | (-)            | (-)                    | (+)            |

After three steps of fractionation, the highest iron concentration was observed in the ethyl acetate fraction, compared to the lipid and water fractions whilst iron concentration in water fraction was higher than that of lipid fraction (Table 1). In terms of tannin content, only water fraction was positive and the fat and ethyl acetate fractions were negative. From this findings, it clearly indicates that SFS iron has greater solubility in a mixture of ethyl acetate and diethyl ether reagents, compared to tetrahydrofuran and methanol-acetic acid-water mixtures [17]. Ethyl acetate is a semi-polar solvent that can dissolve both polar and non-polar compounds in SFS simplicia to produce high iron concentration.

Furthermore, tannins have more than one hydroxyl groups and unequal dipole moment which result in polar charge so that it can completely be dissolved with polar solvents. Based on the Ummah study, more tannin levels (10.92%) were obtained using using a mixture of acetone: water (7: 3 v/v) solvents [18]. The acetone solvent can minimize the interaction between tannins and proteins from which the protein will be dissolved in acetone while tannin remains in the water phase [19]. Therefore, the ethyl acetate SFS fraction become the best candidate of iron supplement for anemia therapy in near future.

However, this study has a limitation because we have not measured yet protein, zinc and vitamin C concentrations and active compounds as well in the ethyl acetate SFS fraction, which improve or reduce its anti-anemia properties.
4. Conclusion
Whole seeds of snake fruits which were dried under direct sunlight and in oven have a high quality of simplicia with high iron, zinc, protein and vitamin C concentrations. Fractionation of the SFS simplicia using (1:1) ethyl acetate and diethyl ether solvents produces the highest iron concentration without tannin content and it becomes a potential iron supplement for anemia therapy. In vivo study in anemia rat model is required to investigate the beneficial effects of that SFS fraction.

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References
[1] World Health Organization 2014 WHO Global Nutrition Targets 2025: Anemia Policy Brief Geneva World Health Organization
[2] World Health Organization 2015 The Global Prevalence of Anemia in 2011 Geneva World Health Organization
[3] Ministry of Health of the Republic Indonesia 2014 Regulation of the Minister of Health of the Republic of Indonesia Number 88 of 2014 concerning Standard Blood Plus Tablets for Women of Fertile Age and Pregnant Women DKI Jakarta Ministry of Health of the Republic Indonesia
[4] Suskendriyati H, Wijayati A, Hidayah N and Cahyuning from D 2000 Study of Morphology and Relationship between Salak Pondoh (Salacca zalacca (Gaert.) Voss.) Varieties in the Sleman Highlands Biodiversity 1(2): 59-64 1412-033x
[5] Hidayati N 2013 Physical and Chemical Properties of Salak Pondoh Fruits in Sleman Regency. Agros 15(1): 166-173 1411-0172
[6] Ministry of Health of the Republic Indonesia and GAIN 2018 List of Indonesian Food Composition. Retrieved 27 August 2018, from page http://www.panganku.org/id-ID/view
[7] Dhyanaputri IGAS, Karta IW and Krisna LAW 2016 Analysis of Nutritional Content of Snake fruit Skin Extract Production by Farmers Group Abian Snake fruit in Sibetan Village as Efforts to Develop Potential of Local Food Products Meditory 4(2)
[8] Karta IW, Susila LA, Mastra IN and Dikta PG 2015 Nutritional Content in Salak Beans Coffee (Salacca zalacca) Produced by Abian Salak Farmer Group in Sibetan Village, Potential as Local Food Products with Antioxidants and Competitiveness, Virgin Journal 1(2): 123-133 2442-2509
[9] Susanti T 2018 Effect of Dose and duration of administration of Wheat Seeds Of Hemoglobin And Salak erythrocytes Index Anemia In Rat Model Thesis S2 Nutrition Science Postgraduate UNS Surakarta
[10] Werdyani S, Jumaryatno P and Khasanah N 2017 Antioxidant Activity of Ethanolic Extract and Fraction of Snake fruit Fruit Seeds ( Snake fruit zalacca (Gaertn.) Voss.) Using DPPH (2,2-diphenyl-1- picrylhydrazyl) Method Eksakta: Jurnal Ilmu-Ilmu MIPA
[11] Murtini ES and Sutrisno A 2011 Characteristics of Chemical Ingredients and Digestibility of Tempe Chocolate Sorghum (Sorghum bicolor) Journal of Food Technology and Industry 22(2): 150 - 155
[12] Fahreza T 2016 Anti-Nutritional Substances Malang Universitas Brawijaya
[13] Nugraheni MA 2020 Effect of Dosage and Duration of Giving Salak Seed Jelly on Hematological Status and Nutritional Status of Anemic Adolescent Girls Postgraduate Thesis of Nutrition Science UNS Surakarta
[14] Tang G-Y, Zhao C-N, Liu Q, Feng X-L, Xu X-Y, Cao S-Y, Meng X, Li S, Gan RY and Li H-B 2018 Potential of Grape Wastes as a Natural Source of Bioactive Compound Molecules 23
2598

[15] Denis M 2019 The Effect of Giving Salak Seed Extract on Body Weight, Hematological Profile, Serum Ferritin and Transferrin Levels in Anemia Model Mice Thesis S2 Nutrition Science Postgraduate UNS Surakarta

[16] Ristanti IK 2019 Effect of salak seed extract on body weight, hematological profile, levels hepcidin and matriptase-2 in the rat model of anemia Thesis S2 Nutrition Science Degree UNS Surakarta

[17] Atisanto VS, Mulyani S and Triani I 2017 Effect of type Solvents and temperature Drying of the characteristics Extract on Fruit Kelubi Journal of Engineering and Management of Agro-Industry 5(3): 35 - 44

[18] Ummah MK 2010 Extraction and Testing of Antibacterial Activity of Tannin Compounds in Blimbing Wuluh (Averrhoa blimbi L.) (Study of solvent variation) Malang Department of Chemical Engineering Malang

[19] Sa'adah L 2010 Isolation and Identification Tannin Compounds from Wuluh Starfruit Leaves (Averrhoa Bilimbi L.) Malang Department of Chemical Engineering UIN Malang