EKSAKTA
Journal of Sciences and Data Analysis

E-ISSN: 2720-9326
P-ISSN: 2716-0459
DOI: 10.20885/EKSAKTA.vol1.iss1.art4

Research Article

Effect of Cooking On Iron Availibility in Fortified Homemade Tempeh

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Received: 13 September 2019; Accepted: 30 December 2019; Published: 12 January 2020

Abstract: Iron deficiency anemia is a type of anemia caused by iron deficiency, decreasing in the number of healthy red blood cells. The purpose of this study was to make fortified Fe-EDTA and find out the changes in iron availability after cooking (frying and boiling). The biological availability test for iron was carried out in vitro by simulating human digestion using enzyme pepsin and pancreatin-bile solution. The iron variants added were 0, 0.033, 0.066, 0.099, 0.132, and 0.166 % of EDTA iron on 30 grams of raw soybeans to be homemade tempeh. Test results with Atomic Absorption Spectrometry (AAS) showed that the highest Fe content of 12.54 mg was obtained by adding 0.166% of EDTA iron to raw tempeh. But after cooking, there is a decrease in fried and boiled tempeh. The results obtained from the addition of 0.166% of iron-EDTA are 7.74 mg for fried tempeh, and 8.40 mg for boiled tempeh. The results of the addition of Fe-EDTA 0.166% for raw tempeh and boiled match to the value of daily Fe intake to reduce iron anemia in the amount of 8-15 mg / day according to Recommendation Dietary Allowance (RDA). The addition of 0.166% of Fe-EDTA fortification on dried tempeh, it still has not match to the recommended level.

Keywords: tempeh, iron EDTA, fortification, in vitro

Introduction

Micro nutrient is substance that the body needs in small amount. Lack of intake of that substance will cause health problems, affect the growth and development in physically and mentally. Iron is a micro mineral that is found most in the human body which has the essential functions: as an oxygen carrier from the lungs to the body's tissues, electron carrier within cells, and integrated parts of various enzyme reactions in body tissues. Iron deficiency can cause disruption of those processes and can cause anemia. Iron deficiency during pregnancy can affect fetal growth and increase the risk of iron deficiency in infants. The scientists found that iron deficiency in children can trigger physical and mental growth disorders [1].

The prevalence of toddlers who have anemia in Indonesia is increasing every year. According to the 2013 Basic Health Research (Riskesdas), there were 28.1 percent of children under five years old with anemia, meanwhile in 2018 it increased to 38.5 percent. There are 2 kinds of approaches that can be taken to overcome and prevent iron deficiency, they are: the medical-based approach (supplementation) and the food-based approach (food improvement) and food fortification which is still facing various complex problems. Handling iron deficiency through iron supplementation is the most effective way to increase iron levels in the short term. Supplementation is usually aimed at groups that are vulnarable in iron deficiency (pregnant women and breastfeeding moms). The main obstacle to this method is the high cost and it needs sustainable motivation in consuming supplements [2]. The approach in improving food requires high cost and it is difficult to change someone's eating habit.

Food fortification (adding one or more micronutrients) is the most appropriate strategy in handling the problem of iron deficiency in the long term. Another plus is that the target population is very wide and the level of acceptance and sustainability is high. The food ingredients that are usually used are instant

noodles, milk powder, flour, and food made from soybeans. Food that are made from soybeans (tofu and tempeh) are the most suitable food because those are commonly consumed for all the classes, especially the lower middle class. Fortification that are commonly used are Fe-EDTA, Fe-glycinate, Fe-fumarate, and Fe-succinate. The fortification used must be precise so that sufficient Fe is obtained and is easily absorbed by the body. Fe-EDTA is a better fortification than iron glycinate, fumarate, and succinate which has the highest Fe content in tempeh sample at 5.0709 mg and tofu sample at 1.5313 mg [3]. The use of Fe-EDTA as iron fortification in soybean based food (tempeh, tofu and soy milk) has a higher fortification effectiveness compared to Fe-Fumarat fortification for tempeh, tofu and soy milk samples respectively 98.19%, 65.08 % and 66.27% [4]. However, the result of the research ignores the cooking factor that is usually done by the community. Cooking process can be done by boiling, steaming and frying. The use of heat in the cooking process has an effect on the nutritional value of the food including decrease in minerals with the range between 5-40%, especially calcium, iodine, zinc, and iron [5]. Therefore, research is needed to the cooking process by frying and boiling, reducing the availability of iron from soybean based food.

Methods and Materials

Materials

NaOH, $Na_2H_2EDTA.2H_2O$, $FeCl_3.6H_2O$, aquabides, EDTA solution, soybeans, tempeh yeast, aluminum foil, cooking oil, pepsin, HCl, bile, pancreatin, $NaHCO_3$.

Synthesis of Complex Compounds $Na[Fe(EDTA)].3H_2O$

0.4 g NaOH dissolved in 10 mL aquabides and added as much as 3.8 g $Na_2H_2EDTA.2H_2O$. The solution is stirred and helped by heating until completely dissolved. 2.5 g of $FeCl_3.6H_2O$ are dissolved in 5 mL of water and added to the EDTA solution while stirring and heated slowly until a yellow precipitate is formed. The solution is chilled and decanted to separate the precipitate and the resulting precipitate is washed with cold water and ethanol 2 mL twice. The product result is filtered by vacuum filter and the precipitate is dried at room temperature of the solution.

Characterization in Using FT-IR

$Na_2H_2EDTA.2H_2O$ and precipitate of $Na[Fe(EDTA)].3H_2O$ from the previous synthesis then characterized using FT-IR.

Fortification by Adding Fortification Sample and The Cooking Process

Tempeh: A total of 30 g of soybeans are washed and boiled for 20 minutes. Soybeans soaked for 10-12 hours and peeled the skin clean and put into a steamer. Soybeans are steamed for 30 minutes, chilled, and drained. Soybeans that have been chilled and drained are added 0.1 g of tempeh yeast and EDTA iron fortification variations (0; 0.033; 0.066; 0.099; 0.132 and 0.166%) while stirring and put into plastic with holes. Soybeans are fermented for 1-2 days until they become tempeh. This procedure repeat by 18 times for 6 raw tempehs, 6 boiled tempehs, and 6 fried tempehs. Tempeh then cut thinly in aluminum foil and dried in an oven to evaporate water for 1-2 days then mashed.

The Making Pepsin Solution and Pancreatin-bile Solution

Pepsin Solution: 3 g of pepsin dissolved into 500 mL HCl 0.1 M

Pancreatin-bile Solution: 2.5 g of bile and 0.4 g pancreatin dissolved into 100 mL $NaHCO_3$ 0,1 M solution.

Iron Availability Test

1 g of Tempeh samples (raw, fried, or boiled) that have been added fortification substances then added 10 mL of pepsin solution. Adjust the pH to pH 2 by adding 0.1 M NaOH. The mixture is incubated and stirred in a hot plate at 37 ° C for 90 minutes. After 90 minutes 3 mL of pancreatin-bile was added. The pH value is adjusted to 5 with 0.1 M NaOH and the mixture is centrifuged at 5000 rotation/min for 20 minutes. The precipitate was filtered using a 45 filter.

E-ISSN: 2720-9326
P-ISSN: 2716-0459

Results and Discussions

The Making of Fortifican Iron-EDTA

In this study the fortification used was Iron-EDTA which was synthesized by reacting $FeCl_3.6H_2O$ with $Na_2H_2EDTA.2H_2O$ according to the reaction equation as follows:

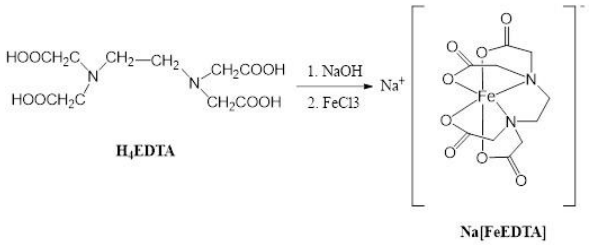

Figure 1. Synthetic reaction $Na[Fe(EDTA)].3H_2O$

An amount of $Na_2H_2EDTA.2H_2O$ is added to the NaOH solution, so it will form four carboxylic functional groups, which cause the $EDTA^{4-}$ ionization reaction as in the following reaction equation

$$EDTA + 4OH^- \longrightarrow EDTA^{4-} + 4H_2O$$

Heating is needed in this process to accelerate the rate of $Na_2H_2EDTA.2H_2O$ reaction of solution.

Ion $EDTA^{4-}$ is a hexadentate metal which can form complex compounds with metal ions. Then $FeCl_3.6H_2O$ is dissolved with aquabides, so it will form $[Fe(H_2O)_6)]^{3+}$. $[Fe(H_2O)_6)]^{3+}$ that has been formed is then reacted with $EDTA^{4-}$ so that solution which is brownish yellow in colour, according to the following reaction:

$$[Fe(H_2O)_6]^{3+}(aq) + EDTA^{4-}(aq) \longrightarrow [Fe(EDTA)]^-(aq) + 6H_2O(l)$$

Solution $[Fe(EDTA)]^-$ is then heated until a brown precipitate is formed. Then the solution is chilled while decanted, then the solution and the sludge are filtered with a Buchner. The precipitate was then washed with 2 mL cold aquabides and 2 mL ethanol twice. After being washed, the precipitate is dried at room temperature. The precipitate is Iron-EDTA (Figure 2) which will be used in this research.

Figure 2. Fe-EDTA synthetic result

Characterization using FT-IR

Before being synthesized into Fe-EDTA, $Na_2H_2EDTA.2H_2O$ was characterized using FT-IR as a comparison for the results of FT-IR Fe-EDTA complexes. FT-IR results for $Na_2H_2EDTA.2H_2O$ compounds can be seen in Figure 3 (a), while for FT-IR results for Fe-EDTA compounds can be seen in Figure 3 (b).

From the two FT-IR results that can be seen in Figure 3, it looks the same at a brief. The most stand out difference is the change in numbers of the range 3400-3500 cm^{-1}. This is suspected due to a shift caused by the change in bonding from O-H in the $Na_2H_2EDTA.2H_2O$ compound to the O-Fe bond in Fe-EDTA. Peak at

E-ISSN: 2720-9326
P-ISSN: 2716-0459

1500cm-1 at Figure 3b wider than Figure 3a, because the change in bonding from C-N $Na_2H_2EDTA.2H_2O$ to C-Fe bond on Fe-EDTA.

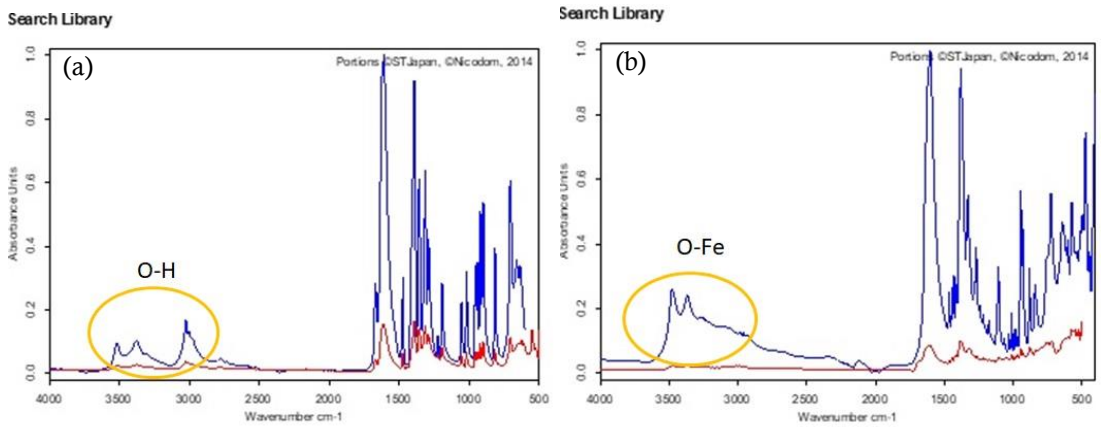

Figure 3. The result FT-IR (a) $Na_2H_2EDTA.2H_2O$ compound, and (b) Fe-EDTA compound.

Iron Availability Test Result

This research tests the biological levels of iron in vitro. The in vitro method is a simulation of the body digestive system and absorption of iron from food complexes. Both inhibiting factors and increasing absorption of iron absorption show the same response as indicated by iron absorption by the in vivo method. The significant correlation between absorption of iron by the body with the in vivo method and the availability of iron in vitro. Therefore, vitro methods are used because the analysis is easier, faster, and cheaper in analyzing the availability of iron in food [3]. The in vitro method allows for proper control of conditions during testing and reduces the diversity that occurs in - in vivo determination [6]. Besides that, when using the in vivo method, individual physiological factors will greatly affect the absorption or absorption of nutrients, while in vitro methods the individual physiological factors are assumed to be the same [7].

To make fortified iron tempeh, raw soybeans are weighed 30 grams each for each sample. From 30 grams of raw soybean sample produces tempeh with an average weight of 35 grams of raw tempeh. Then the tempeh is dried and mashed, so that 25 grams of tempeh flour is obtained. Tempeh flour is then added to the fortified variation of Fe-EDTA and then pepsin solution is added. Pepsin-HCl is a simulation of gastric sap / gastric fluid [3]. Made into acidic because the human stomach also has acidic condition. This is due to the presence of gastric acid secretion by gastric mucosal cells. Gastric acid will make the pH in the lumen around 2-2.5 which is the optimum pH for the activity of the enzyme pepsin to catalyze the hydrolysis of some proteins in the sample. Beside that, acidic conditions in the stomach are also needed to convert pepsinogen that's not active into active pepsin.

Tempeh flour that has been added to the fortified variation and pepsin solution is then added to the pancreatin enzyme and bile solution. Pancreatin bile mixture is a simulation that resembles gas released by the pancreas gland. This liquid contains sodium bicarbonate which neutralizes fluid from the stomach and its function is to break the binding of sample proteins. The sample mixture is then centrifuged and the filtrate taken which will then be analyzed by AAS. The purpose of this AAS analysis is to determine the Fe concentration in the tempeh sample.

The results of the analysis of iron content (Fe) for each sample are presented in Table 1. The sample consisted of raw tempeh and tempeh processed by frying and boiling. Analysis was conducted on tempeh added with Fe-EDTA and without the addition of Fe-EDTA.

E-ISSN: 2720-9326
P-ISSN: 2716-0459

Table 1. Tempeh Sample Test with Fortified Variations

Treatment	Amount of Addition Fe-EDTA (mg)	Amount of Addition Fe-EDTA(%)	Con-tent/ Level Fe (mg)
Raw	0	0	0.67
	10	0.033	2.26
	20	0.066	2.78
	30	0.099	4.77
	40	0.132	10.28
	50	0.166	12.54
Fried	0	0	0.59
	10	0.033	0.77
	20	0.066	1.28
	30	0.099	4.41
	40	0.132	5.24
	50	0.166	7.74
Boiled	0	0	0.09
	10	0.033	1.28
	20	0.066	1.74
	30	0.099	4.29
	40	0.132	6.00
	50	0.166	8.40

The test results showed that with the addition of iron EDTA fortification there was an increase in Fe levels compared to without the addition of fortified substances (0%). Tempeh without the addition of fortified substances still has Fe content because raw soybean has Fe content. The highest Fe content of 12.54 mg was obtained by adding 0.166% iron EDTA to raw tempeh. But after the cooking process, there is a decrease in tempeh and boiled tempeh. Where the results obtained from the addition of 0.166% EDTA iron is 7.74 mg for fried tempeh, and 8.4 mg for boiled tempeh. The results of iron EDTA fortified 0.166% for raw tempeh and boiled tempeh reached the right range for the daily intake of Fe to reduce iron anemia in the amount of 8-15 mg/day according to Recommendation Dietary Allowance (RDA). Meanwhile, for fried tempeh with the addition of EDTA iron fortification as much as 0.166% still did not reach the recommended levels.

Test results on the availability of iron with variations in the amount of fortification, in general, can be seen in Figure 4 which shows that the cooking process such as boiling and frying can reduce the levels of iron fortified. This matches the research of [8] which states that fortified iron fortified tempeh, the higher iron fortified added, the tempeh iron content increases both raw tempeh and cooked tempeh. The effect of cooking can significantly reduce iron levels. Where heat treatment can cause bonding to break and affect the absorption or use of some minerals [9].

E-ISSN: 2720-9326
P-ISSN: 2716-0459

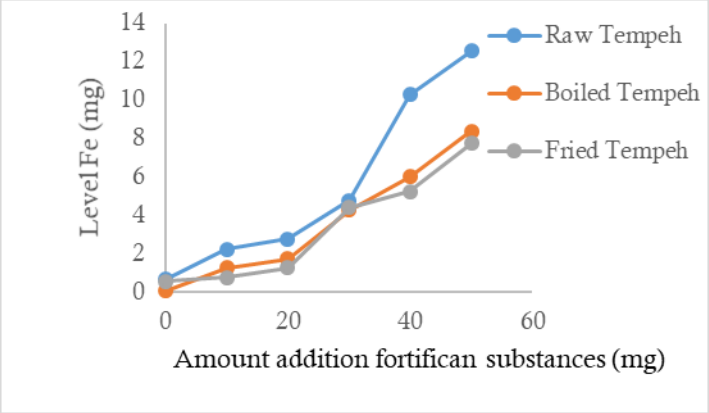

Figure 4. In vitro test on tempeh with various treatments

From the research, we can see that the most decreased iron is tempeh which is cooked by boiling. It is suspected that there is damage to the matrix that binds minerals to foodstuff during high temperature processing such as boiling and frying. This causes the mineral to be free of the matrix and can pass through the membrane of the dialysis bag because it has smaller molecules compared to the matrix complex with the mineral. Heating can reduce the attracting energy between the water molecules and will provide enough energy to the water molecules to overcome the attracting energy between the molecules in the food. So, the solubility of minerals in the material will increase with increasing temperature [10]. The biological availability of minerals, including iron, is closely related to their solubility in the digestive tract [11]. With increasing solubility due to heat, it will also increase the biological availability of minerals, including iron. According to Palupi et al [9] beside the appearance of undesirable things because it damages the nutrients contained in foodstuffs a lot, the processing can be beneficial to some components of the nutrients contained in these foodstuffs, such as: changes in levels of nutrient content, increased digestibility and the availability of nutrients as well as the reduction of various anti-nutrition compounds contained in it. The process of heating food can increase the availability of nutrients contained in it, for example, heating raw nuts (soybeans) can increase the digestibility and the availability of protein contained in it.

Conclusion

Tempeh that has been fortified with iron EDTA has increased its availability of both raw and cooked iron. The addition of 0.166% variant of EDTA iron to raw tempeh (12.54 mg) and boiled tempeh (8.40 mg) is an ideal amount for EDTA iron fortification because it has reached the recommended value of iron availability (8-15 mg/day). Meanwhile, fried tempeh (7.74 mg) still has not reached the recommended value. Cooking processes such as boiling and frying can reduce iron fortification EDTA.

Acknowledgement

We thank Ristekdikti as a funding source of this research and Sekolah Tinggi Analis Kimia Cilegon for the place to us to conduct research.

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