

## Fortification of ferrous fumarate on tempeh and drinks of *Vigna unguiculata* (L.) and *Phaseolus vulgaris* (L.)

<sup>1,\*</sup>Fadly, D., <sup>2</sup>Angkasa, D. and <sup>2</sup>Friawan, A.

<sup>1</sup>Department of Food Technology, Universitas Tanjungpura, Pontianak 78124, Indonesia

<sup>2</sup>Department of Nutrition, Universitas Esa Unggul, Jakarta 11510, Indonesia

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### Abstract

Food fortification is one way to prevent micro-nutrient deficiency, including anaemia. Ferrous fumarate can be an optional fortification for legume-based food products. This study aimed to analyse the effect of ferrous fumarate fortification on tempeh and drinks made with black-eyed peas (*Vigna unguiculata* (L.)) and kidney beans (*Phaseolus vulgaris* (L.)). It was an experimental study with a completely randomised design (CRD). This study had reformulated black-eyed peas and kidney beans food products with several levels of ferrous fumarate fortification, such as tempeh were F1 = 0 mg/100 g, F2 = 100 mg/100 g, and F3 = 200 mg/100 g, and the drinks were F1 = 0 mg/500 mL, F2 = 100 mg/500 mL, and F3 = 200 mg/500 mL. The results showed that fortification of Fe fumarate significantly affected the texture of black-eyed peas tempeh, the colour of black-eyed peas drink, and the texture of kidney beans drink ( $p < 0.05$ ), based on sensory acceptance rate. The highest sensory acceptances in tempeh products included black-eyed peas tempeh F3 and kidney beans tempeh F2. They contained  $48.53 \pm 0.03$  and  $41.23 \pm 0.10$  mg/g of Fe, respectively. For the drinks, fortification of ferrous fumarate at a dose of 100 mg/g had the highest sensory acceptance rate, both black-eyed peas and kidney beans drinks, and Fe content of about  $63.77 \pm 0.02$  and  $74.64 \pm 0.01$  mg/g, respectively. The Fe content in those legume-based food products with ferrous fumarate fortification already met the nutritional label reference. Thus, fortification of ferrous fumarate in food products developed from black-eyed peas and kidney beans can be contributed to the improvement of iron intake.

## 1. Introduction

One of the most common malnutrition conditions in society is iron deficiency anaemia, experienced by many developing countries. Due to the monthly menstruation cycle, women may risk this type of anaemia more than men (Briawan *et al.*, 2013). According to WHO, globally, anaemia cases affect 1.62 billion people or 24.8% of the population; about 25–40% of young women in Southeast Asia suffer from mild to severe anaemia. In Indonesia, the prevalence of anaemia reaches 21.7%, and the prevalence of anaemia in women is 23.9%. The main factor causing anaemia is insufficient iron intake. About two-thirds of the iron in the body is found in haemoglobin red blood cells. One of the causes of iron deficiency anaemia is iron deficiency in food (nutritional factors).

Iron is one of the essential nutrients for living cells, both plants, and animals. In the body, it is mostly present in the blood as haemoglobin. In foodstuffs, there are two

types of iron, heme iron and non-heme iron. Heme iron in foodstuffs is composed of haemoglobin or myoglobin, including in animal-based foods such as meat, liver, and fish. Meanwhile, non-heme iron can be found in plant-based foods, such as legumes. The need for iron in humans is relatively high so it is classified as a macromineral. However, generally, in developing countries, there are difficulties in fulfilling the iron needs from available foodstuffs. Therefore, several attempts were made to improve this situation.

There are two approaches to overcoming and preventing iron deficiency, a pharmaceutical-based approach (supplementation) and a food-based approach (food improvement/fortification). Food fortification is the most appropriate strategy for dealing with iron deficiency problems in the short and long term (Rawat *et al.*, 2013; Shubham *et al.*, 2020). Food derived from legumes is the most suitable food material for fortification because everyone commonly consumes it,

\*Corresponding author.

Email: [dzul.fadly@faperta.untan.ac.id](mailto:dzul.fadly@faperta.untan.ac.id)

especially the lower middle class.

Ferrous fumarate/ Fe fumarate is an iron compound, slightly water-soluble but soluble in weak acids. It is most widely used in fortification, and in adults, it has the same bioavailability as Fe-sulphate (Pérez-Expósito *et al.*, 2005; Harrington *et al.*, 2011). Also, it is widely used for the fortification of baby cereals and chocolate drinks.

According to Adriana (2015), the organoleptic test of soy-based foods (milk, tofu, tempeh) with Ferro fumarate has a better value than the addition of NaFeEDTA products. The bioavailability test in that study showed that both fortifications, NaFeEDTA and ferrous fumarate, were well absorbed. Yusrina (2016) also revealed that the bioavailability of fortified iron in soy-based foods could be well absorbed in milk with an effectiveness value of 94.86% for ferrous fumarate and 77.14% for ferrous glycinate.

This study aimed to determine the effect of ferrous fumarate fortification on sensory responses, proximate composition, and iron content in tempeh and drinks made with black-eyed peas (*Vigna Unguiculata* L.) and kidney beans (*Phaseolus vulgaris* L.).

## 2. Materials and methods

### 2.1 Design and materials

This type of research was experimental with a completely randomised design (CRD), with 3 level concentration of ferrous fumarate. The fortification was carried out on black-eyed peas tempeh, kidney beans tempeh, black-eyed peas drinks, and kidney beans drinks. The treatment levels used in this study are listed in Table 1.

### 2.2 Product preparations

#### 2.2.1 Tempeh formulation

The black-eyed peas were washed and soaked in water for 12 hrs, then removed the skin/epidermis of the beans. The cleaned beans were then soaked again for 12 hrs and boiled in the water for 10 mins. Then, the beans were soaked in the water for one night to lower the pH, washed off, and then steamed for 30 mins. A total of 200 g of black-eyed peas was mixed with 1 g of tempeh yeast (Aminudin, 2007). Later, the ferrous fumarate was added. Then, the fermentation process was continued for 24 hrs.

The formulation of kidney bean tempeh began with cleaning and sorting the beans. The beans were soaked in the water for 7 - 9 hrs and boiled for 15 mins. Then, the beans were soaked again with water for 5 hrs. After that, the epidermis of the beans was removed. The cleaned beans were then steamed for 30 mins. About 100 g of kidney beans were mixed with 1.5 g of tempeh yeast (Kusnandar *et al.*, 2020). Then, the ferrous fumarate was added. After that, the fermentation was continued for 60 hrs.

#### 2.2.2 Drinks formulation

About 150 g of the black-eyed peas were cleaned and sorted. The beans were soaked in the water for 12 hrs. Then, it was boiled for 15 mins. After that, the beans were blended with 500 mL of water and filtered (Barokah, 2017). The drinks obtained were added ferrous fumarate.

About 150 g of the kidney bean was cleaned and soaked in the water for 12 hrs. The beans were then boiled for 30 mins and blended with 500 mL of water. After that, it was filtered (Sandi, 2015). The drinks obtained had ferrous fumarate added to them.

### 2.3 Sensory analysis

Sensory acceptance was measured based on the assessment of 25 semi-trained panellists using the Visual Analog Scale (VAS). The parameters tested included colour, texture, aroma, and texture rate, ranging from very dislike to very like (0 - 10). According to the sensory analysis results, the product with the highest sensory value would be followed by proximate and iron analysis.

### 2.4 Proximate and Fe content analysis

Determination of water, ash, fat, and protein was carried out using the (AOAC, 2012). Then, the determination of carbohydrates was conducted by the difference method as referred to Winarno (2008). Analysis of iron levels was carried out using the Atomic Absorption Spectrophotometry (AAS) method (AOAC, 2012).

### 2.5 Data analysis

The data obtained were processed using Microsoft Excel 2007, and SPSS 16.0 for Windows. The statistical test on the panellists used the One Way Anova test,

Table 1. Ferrous fumarate fortification level in various formulations

Formulations	Ferrous fumarate dosages	Food products			
		Black-eyed peas tempeh	Kidney beans tempeh	Black-eyed peas drinks	Kidney beans drinks
F1	0 mg	100 g	100 g	500 mL	500 mL
F2	100 mg	100 g	100 g	500 mL	500 mL
F3	200 mg	100 g	100 g	500 mL	500 mL

which was followed by the posthoc test of Duncan's Multiple Range Test (DMRT) to determine the real difference between treatments at  $\alpha = 0.05$ .

### 3. Results

#### 3.1 Sensory acceptance

The acceptance rates of tempeh and drinks made from black-eyed peas and kidney beans fortified with ferrous fumarate are presented in Table 2. In general, the acceptance rates of tempeh and drinks made from black-eyed peas and kidney beans were not significantly different at  $\alpha = 0.05$ . However, the fortification of ferrous fumarate affected only the texture of black-eyed peas tempeh, the colour of black-eyed peas drink, and the texture of kidney bean drink ( $p < 0.01$ ). In this study, the products were decided as the most favoured by the panellist according to the value of sensory acceptance determination on colour, taste, aroma, and texture. The higher value means better sensory acceptance. Furthermore, the four chosen products would be going for further analysis.

#### 3.2 Proximate value and iron content

The proximate value was conducted to the chosen products with better sensory acceptance. The products included black-eyed peas tempeh Formulation F3 and kidney bean tempeh Formulation F2, black-eyed peas drink Formulation F2, and kidney beans drink Formulation F2. The determination of ash, water, protein, fat, carbohydrate and Fe are presented in Table 3.

In black-eyed peas tempeh fortified with ferrous fumarate of 200 mg/100 g contained ash, water, protein, fat, carbohydrates, and Fe of  $1.24 \pm 0.01\%$ ,  $69.28 \pm 0.00\%$ ,  $17.93 \pm 0.08\%$ ,  $6.21 \pm 0.00\%$ ,  $5.34 \pm 0.02\%$ , and  $48.53 \pm 0.03$  mg/g, respectively. However, kidney beans tempeh with the fortification of ferrous fumarate of 100 mg/100 g contained ash, water, protein, fat, carbohydrates, and Fe of  $1.04 \pm 0.03\%$ ,  $1.04 \pm 0.03\%$ ,  $17.71 \pm 0.04\%$ ,  $9.00 \pm 0.00\%$ ,  $5.47 \pm 0.07\%$ , and  $41.23 \pm 0.10$  mg/g, respectively.

Furthermore, in black-eyed peas drink fortified with ferrous fumarate 100 g/500 mL contained ash, water, protein, fat, carbohydrates, and Fe of  $0.34 \pm 0.13\%$ ,

Table 2. Sensory acceptance of tempeh and drinks of black-eyed peas and kidney beans fortified with ferrous fumarate

Products	Parameters	Formulations			Sig.*
		F1	F2	F3	
Black-eyed peas tempeh	Colour	6.21±1.59	6.30±1.28	7.02±1.58	0.11
	Taste	6.78±1.90	7.20±1.43	6.87±1.61	0.44
	Aroma	6.80±1.64	6.69±1.55	6.01±1.72	0.18
	Texture	6.38 ±1.46 <sup>ab</sup>	6.52±1.10 <sup>a</sup>	7.06±1.40 <sup>b</sup>	0.02
Kidney beans tempeh	Colour	7.15±1.42	6.66±0.60	6.42±1.35	0.20
	Taste	6.77±1.33	6.50±1.51	6.02±1.51	0.21
	Aroma	7.03±1.30	6.83±1.58	6.07±1.18	0.93
	Texture	6.42±1.64	6.47±1.15	7.11±1.19	0.13
Black-eyed peas drink	Colour	6.60±1.40 <sup>a</sup>	6.25 ±1.48 <sup>a</sup>	7.45±1.34 <sup>b</sup>	0.01
	Taste	6.35±1.41	6.92 ±1.46	6.20±1.49	0.18
	Aroma	6.50±1.83	7.14 ±1.45	6.03±1.55	0.05
	Texture	6.54±1.32	6.87 ±1.17	6.13±1.08	0.09
Kidney beans drink	Colour	7.15±1.42	6.66 ±1.60	6.42±1.35	0.20
	Taste	6.77±1.33	6.50 ±1.51	6.05±1.51	0.21
	Aroma	6.89±1.49	6.69 ±1.55	6.01±1.72	0.16
	Texture	6.38±1.46	6.02 ±1.10	6.70±1.22	0.17

\* $\alpha = 0.05$

Values with different superscript within the same row are significantly different at  $\alpha = 0.05$ .

Table 3. Proximate values and iron content of tempeh and drinks of black-eyed peas and kidney beans fortified with ferrous fumarate

Parameters	Tempeh		Drinks	
	Black-eyed peas - F3	Kidney beans - F2	Black-eyed peas - F2	Kidney beans - F2
Ash (%)	1.24±0.01	1.04±0.03	0.34±0.13	0.24±0.00
Water (%)	69.28±0.00	66.78±0.00	91.44±0.50	93.24±0.43
Protein (%)	17.93±0.08	17.71±0.04	2.38±0.01	1.61±0.02
Fat (%)	6.21±0.00	9.00±0.00	0.51±0.00	0.12±0.00
Carbohydrate (%)	5.34±0.02	5.47±0.07	5.33±0.01	4.79±0.00
Fe (mg/g)	48.53±0.03	41.23±0.10	63.77±0.02	74.64±0.01

91.44±0.50%, 2.38±0.01%, 0.51±0.00%, 5.33±0.01%, and 63.77±0.02 mg/g, respectively. Then, the kidney beans drink fortified with ferrous fumarate 100 g/500 mL contained ash, water, protein, fat, carbohydrates, and Fe of 0.24±0.00%, 93.24±0.43%, 1.61±0.02%, 0.12±0.00%, 4.79±0.00%, and 74.64±0.01 mg/g, respectively.

#### 4. Discussion

Anaemia is known as a common malnutrition incidence in developing countries. Children, young women, pregnant women, and breastfeeding mothers are at high risk of anaemia which will significantly impact the quality of human resources and life expectancy. Lack of Fe results in a decrease in the number of erythrocyte/Red blood cells, the oxygen transporter within the body. In children and young women, long-term anaemia will disrupt physical activity and speed of thinking, which may affect intelligence levels. Then, iron deficiency in maternal pregnancy can cause disruption or inhibition of fetal growth, in both body cells and brain cells. This situation leads to fetal death in the womb, abortion, congenital disabilities, low birth weight (LBW), and anaemia in babies born, which is a cause of the child's premature birth and maternal morbidity/mortality and perinatal mortality. Fortification is needed to improve health quality by increasing iron intake.

According to Allen *et al.* (2006), fortification is a deliberate attempt to add essential micronutrients, namely vitamins and minerals in food, to improve the nutritional quality of the food supply and benefit public health with minimal risk of health. Compared with other strategies used to improve iron nutrient anaemia, some researchers have seen iron fortification as the cheapest strategy to initiate, maintain, reach/cover the most significant population size, and guarantee a long-term approach. Iron fortification does not cause gastrointestinal side effects. It is a significant advantage in terms of consumer acceptability and marketing of iron-fortified products. Targeting iron fortification recipients prone to iron deficiency is a safe and effective strategy for treating iron anaemia. A critical step in planning an iron fortification program is selecting the acceptable bioavailability of iron compounds (Uauy *et al.*, 2002; Huma *et al.*, 2007; Hurrell, 2021).

Ferrous fumarate is an iron supplement often fortified in foodstuffs that aim to increase iron content in food. It is slightly water-soluble but soluble in weak acids, odourless, reddish-orange powder colour and white, almost insoluble in water, with a solubility of 0.14 g/100 cm<sup>3</sup> and very low solubility in alcohol, less than 0.01 g/100 cm<sup>3</sup>, and stable even at temperatures above 200°C (Skuban *et al.*, 2007). Moreover, this mineral is most widely used in fortification, and it has

bioavailability equal to Fe-sulphate in adults. It is also known that ferrous fumarate is widely used to fortify infant cereals Davidsson *et al.* (2000), and iron chloride is widely used for chocolate drink powder (Allen *et al.*, 2006) since it is organoleptically acceptable and is more stable in the vacuum drying process at 100°C (Surahman, 2014). Some infant cereals now fortified with ferrous fumarate are reported to have a bioavailability comparable to ferrous sulphate. This mineral has good absorption in the human body, does not cause changes in colour or flavours, and causes fewer sensory problems than Fe-sulphate. This substance is listed in the Codex Alimentarius of mineral salts recommended for babies and children. In the United States, Fe-fumarate includes GAS additives (generally recognised as safe).

The fortification of iron in food can increase its value and bioavailability. Ingredients that can inhibit iron availability in nuts, namely phytate and oxalate, phytate and oxalate content, can be overcome by washing food, processing, and fortifying food ingredients such as FeSO<sub>4</sub>, ferrous fumarate, and Na<sub>2</sub>EDTA. The effectiveness of iron fortification in liquid soy milk by invitro is 78.19% (Adriana, 2015).

Iron levels are contained in black-eyed peas tempeh, kidney beans tempeh, black-eyed peas tempeh, and kidney beans tempeh with the addition of ferrous fumarate. It can be said that those fortified product has met the need for iron by as much as 26 mg/day. Based on the National Food and Drug Agency, Republic of Indonesia, the iron contained claimed in the processed food nutrition label reference is 22 mg. The result shows that black-eyed peas and kidney bean products already met the nutrition label reference for processed food.

In this study, ferrous fumarate showed a good effect on sensory parameters at the fortification level used in this study. The ferrous fumarate fortification of 100 mg/100 g and 200 mg/100 g, both black-eyed peas and kidney beans products, showed non-differences in sensory acceptance. Although, 200 mg/100 g dosage of ferrous fumarate to the black-eyed peas tempeh might have a higher sensory acceptance value than the 100 mg/100 g fortification dosage. On the other hand, in kidney bean tempeh, fortification of 100 mg/100 g dosage of ferrous fumarate showed a higher level of sensory acceptance than fortification of 200 mg/100 g. Then, for drink products, fortifying 100 mg/500 mL of black-eyed peas and kidney beans drinks provided a higher sensory effect than the dose of 200 mg/500 mL of each legume. Thus, although sensory evaluation results showed insignificant difference values, it seems that the dose of ferrous fumarate in tempeh and drinks of black-eyed peas and kidney beans should be considered in

carrying out Fe fortification.

## 5. Conclusion

The use of ferrous fumarate in fortification can be used to increase iron intake. The acceptance value and iron content showed that the products made from black-eyed peas and kidney beans fortified with ferrous fumarate potentially become nutritious food to support nutritional fulfilment, especially the mineral Fe.

## Conflict of interest

The authors declare there is no conflict of interest.

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