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## Minerals Improvement of Soy Milk by Addition of Drumstick Tree (*Moringa oleifera*) Extract

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**Abstract.** Soy milk is a traditional beverage widely consumed in Asian countries, one of which is Indonesian. Soy milk has a balanced nutrient combination which is more or less similar to cow milk, excluding its vitamins (vitamins A and B2) and minerals (K, P, and Na). Behind its limitation, soy milk is free of cholesterol, lactose, gluten, and rich of phytochemical compounds. Soy milk has been consumed as an alternative to milk for people with lactose-intolerant problem, and for vegetarian as well. Therefore, a fortified soy milk using water extract of drumstick tree (*Moringa oleifera*) leaves was developed. A water extract of pandan leaves (*Pandanus amaryllifolius*) was also added to minimize the strong aroma of leaves/sepat from the *M. oleifera* extract and to appeal the appetite. Hedonic ranking test as an organoleptic testing method was conducted to identify consumer preferences. The test revealed the soy milk with low addition of leaves *M. oleifera* extract (0.26 g/L) is correspondents' more preferable options than higher concentration of extract, i.e. 0.50 and 0.74 g/L. Minerals contents of Ca and Fe were increased to 9% and 32%, respectively, with the addition of 0.26 g/L of the extract. Unfortunately, no vitamins B and C were detected in all variables of added extract concentrations to soy milk.

### 1. Introduction

Micronutrients are essential for the human body to perform physiological functions. Micronutrient deficiencies are critical especially in pregnant women and young children, as it affect fetal and child growth in cognitive development (such learning disabilities, mental retardation), and resistance to infection. Therefore, nutritional interventions, able to ensure the fulfilment of the adequacy of micronutrients, need to be developed, especially for poor and rural areas. Empowering the diversity of local sources to improve the micronutrient status of the community is one of the answers.

In 2006, the Ministry of Health of the Republic of Indonesia that malnutrition in Indonesia is still a major problem and may cause death, especially in high risk groups of infants and toddlers. Malnutrition cases found in Indonesia are quite high, reached out 1,401 cases. Recently, there have been extraordinary outbreaks of malnutrition in Papua, especially in Asmat District. Therefore, local food fortification in rural areas is one of the strategies to overcome the problem.



Food fortification programs have proven their effectiveness in eliminating pellagra, beriberi, ariboflavinosis, rickets, and goiter. It can also be made by adding key nutrients to foods, such as flour and bread which are economically priced and widely consumed by local population. One of the nutrient-rich sources is *Moringa oleifera*. *Moringa oleifera* leaves are reported to contain 7 times as much vitamin C as oranges, 10 times as much vitamin A as carrots, 17 times as much calcium as milk, 9 times as much protein as yoghurt, 15 times as much potassium as bananas and 25 times as much iron as spinach [1]. It is also rich in phytosterols like stigmasterol, sitosterol, and cholesterol which are precursors of hormones. It is used to treat malnutrition in children younger than 3 years [2].

The use of *M. oleifera* as a food fortificant is on the increase. For instance, both fresh and dried Moringa leaves are included in meals in African countries such Ghana, Nigeria, Ethiopia, East Africa, and Malawi [3]. The potential use of *M. oleifera* in food preparation such in making amala (dough made from yam and plantain (*Musa paradisica*) flour), bread [4], cake [5], yoghurt [6], jelly [7], and drinking tea preparation [8] were reported. *M. oleifera* is well known as kelor leaves in Indonesia. One of the traditional recipes from Indonesia, uses processed *M. oleifera* leaves in soup and other processed food. Utilization of *M. oleifera* such as natural coagulant [9] and for biodiesel production [10] were also reported.

Tofu and soy milk, the main products of soy bean, are favorites especially in Asian countries. Soy milk is widely used as substitute for milk instead of cow milk due to possible allergenic problems as well as for vegetarian [11]. Soy milk has a balanced nutrient combination which is more or less similar to cow milk, excluding its vitamins (vitamins A and B2) and minerals (K, P, and Na) content. It contains no cholesterol or lactose and only small quantities of saturated fatty acids [12]. In fact, it must be a healthier protein source than milk because it does not contain cholesterol and lactose [13]. Thus, soy milk is a good candidate for nutritional sources especially for rural areas with less expensive prices and more affordability. Combining extracts of *M. oleifera* leaves as a food fortificant to increase the nutritional value of soy milk is a good strategy to raise an alternative food sources to combat malnutrition, especially among children, infants, and lactating mothers in Indonesia.

## 2. Experimental Section

### 2.1. Materials

All the ingredients for soy milk production was bought from the local market in Rungkut, Surabaya, Indonesia. The high quality of soy bean (FNXX brand) was used as a raw ingredient. The Soy milk was processed and produced in collaboration with a small-medium sized enterprise (Usaha Kecil Menengah, UKM) of Soy Milk, Rungkut, Surabaya, Indonesia using a conventional method. While all the chemicals for analysis were provided by Laboratorium Dasar Bersama (LDB), Universitas Airlangga, Surabaya, Indonesia.

### 2.2. Extraction of Leaves of Drumstick Tree (*Moringa oleifera*)

Fresh leaves of drumstick tree (*Moringa oleifera*) were supplied from Jemur Wonosari, Wonocolo, RT 6, RW 4, Surabaya, Indonesia. Subsequently after the leaves had been received, 100 g of the fresh leaves was washed with water, and chopped with a kitchen blender using 100 mL of water addition (1:1, w/v). The obtained extract was filtered twice using cloth filter. The obtained filtrate was mixed. The solid residue from the first step of filtration was denoted as a rough cake, and the residue from the second step as a fine cake. All the obtained filtrate and residues were put into freeze dryer for 3 days.

### 2.3. Soy Milk Production

Sorted soybeans (500 g) were soaked in water (3 L) for 10-12 hours. Afterwards, the soybeans were cleaned, washed, and drained. Subsequently, the soybeans were blended with water at 1:10 (w/w), boiled at 100 °C for 30 minutes, and filtered to separate the pulp from the juice of the soybeans. The produced soy milk was reheated, and sugar was added. A preservative (0.2 g/L) was added as optionally. Furthermore, the extract of *M. oleifera* leaves was added to the soy milk according to the pre-determined

variables. The soy milk was immediately packed (330 mL capacity) and should be kept in cold temperature ca. 4-5 °C for further consumption and analysis.

#### 2.4. Analysis of Minerals

The analysis of all the minerals of Ca, Mg, and Fe were conducted using Atomic Absorption Spectroscopy (AAS) with dry destruction method. The sample was accurately weighed (in Duplo), ca.  $10 \pm 0.0001$  g, in curs porcelain. Sample was ashes in muffle furnace at 450-500 °C overnight. Subsequently, after the sample temperature reached room temperature, 2 mL of concentrated  $\text{HNO}_3$  was added till all of the sample was dissolved and homogenized carefully in fume hood. The vaporization process of the sample was slowly done using hot plate or water bath until the sample was dried; directly put it in to the muffle furnace at 450-500 °C for an hour. Make sure that all the volatile carbon was vaporized by repeating the addition step of  $\text{HNO}_3$  (two or three times). The ash was transfer quantitatively to 25 mL volumetric flask, and La was added until its concentration reached 0.1 or 1% La; add 1% (v/v)  $\text{HNO}_3$  up to the volumetric mark. Finally, the filtration was done and the filtrate was analyzed using polyethylene bottle at AAS.

#### 2.5. Organoleptic Test of The Fortified Soy Milk

The consumer acceptance was tested using an organoleptic test form which was distributed and filled by 75 respondents in the age range of 20-30 years. Each respondent assess 4 types of corresponding fortified soy milk including a Blanco of soy milk (soy milk without any addition of extract leaves of *M. oleifera*) and soy milk with addition of 0.24, 0.5, and 0.74 g/L of filtrate. The assessments of the consumer preferences was scored between 0 and 7 with 0 (neutral/undefined), 1 (extremely dislike) to 7 (I extremely like). The detailed scores as follows: 0 = zero/undefined, 1 = extremely dislike, 2 = I dislike, 3 = barely dislike, 4 = moderately like, 5 = I like, 6 = I like very much, and 7 = really like.

One way-ANOVA analysis was applied to analyse the data of the consumers' overall acceptance of organoleptic test for soybean milk fortified using the extract of *M. oleifera* leaves with p value <0.05. Meanwhile for the homogeneity test, a Levine statistics (based on mean) with p value > 0.05 was applied. A sample of dataset were consisted of the following 14 attributes (colour, texture, uniformity, viscosity, appearance, tastefulness, pleasantness, sweetness, bitterness, sourness, saltiness, aroma of leaves/sepat, fresh, and texture) and 4 types of samples (blank of soy milk, soy milk with addition of 0.24, 0.5, and 0.74 g/L of filtrate).

#### 2.6. Analysis of Vitamin C

Vitamin C analysis was conducted using high performance chromatography (HPLC) with ascorbic acid as a standard for Vitamin C and tartaric acid as an internal standard. A sample of 2.0 g was accurately weighed and diluted up to 50 mL using methanol p.a. in HPLC grade addition in corresponding volumetric flask. A sample was filtered with 0.2  $\mu\text{m}$  of nylon membrane syringe filter. Then this sample was ready to be injected into HPLC-DAD at 260 nm using the LiChrospher 100 RP-18 HPLC column (5  $\mu\text{m}$ , 250 x 4 mm ID). A series of Agilent HPLC 1100 equipped with a PDA auto sampler and detector was used. An isocratic mobile phase system with  $\text{KH}_2\text{PO}_4$  composition (0.02 M): methanol = 40:60 (v/v) is used at a flow rate of 0.6 mL/min, injection volume = 10  $\mu\text{L}$ , 22 °C column temperature, and total running time = 10 minutes. Each sample was made in Duplo and triplet analysis.

#### 2.7. Analysis of Vitamin B

The sample was accurately weighed and diluted up to 10 mL using methanol p.a. in HPLC grade addition in corresponding volumetric flask. The sample solution was further ultra-sonicated for 30 minutes and 15 minutes in vortex. It was subjected to stand for about 1 hour; filtered with 0.2  $\mu\text{m}$  of nylon membrane syringe filter. Then it was ready to be injected into the HPLC at 264 nm. A similar HPLC systems was used (subsection 2.4 analysis of vitamin C). The Luna C8 HPLC column (100 Å, 150 x 4.6 mm ID) was used with mixture of mobile phase as follow: acetonitrile (eluent A) and  $\text{KH}_2\text{PO}_4$  (pH = 3) (eluent B) at a flow rate of 0.5 mL/min, injection volume = 10  $\mu\text{L}$ , 25 °C column temperature, and total running

time = 25 minutes. Each sample was made in Duplo and triplet analysis. Gradients of the mobile phase are as shown in Table 1.

**Table 1.** Gradients of the HPLC mobile phase system for analysis of vitamin B

Time (min)	Mobile phase composition (v/v)	
	Eluent A	Eluent B
7	10	90
8	40	60
10	30	70
15	40	60
20	10	90

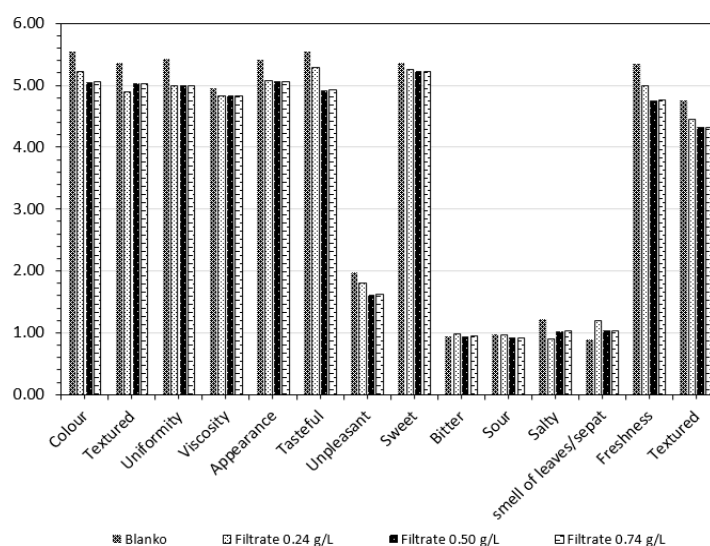
### 3. Results and Discussion

Consumer acceptance generally depends on the attributes were tested at the organoleptic or hedonic test (subsection 2.5). Test was conducted to determine the quality of the fortified soy milk with extract leaves of *M. oleifera*, i.e consumer preferences. The test sheet dividing sensory parameters in three categories: appearance, smell, and flavour which is very important for the food industry. The appearance/sight categories were based on colour, texture, uniformity, viscosity, and appearance. The smell categories were based on the degree of tastefulness and pleasantness. The flavour categories included sweetness, bitterness, sourness, saltiness, aroma of leaves/sepat, freshness, and texture.

#### 3.1. Organoleptic Test Analysis of Produced Soy Milk

Data analysis of the organoleptic dataset of the fortified soy milk with filtrate of *M. oleifera* leaves showed fortified soybean milk was not superior to soy milk in all categories of appearance/sight, smell, and flavor (Figure 1). Increasing the added concentration of filtrate of *M. oleifera* leaves affected the scores of color and texture (appearance category); tastefulness and pleasantness (smell category); and freshness and texture (flavor category). The extract addition did not affect the sweetness of the soy milk but tended to decrease the score of the tastefulness of the soy milk, especially when high concentrations of filtrate was added, i.e. 0.74 g/L. Overall, organoleptic test results suggested that an 0.24 g/L concentration of *M. oleifera* leaves be acceptable to be added to the soy milk without interfering the tastefulness of the consumer.

One way-ANOVA analysis of the overall acceptance of organoleptic test for soy milk fortified with filtrate of the extract of *M. oleifera* leaves, at concentration of 0.24 and 0.50 g/L, gave a significance of 0.008 (p value <0.05). There was a significant difference in the overall acceptance of the organoleptic test between the compared sample groups (Blanco, soy milk fortified with extract of *M. oleifera* leaves at filtrate of concentration 0.24 g/L (F2) and 0.50 g/L (F1)). While the homogeneity test using Levine statistics (based on mean) gave p value 0.226 (p value > 0.05), meaning that there was no significant variation between the groups of compared samples (results are not shown here). In other words, the data used were uniform. A post hoc test-multiple comparison (Tukey HSD) was also applied to find out which sample group was superior (Table 2). The F1 sample and Blanco gave significant differences on the overall acceptance results (Table 2) indicated by the different locations of F1 (in column 1) and B (in column 2). The overall acceptance for F1 and F2; however was not significantly different. The acceptance is similar for F2 and B. Overall, respondents prefer Blanco with a hedonic value scale of 5.26 (medium). The Blanco was superior to the F2, with a value of 4.93 (Table 2).



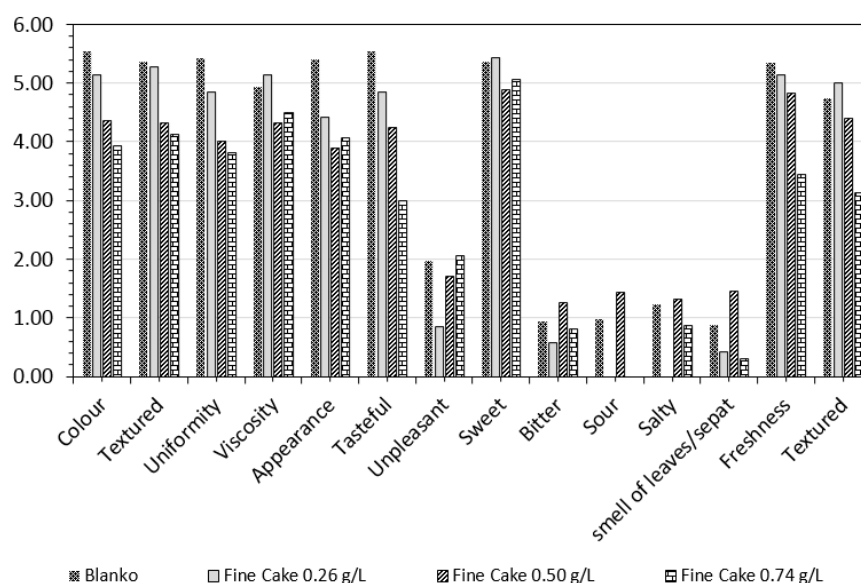
**Figure 1.** Organoleptic test results of the soy milk fortified with the extract of *M. oleifera* leaves (filtrate) at concentration level of 0.24, 0.50, and 0.74 g/L

**Table 2.** Overall acceptance of compared samples of soy milk

Sample	N	$\alpha = 0.05$	
		1	2
Soy milk fortified with filtrate of the <i>M. oleifera</i> leaves 0.50 g/L (F1)	69	4.81	
Soy milk fortified with filtrate of the <i>M. oleifera</i> leaves 0.24 g/L (F2)	69	4.93	4.93
Blanco (Soy milk no fortification) (B)	69		5.26
Significantly		0.714	0.065

Recapitulation of the organoleptic test of the respondent to the fortified soybean milk with fine cake of the *M. oleifera* leaves showed that fortified soy milk could not beat the Blanco in all categories (appearance/sight, smell, and/or flavor) (Figure 2). Increasing the concentration of *M. oleifera* leaves (fine cake) will further lower the score to dislike category. On all aspects of the assessment, the fortified soy milk with fine cake of the *M. oleifera* leaves at the concentration of 0.24 g/L yielded a better test value than the one with the higher concentrations (0.5 and 0.74 g/L). The effect of addition of the *M. oleifera* leaves (fine cake) in soy milk yielded more significant compared to the filtrate addition (Figure 1). Hence, the filtrate of *M. oleifera* leaves was the preferred addition to soy milk compared to the fine cake of *M. oleifera* leaves.

The addition of a rough cake of the *M. oleifera* leaves as well as the addition of fine cake of the *M. oleifera* leaves to the soy milk was also significant (result not shown). The addition of both fine and rough cake greatly affects the assessment of appearance/sight and taste categories. The fine cake is more preferable than the rough cake. The filtrate and fine cake of 0.24 g/L of the *M. oleifera* leaves can be used as the soy milk fortificant with acceptable organoleptic test result (Table 3). However, the filtrate of the *M. oleifera* leaves 0.24 g/L was selected as the most preferred fortificant over all other extract types of *M. oleifera* leaves. Thus, concentration apparently lower than standardized addition portion of Moringa powder with classifications of acceptability “liked moderately” and “liked very much”, ca. 5-7% [14].



**Figure 2.** Organoleptic test results of the soy milk fortified with the extract of *M. oleifera* leaves (fine cake) at concentration level of 0.24, 0.50, and 0.74 g/L.

**Table 3.** Organoleptic score of Blanco, fortified soy milk with filtrate and fine cake (0.24 g/L).

		Fortified Soy milk (at 0.24 g/L)		
		Blanco	Filtrate	Fine cake
<b>Appearance/ Sight Test</b>	Color	5.54	5.23	5.14
	Texture	5.37	4.89	5.29
	Uniformity	5.42	4.99	4.86
	Viscosity	4.94	4.83	5.14
	Appearance	5.41	5.07	4.43
<b>Smell Test</b>	Tastefulness	5.54	5.29	4.86
	Pleasantness	1.97	1.80	0.86
<b>Flavor Test</b>	Sweetness	5.37	5.26	5.43
	Bitterness	0.94	0.99	0.57
	Sourness	0.97	0.97	0
	Saltiness	1.23	0.90	0
	aroma of leaves/langu	0.89	1.20	0.43
	Freshness	5.35	5.00	5.14
	Texture	4.75	4.44	5.000
	<b>average score</b>	<b>3.84</b>	<b>3.63</b>	<b>3.37</b>

### 3.2. Nutrition Analysis of the Produced Soy Milk

To find the best product of the soy milk, the nutrition analysis of the produced soy milk was also conducted, focusing on vitamin B and C and minerals (Ca, Mg, and Fe). Moyo et al. [15] reported vitamin C content of *M. oleifera* leaves was 34.78–40.64 mg/100 g, while 0.06 mg/100 g of vitamin B1 (thiamine), 0.05 mg/100 g of vitamin B2 (riboflavin), 0.8 mg/100 g of vitamin B3 (niacin), 220 mg/100 g of vitamin C, 440 mg/100 g of calcium, 7 mg/100 g of iron, and Mg content of 0.5% weight of dry leaves of *M. oleifera*. Lower minerals composition from *M. oleifera* leaves flour was reported by

Teixeira et al. [16]. They contained 2.97 mg/100 g of calcium, 103.12 mg/100 g of iron, 1.9 mg/100 g of magnesium, 1.58 mg/100 g of zinc, 4.16 mg/100 g of potassium, and 3.38 mg/100 g of copper.

Mineral contents of fortified soy milk were shown in Table 4. The Blanco of soy milk itself contained more Mg and less Ca and Fe. The fortified soy milk with filtrate of *M. oleifera* leaves (no preservative addition) yielded higher content of minerals than the fortified soy milk with fresh extract of *M. oleifera* leaves. The preservative, however, cannot store the minerals.

**Table 4.** Minerals in the Fortified Soy Milk

Minerals	Type of Fortified Soy Milk				
	Soy Milk (Blanco)		freeze dry	fresh extract (0.26 g/L)	
	no preservative	+ preservative	+ filtrate (0.26 g/L)	no preservative	+ preservative
Ca (ppm $\pm$ RSD)	5.12 $\pm$ 0.4	2.53 $\pm$ 3.1	5.58 $\pm$ 4.4	2.84 $\pm$ 1.4	2.35 $\pm$ 0.3
Mg (ppm $\pm$ RSD)	152.30 $\pm$ 4.2	80.00 $\pm$ 3.6	144.70 $\pm$ 7.0	75.80 $\pm$ 0.1	82.50 $\pm$ 1.8
Fe (ppm $\pm$ RSD)	4.13 $\pm$ 1.8	1.27 $\pm$ 0.59	6.09 $\pm$ 1.6	1.35 $\pm$ 1.41	1.25 $\pm$ 1.51

No vitamins B and C were detected in all fortified soy milk including it Blanco itself. Soymilk fortified by a fresh filtrate of *M. oleifera* (without freeze dried process) was also analyzed. Preservation addition to the soy milk still could not preserve both of vitamins B and C. No vitamins were detected in all soy milk samples though the limit of detection in the analysis of both vitamins was very low, ca. 0.0125% w/w. Vitamins B and C were absent presumably due to the high temperature process of soy milk production, ca. 100 °C, 30 minutes (subsection 2.3). This temperature might be damaging the vitamins. However, this step is needed to sterilize the milk. Therefore, a conventional method is not appropriate to preserve the vitamins of the extract of *M. oleifera* leaves.

#### 4. Conclusions

The filtrate of *Moringa oleifera* leaves extract at the concentration of 0.24 g/L is the most preferable fortificant for the soy milk, based on the organoleptic test. Water extraction of *M. oleifera* leaves as well as freeze dried processed cannot preserve the vitamins B and C in *M. oleifera* leaves. Hence, the vitamins B and C in the soy milk cannot be substituted either. Fortified soy milk with filtrate of *M. oleifera* leaves (0.24 g/L) increase the minerals content of Ca and Fe to 9% and 32%, respectively. More work is still needed, though, to find the suitable extraction process of *M. oleifera* leaves, especially in the process to preserve the high content of vitamins of *M. oleifera*. This work will eventually result in more competitive soy milk products, in both quality and price.

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