Nutrient and saponin content of *Moringa oleifera* leaves under different blanching methods

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Abstract. *Moringa oleifera* leaves have high nutritional value and potentially used as food material addition. However, the addition of *M. oleifera* leaves may increase the bitter taste and unpleasant odor caused by saponin. Therefore, this research aimed to investigate the optimum blanching methods of *M. oleifera* leaves, which can decrease the saponin content and maintain the nutritional value. The *M. oleifera* leaves were blanched in four different types namely boil blanching, boil blanching with the addition of (sodium bicarbonate, in-activated bentonite, and activated bentonite). Boil blanching with the addition of activated bentonite have been decreased saponins content of *M. oleifera* leaves to the lowest content of 0.35 %, with 26.03 % protein content, and 86.87 mg 100g–1 vitamin C. These results indicate that the addition of activated bentonite in the boil blanching process was able to decrease the saponin content by 80%, and maintain the nutritional value.

1. Introduction

*Moringa oleifera* leaves are high nutritive value which are contain vitamins, carotenoids, proteins, minerals and bioactive compounds [1, 2]. In another research the *M. oleifera* leaves contain balanced levels of essential amino acids, calcium, vitamin A, C and E as well as proteins [3]. The leaves of *M. oleifera* also contain mineral such as potassium, magnesium, iron, manganese and copper [4]. The *M. oleifera* leaves are known as source of a wide range of dietary antioxidants [5].

The nutritional value of *M. oleifera* leaves may vary with source and cultivar. For instance, Mutiara et al. [6] reported variation in the protein contents (approx. 23-30%) grown in Indonesia, Thailand (approx. 19-29%) [7], Brazil 28% [8], and South Africa 30% [9]. Fresh *M. oleifera* leaves reported contain ascorbic acid (Vitamin C) of 271 mg/100 g, tocopherol of 36.9 mg/100 g, and β-carotene 18 mg/100 g [10]. *M. oleifera* leaves also contain essential amino acid namely alpha linoleic acid [9]. Many of the nutritional benefits of *M. oleifera* leaves suggest that these leaves can be used as a functional ingredient in the food and derivative industries.

The additional of *M. oleifera* leaves may increase the nutritional value of the food. However, the product may poorly rate because the low sensory attributes such as color, taste and odor [11, 12]. Another research reported the problem in the fortification of *M. oleifera* leaves was the increase of unpleasant odor and bitter taste that reduce the consumer acceptances of the product [14]. Unpleasant odor in the *M. oleifera* leaves may be caused by enzymatic substance and secondary metabolite...
namely saponin [11]. Therefore in order to increase the consumer acceptance, the saponin and enzymatic substance must be decreased prior to fortification.

Enzymatic substance can be decreased using blanching [6, 13]. Various researches have been reported antinutrition compound including saponin can be decreased during blanching. Blanching can decrease the saponin content until 0.39% from initial value of 0.65% [11]. There is significant decrease in the saponin content using blanching, but there also an increase in saponin content after further blanching process. Therefore, the blanching process needs a modification to improve the decreasing of antinutrition and maintain the nutrient compounds.

Blanching itself also reduce nutrient from vegetables mainly because high temperature. Modification in the blanching includes the addition of sodium bicarbonate that can soften the texture and increase the destruction of thiamine [14]. In the other hand, there is no specific research about the addition of adsorbent during blanching, which is generally used in the traditional processing of leafy vegetables. The objectives of this study were to investigate the effect of various blanching process on the nutritional properties of M. oleifera leaves powder.

2. Materials and Methods

2.1. Sample Preparation

M. oleifera leaves were harvested from Lawang, Malang, Indonesia with cutting the branches of the tree in 15-30 cm. The branches then withered in the laboratory for 24 hours before stripping the leaves. The leaves then washed using fresh water and stored for further experiments. The other materials namely sodium bicarbonate and adsorbent were obtained from local stores.

Two types of adsorbent were used, namely in-activated bentonite and activated bentonite clay. The activated bentonite was processed using chemical and physical activation adopted from Toor et al. [15] method. HCl 0.1 M were used for chemical activation in 24 hours, then washed until Cl⁻ was undetectable in the supernatant. Hydrothermal pressure of autoclave was used for physical activation in 1.5 atm after chemical activation. Lastly, dehydration of supernatant were carried out using oven in the 150°C for 20 min, and then the samples were cooled in desiccator.

2.2. Blanching process

A complete randomized block design was carried out using 4 types of blanching treatments and 5 time periods of blanching as the first and another factor. M. oleifera leaves were randomly divided into 4 treatments of blanching (boil, boil with addition of 1500 ppm sodium bicarbonate, boil with addition of 1500 ppm in-activated bentonite and boil with addition of 1500 ppm activated bentonite) in 5 periods of time (2, 4, 6, 8 and 10 minutes). The result was subjected to statistical Analysis of Variance (ANOVA) and the significant difference among the means was compared with the Tuckey tests with a probability p ≤ 0.05 using Minitab 18.

100 g of M. oleifera leaves were immersed in boiling water at 85°C for 5 periods of times according to the treatments for the blanching process. The other blanching treatment was carried out with addition of 1500 ppm in the boiling water for each compound namely sodium bicarbonate, in-activated bentonite, and activated bentonite. The samples were drained and then weighed.

The blanched M. oleifera leaves were loaded on the trays and dried in the cabinet dryer. The cabinet dryer was preheated and maintained at 40°C. The leaves were sufficiently dried until they became crisp and brittle to touch after four hours. The dried leaves were milled using stainless Kenwood Chef Warring Blender, Model KM001 (0067078) series and sieved through 0.25 mm laboratory sieve to obtain uniform particle size. The leaves powder samples were then analyzed for (i) Crude protein (ii) Vitamin C and (iii) Saponin.

2.3. Protein measurement

The Kjeldahl method AOAC [16] was carried out to determine the protein content. 1g of dry sample was transferred in digestion tubes 250 ml as the standard sample using blank tube. Half of a tablet of
catalyst and 13 ml of concentrated sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) were added into blank tube. The rack with 20 tubes, including blank and standard sample were inserted into digestion block heater under the fume hood, and install exhaust manifold connected to water aspirator. The digester was kipped at 420°C until liquid becomes transparent. The rack was removed with exhaust manifold from digester and cooled to room temperature. The exhaust manifold and transfer tubes were removed separately to distillation unit. Automatic distillation was carried out using 65 ml distilled water ±35 ml of 40% sodium hydroxide solution. The condensed liquid was collected in Erlenmeyer flask with 10 ml indicator solution and titrated with 0.1142 N sulfuric acid until color turns purple. The protein content was calculated as follows:

\[
\% \text{Protein} = \left( \frac{14007 \times (V \text{ acid titration} - V \text{ acid blank}) \times N}{\text{weight of the sample}} \right) \times 6.25
\]  

(1)

2.4. Vitamin C Measurement
Ascorbic acid was determined using titration methods with the procedure described by Adebayo [17]. Standard indophenol’s solution was prepared by dissolving 0.05g 2,6-dichloro Indophenol in 100ml of water and filtered. To standardize, 0.053g of ascorbic acid was dissolved in 90 ml of 20% metaphosphoric acid and diluted with water to 100 ml. 10 ml of this standard solution was pipette into a conical flask and titrated with indophenol’s solution until a faint pink colour persists for 15 seconds.

2 ml of the extracted juice from the calyces was pipette into a conical flask and 5ml of 20% metaphosphoric acid (as stabilizing agent) was added and made up to 10ml mark with water. It was titrated with the indophenols solution a faint pink colour persists for 15 seconds. The vitamin content in the calyces was calculated using the following equation:

\[
Vitamin \ C (\frac{mg}{100 \ g}) = \frac{\text{tibrate value} \times 0.212 \times 100}{\text{weight of the sample}}
\]  

(2)

2.5. Saponin Measurement
The saponin content was determined using gravimetric methods as described by Eleazu and Elazu [18]. 100 ml of 20% ethanol was added to 10 grams of M. oleifera leaves powder sample in a 250 ml conical flask. The mixture was heated over a hot plate at 55°C for 4 hours with continuous stirring. The residue of the mixture was re-extracted with another 100 ml of 20% aqueous ethanol after filtration and heated for 4 hours at55°C with constant stirring. The combined extract was evaporated to 40 ml over water bath at 90°C. 20 ml of diethyl ether was added to the concentration a 250 ml separator funnel and vigorously agitated from which the ether layer was discarded and the aqueous layer was recovered. 60 ml of n-butanol was added and extracted twice with10 ml of 5% sodium chloride. The remaining solution after discarding the sodium chloride layer was heated in a water bath for 30 minutes, and the solution was transferred into a crucible then dried in an oven to a constant weight. The saponin content was calculated as a percentage:

\[
\% \text{ saponin} = \frac{\text{saponin sample}}{\text{sample}} \times 100
\]  

(3)

3. Results and Discussion
3.1. Nutritional Content
All the blanching treatments caused dramatic loss of the nutrient either protein or vitamin C (p<0.05) compared to the unblanched sample as depicted in Table 1. The greatest loss of nutrient was observed in M. oleifera powder after boil blanching with addition of activated bentonite and the lowest one was the boil blanching without compound addition. However, the most significant loss of nutrient in the M. oleifera leaves powder was caused by blanching process. Boil blanching seriously destroyed the nutrient due to its instability at high temperatures [14, 19].
Various researches indicated that the loss of vitamin C during blanching was caused due to its very soluble in water and instability at high temperatures. This finding was consistent with the observation of Suttikomin [19] that among blanching methods produces higher percent vitamin C loss (60–94%) when Thai vegetables such as Chinese white cabbage (Brassica pekinensis Rupr) (60%), ivy-gourd leaves (C. grandis Voigt) (94%), Chinese swamp cabbage (I. reptans) (88%), fruit producing plant (71%), egg plant (S. melongena Linn) and young-pod wing beans (Psophocarpus tetragonolobus) (58%). The instability of vitamin C in the high temperature was caused high precipitation in the water and then adsorbed by bentonite which is caused the greatest loss of vitamin C.

### Table 1. Nutritional content of M. oleifera leaves under different blanching methods.

| Treatment* | Protein (%)** | Vitamin C (mg 100 g⁻¹)** |
|------------|---------------|--------------------------|
| UB         | 27.35±0.01ᵃ   | 193.04±0.01ᵃ             |
| BIT1       | 27.31±0.03ᵃ   | 90.89±0.39ᵇ             |
| BT2        | 27.29±0.02ᵃ   | 90.11±0.13ᵇᶜ         |
| BT3        | 27.26±0.01ᵃ   | 89.85±0.23ᶜ             |
| BT4        | 27.22±0.02ᵇᵃ  | 88.33±0.38ᵉᶠ     |
| BT5        | 27.19±0.01ᵇᵃ  | 87.61±0.48ᵉᶠᵍʰ       |
| BSBT1      | 27.29±0.02ᵃ   | 90.12±0.12ᵇᶜ       |
| BSBT2      | 27.26±0.01ᵃ   | 89.85±0.23ᶜ         |
| BSBT3      | 27.22±0.02ᵇᵃ  | 88.34±0.38ᵈᵉʳ       |
| BSBT4      | 27.19±0.01ᵇᵃ  | 87.51±0.44ᵍʰ       |
| BSBT5      | 27.19±0.02ᵇᵃ  | 87.16±0.22ᵃᵇ       |
| BIBT1      | 27.25±0.01ᵇᵃ  | 89.33±0.38ᵃᵈ       |
| BIBT2      | 27.22±0.02ᵇᵃ  | 88.33±0.38ᵉᶠ     |
| BIBT3      | 27.20±0.02ᵇᵃ  | 87.16±0.10ᵃᵇ       |
| BIBT4      | 27.18±0.02ᵇᵃ  | 87.14±0.09ᵃᵇ       |
| BIBT5      | 27.15±0.01ᵇᵃ  | 87.13±0.30ᵃᵇ       |
| BABT1      | 27.04±0.05ᵇᶜ  | 88.51±0.44ᵃᵉ       |
| BABT2      | 26.88±0.09ᶜᵉ  | 88.00±0.07ᵉᶠᵍ     |
| BABT3      | 26.58±0.25ᵈ   | 87.32±0.50ᵉʰ       |
| BABT4      | 26.03±0.02ᵉᶜ  | 86.87±0.14ᵇ       |
| BABT5      | 25.56±0.11ᶠ  | 86.86±0.14ᵇ       |

* Treatment with various type of blanching; unblanched (UB) and blanched M. oleifera leaves; boil blanching (B), boil blanching with addition of sodium bicarbonate (BSB), boil blanching with addition of in-activated bentonite (BIB) and boil blanching with addition of activated bentonite (BAB) and 5 level of blanching time 2, 4, 6, 8 and 10 minutes for T1, T2, T3, T4 and T5 respectively.

** Values are means of triplicate determinations. Within column values with different superscripts are statistically significant.

Protein content also significantly decreased in all the blanching treatments (P<0.05). There was structural change in the cell of blanched M. oleifera leaves powder because the opened hydrogen bound, the ability of protein to bound with water decreased and a part of protein coagulated, precipitated and then wasted with the water. This result was consistent with the finding of Mutiara et
al. [6] which is a significant decrease of protein in the blanched M. oleifera leaves powder. The precipitated protein was then adsorbed by bentonite which is caused the greatest loss of protein in the blanching process.

3.2. Saponin content
The main assumption in this study was the decrease in the saponin content caused by various type of thermal stress in the various types of blanching. Measurement of saponin content in this study was done three times for each treatments; boil blanching (B), boil blanching with addition of sodium bicarbonate (BSB), boil blanching with addition of in-activated bentonite (BIB) and boil blanching with addition of activated bentonite (BAB). The average of saponin content of each treatment was depicted in Figure 1.

![Saponin content graph](image)

**Figure 1.** Saponin content of unblanched (UB) and blanched M. oleifera leaves; boil blanching (B), boil blanching with addition of sodium bicarbonate (BSB), boil blanching with addition of in-activated bentonite (BIB) and boil blanching with addition of activated bentonite (BAB).

The result showed that saponin content of fresh M. oleifera leaves was 0.67%, and decreased after blanching, around 0.35% to 0.58%. Therefore there is a statistically significant (p<0.05) different in the saponin content of 4 treatment of blanching compared to the unblanched M. oleifera leaves. According to Eleazu and Eleazu [21], blanching process softened the leaves tissues so the water and the compound that contains in it can fill in osmosis because of the very high permeability of membrane then the compound was precipitated in the solution. The greatest saponin reduction was in the boil blanching with the addition of activated bentonite. There was a relative stagnant decrease in the last two trials of the boil blanching with the addition of activated bentonite. This was possibly due to the adsorption capacity of this adsorbent has been fully filled.

4. Conclusion
The current study clearly shows that nutrient and saponin compounds in M. oleifera powder are significantly affected by blanching. Boil blanching with the addition of activated bentonite has been decreased saponins content of M.oleifera leaves to the lowest content of 0.35 %, with maintain 26.03 % protein content, and 86.87 mg 100g\(^{-1}\)vitamin C. Thus it can be said that the boil blanching with the addition of activated bentonite was more efficiently used than the other type of blanching.
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