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Effect of Blanching Treatments against Protein Content and Amino Acid Drumstick Leaves (*Moringa oleifera*)

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Abstract

Three blanching methods, namely boiling, steaming, and boiling+sodium bicarbonate, were used to evaluate amino acids contens and score of Moringa leaves under different blanching. Results showed that blanching had a great effect on amino acids and scores of Moringa leaves and their digestibility increased. Different methods of blanching had variable effects on Moringa leaves' amino acids and digestibility significantly ($p \leq 0.05$). Steam blanching Moringa leaves had the highest amino acid content by 31.49%. Results also revealed that under different blanching Moringa leaves at levels of leucine of steam and boil+sodium bicarbonate samples, lysine content of boil and steam blanching are higher than those of FAO/WHO requirement pattern. The in vitro protein digestibility under study ranged from 49.6 to 52.3%.

Keywords: kelor, *Moringa oleifera*, blanching, protein, amino acid

1. Introduction

Protein and calorie malnutrition are widespread in developing countries including Indonesia. At present growth rate (2.34%), Indonesian population will double in the next 30 to 40 years. Undoubtedly this population will burden the country in providing enough food supply for the people, and protein in particular. Currently, the major dietary protein sources are from plants and fish. With increasing income of people, there is a trend to consume more animal protein than plant protein.

Moringa leaves are edible and are of high nutritive value (Tetteh, 2006). Dried or fresh leaves are also used in foods such as soups and porridges, curry gravy and in noodles, rice or wheat (Abdelatief et al., 2011).

Moringa oleifera Lam. is a type of vegetable plant shrubs with 5-12 meters height and sometimes it reaches 15 meters with a diameter of about 30 cm. Tree grows in areas 800-1200 m above sea level with rainfall of 400 mm per year (Odee, 1998; Morton, 1991). Moringa leaves are compound, pinnate double, and small rounded oval at the fingertips. Flowering plants all year round yellowish white. The fruit, called "drumstick", is long and angular, its sides form a triangle, at the length of 15-45 cm, with the number of seeds about 20 (Sengupta & Gupta, 1970), soft and brittle stems (Roloff et al., 2009).

It has been reported that ounce-for-ounce, *Moringa oleifera* leaves noting, in Ghana, Moringa leaf products especially leaf powder becomes increasingly popular because of its outstanding indigenous nutritive value. However, limited studies have been documented on the effects of processing and preservation on the nutritional, physicochemical and sensory characteristics of these products (Tetteh, 2008). Moreover, total protein digestibility of these leaves is high (85 % to 90 %). The leaves are also free of anti nutritive factors such as phenols, tannins and saponins (Fuglie, 2001).

Moringa leaves contain protein (in dry weight) 19.34 - 22.42%, and they are balanced in all essential amino acids (Machado et al., 2009; Coppin, 2008). This is supported by the results of Fuglie's research (2001) which reveal that Moringa plant or "drumstick" (*Moringa oleifera*) is a common plant, cheap and multi-vitamin, (Machado et al., 2009).

Protein is essential for growth and development of living organisms and it constitutes 80-90% of all organic substances in animal body. Protein quality is measured by the type of amino acids present. There are twenty different types of amino acids. The 8 classically indispensable amino acids cannot be made by humans. Hence, they must be obtained from the diet, and dietary requirements have been defined for them (Pencharz & Ball, 2006).

A brief assessment is made of the strengths and weaknesses of current estimates of the dietary requirements for the indispensable (essential) amino acids in humans throughout the life cycle, with particular emphasis on the requirements in pre-school children and adults **(**Young and El-Khoury).

Deficiency of protein in the diet constitutes the most serious nutritional problems in cases of malnutrition, which is often known by the term-Protein Energy Malnutrition (PEM). Children and toddlers needing more protein for growth and a more active exchange of energy. Therefore, children are more prone to cases of PEM. The impact of PEM in infants causes abnormal growth, reduced immunity, and a low level of intelligence. In severe stages, PEM in infants can cause a kwarshiorkor until death. Malnutrition infants need high quality and quantity protein intake. So far, protein source for infant and children food product is limited in milk and its derivates which is an expensive material.

Protein is a source of amino acids that contain the elements C, H, N and O are not owned by fat or carbohydrates. The digestibility of protein is the amount of the protein that is absorbed by the body than the protein consumed. Power is the ability to digest protein digestive enzymes in converting protein solvers into smaller units (amino acids). According Ndong et al. (2007) in the protein content of Moringa leaf powder can reach 35%. However, protein digestibility values of Moringa leaf powder is still quite low at 56.1 ± 8.9%, which was caused the bound protein component of high fiber in the leaves of Moringa. Therefore, it is necessary to increase the availability (bioavailability) of Moringa protein.

Moringa leaves has been known as a potential protein source. Moringa plants could be an alternative source of protein with the potential to overcome these problems. This is because the flour has a protein content of Moringa leaves are three times higher than for milk powder. Moringa leaves can make a contribution to the dietary diversity and dietary quality of households in need of improving their nutritional intake (Agyepong, 2009).

Plant Moringa (Moringa oleifera (Lam)) the shrubs are often found in Indonesia as a hedge and has a very broad benefits. Moringa leaves and fruit has long been used by the public as a tasty vegetable; while the seeds can be used as a water purifier because it has compounds that are coagulants. The results of the research in Africa shows that the leaves of Moringa contains seven times more vitamin C than many citrus fruits, contains four times more calcium than milk protein content besides the leaves which can reach 43% when extracted with ethanol. Other plant parts like roots and bark are reported to contain such moringin medicinal and nutritious moringinin as “cardiac stimulant”.

Moringa leaves are vegetables that are easily damaged, so we need processing into dried form intended for the public, especially vulnerable groups, such as children, pregnant and lactating women to be stored for a long or applied more widely. In general, every step of the drying process, especially the blanching treatment, affect the characteristics of vegetables, whether desired or not. The impact of heat on the attributes of quality vegetables are complex and not always beneficial. Protein is one of the important nutrients that easily reacts with heat treatment. The purpose of this study was to measure changes in levels of protein and amino acids from the Moringa leaf powder as a result of different blanching treatments.

Browning is a problem arising in drying. This browning can occur due to enzyme activity oxidase in the tissues are destroyed and the conversion of chlorophyll a result of the drying process pheophytins. Browning on drying can be prevented by inactivation enzyme system. Inactivation of the enzyme system was done by blanching in boiling water or steam for 1-3 minutes with a leaf or until vegetables peroxidase assay peroxidase enzyme shows a negative value.

This study aims to determine of amino acid composition in the Moringa oleifera Lam leaves (family: Moringaceae) under different blanching.

2. Materials and Methods

2.1 Materials

Moringa leaves obtained from Beji village, Malang, Indonesia. It was transferred in an airtight containers to the laboratory. Other ingredients were purchased from local stores.

2.2 Methods

2.2.1 Blanching

Boil Blanching: The *Moringa oleifera* leaves were washed, approximately 100 g of fresh *Moringa oleifera* were immersed in boiling water at 100°C for periods of 5 minutes, respectively. The samples were drained on a stainless sieve until cold and then weighed.

Steam Blanching: The *Moringa oleifera* leaves were washed, approximately 100 g of fresh *Moringa oleifera* and steamed for 5 minutes (at a temperature of 97±1°C)

Boil Blanching addition of sodium bicarbonate: The *Moringa oleifera* leaves were washed, approximately 100 g of fresh *Moringa oleifera* were immersed in boiling water with addition of sodium bicarbonate 1500 ppm at 100°C for periods of 5 minutes, respectively.

2.2.2 Experimental Design

The *Moringa oleifera* were grown at Beji village. Moringa leaves were packed in airtight containers and that was cooled to ±4°C with dry ice. Dry ice is used to cool down freezed items. Processing took place at the State University of Malang. Samples were randomly divided to undergo one of 3 treatments blanching (boiling, steaming and boiling addition of sodium bicarbonate).

The samples were drained on a stainless sieve until cold and then weighed. The blanched *Moringa oleiefera* leaves were then dried in a hand spinning kitchen vegetable drier and the blanched leaves were loaded on the trays forming one single layer of the cabinet dryer and were dried in the cabinet dryer. The cabinet dryer was preheated to 40°C and then the loaded tray was added each time, until all the leaves were done. The temperature was maintained at 40°C and the leaves were left for 1 h for their drying. Vegetables were sufficiently dried till they became crisp and brittle to touch. The leaves took four hours for complete drying and milled with food processor to achieve the expected. The resultant powder was sieved using a laboratory size 0.25 mm to obtain uniform particle size.

2.2.2.1 Determination of Crude Protein

The Kjeldahl method was used for the determination of the total nitrogen. Add 1g of dry sample in digestion tubes (250 ml). Consider blank tube. Consider standard sample of known nitrogen contents. Add half a tablet of catalyst. Add 13 ml of concentrated sulfuric acid (H_2SO_4). Insert rack with 20 tubes, including blank and standard sample in digestion block heater under fume hood, and install exhaust manifold connected to water aspirator. Keep in digester at 420°C until liquid becomes transparent. Remove rack with exhaust manifold from digester and allow to cool to room temperature under fume hood. Remove exhaust manifold and transfer tubes separately to distillation unit. Automatic distillation: 65 ml dist. water +35 ml of 40% sodium hydroxide solution. Collect condensed liquid in Erlenmeyer flask with 10 ml indicator solution. Titrate liquid with 0.1142 *N* sulfuric acid until color turns purple.

2.2.2.2 Calculations

$\%N = [1.4007 \times (Va - Vb) \times N]/W$

Va: volume of acid used for sample titration

Vb: volume of acid used for the blank

N: Normality of acid

W: sample weight in grams

1.4007: conversion factor milliequivalent weight of nitrogen and N percent

Calculation Percent Crude Protein (CP) :

$\%\ CP = \%N \times F$

F = 5.70 (Zaklouta et al, 2011).

2.2.2.3 Amino Acid Analysis

Sample Preparation:

Weigh sample ± 0.2 to 0.6 grams, put in a test tube with a lid. Add 3 ml of 6 N HCl, the homogeneous. Hydrolysis at 110ºC for 12 hours. Cool, filtered with filter paper wathman. Adjust pH to normal pH 7 with NaOH ± 6 N. Add to 10.0 ml with aqua bidest. Grab ± 3 ml, filtered with a 0.45 μm millex. For injection into the

HPLC take millex solution as much as 50 ml + 950 ml OPA vortek. Right reaction was 3 minutes. 30 mL of filtrate was injected into the HPLC.

HPLC conditions: Column: Eurospher 100-5 C18, 250x4.6 mm with Eluent A = 0:01 M acetate buffer pH 5.9. B = (MeOH: buffer 00:01 M Acetate pH 5.9: THF -> 80:15:5) ΛExc 340 nm Em: 450 nm

The following formula as proposed by Abdelatief et al. (2011) was used:

$$\text{Amino acid score} = \frac{\text{mg of amino acid per g N in test protein}}{\text{mg of amino acid per g N in reference pattern}}$$

Furthermore, the content of essential amino acids are compared with the pattern of amino acid requirements recommended by the FAO / WHO / UNU (1985) for pre-school children.

Determination of In Vitro Protein Digestibility (IVPD) (Hsu et al., 1977) is:

Prepare samples with finely milled pass 80 mesh sieve. Then suspende them in distilled water samples (glass distilled water) until the concentration of 6.25 mg protein / ml. 50 ml of total sample suspension which was placed in a small beaker, then adjust the pH to 8.0 by adding 0.1 N HCl or NaOH. The sample is placed in water bath temperature of 37˚C and stir it with a magnetic stirrer for 5 minutes. Add 5 ml of multi enzyme (when adding enzyme is recorded as the time to zero, at which the stop watch time is running) into the suspense remains mixed protein samples in a water bath 37˚C. Then the pH of the suspension samples was recorded at minute 10. Protein digestibility was calculated by using the formula:

$$Y = 210.464\text{-}18.103\ X$$

Where: Y = protein digestibility (%), X = pH suspense samples in minute 10

2.2.3 Statistical Analysis

The data obtained was subjected to statistical One Way Analysis of Variance (ANOVA) (Steel & Torrie, 1991). and the significant difference among the means was compared with the LSD tests with a probability $p \leq 0.05$.

3. Result and Discussion

3.1 Protein Content

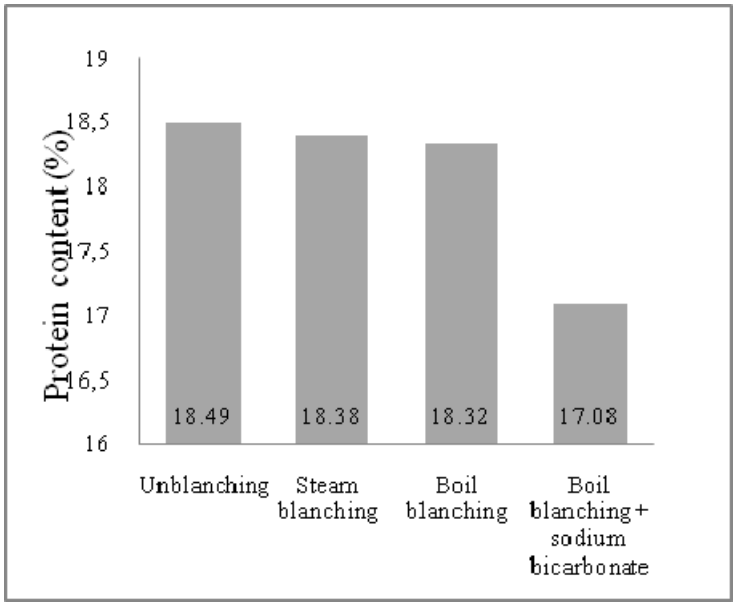

Figure 1. Amino acid content moringa leaves under different blanching

The protein content in four samples of blanched *Moringa oleifera* leaves powder was in the range of 17.08 - 18.49 %. Maximum protein content (18.49%) was in the steam blanching sample and the minimum one was in boil blanching+sodium bicarbonate sample. The protein content in the blanched *Moringa oleifera* leaves powder decreased from the unblanched *Moringa oleifera* leaves powder sample of drumstick leaves. The unblanched

Moringa oleifera leaves contain 18.49. The protein content of the 3 blanching treatment of the Moringa leaves compared to the unblanched leaves was statistically significant ($p<0.05$). Their values are in agreement with the protein content (27.1 %) as Fuglie (2001) reported for *M. oleifera*.

3.1 Amino Acid Content of Moringa Leaves

The quality of the protein depends on the balance and completeness essential amino acids. Misner (2000) adds, that the quality of the protein determined by protein levels and patterns of its constituent amino acids, and each treatment has a different composition and patterns. Protein is needed for growth, production and normal health, so it should have the ideal protein amino acid composition according to the needs and requirement of protein is higher than the young adult age. Tables 1 and 2 show the results of amino acid levels and score of Moringa leaves under different blanching.

Table 1. Essential amino acid content of moringa leaves

	Essential Amino Acid								
	Reference FAO (1985) mg/g protein	Unblanching		Boil blanching		Steam blanching		Boil +sodium bicarbonate	
		mg/g protein	score	mg/g protein	Score	mg/g protein	Score	mg/g protein	Score
Ile	28	19.82	57	34.57	99	38.50	110	30.75	88
Leu	66	35.14	54	23.67	36	72.47	111	52.10	80
Lys	58	16.81	34	98.59	197	30.40	61	23.55	47
Met+Cys	25	64.29	257	28.53	114	34.3	137	28.0	112
Phe+Tyr	65	46.2	71	83.42	128	53.39	82	38.29	69
Thr	34	56.14	225	151.92	608	91.44	366	59.55	238
Val	35	25.16	72	42.3	121	49.81	142	40.39	115
Try	11	5.39	54	17.42	174	34.5	61.1	75.57	756
IVPD		49.61		53.11		53.74		52.29	

Table 2. Non essential amino acid content of moringa leaves powder under different blanching

Amino acid	Unblanching (mg/100 g protein)	Boil blanching (mg/100 g protein)	Steam blanching (mg/100g protein)	Boil +sodium bicarbonate blanching (mg/100g protein
Alanin	34.91	39.49	50.36	37.24
Aspartic	65.38	89.80	112.35	74.38
Glutamic	178.32	87.08	121.99	51.95
Serin	27.07	34.07	42.80	30.32
Histidin	13.54	18.19	25.90	21.53
Glisin	34.37	53.82	66.96	49.97
Arginin	35.10	51.75	70.54	50.44
Total AAE + AA non essential	657.65	854.64	864.67	664.02

The data on the amino acid pattern of total leaves proteins of the investigated moringa reveal that Isoleucine, methionine, cysteine, phenylalanine, tyrosine, threonine, valine, tryptophan content of unblanching, boil blanching, steam blanching and boil blanching+sodium bicarbonate to be higher than requirements recommended FAO/WHO/UNU (1985) for pre-school children.

The results analysis of amino acid content of moringa leaves under different blanching treatments can be seen in Table 2, the highest levels of amino acids contained in the steam blanching treatment (864.67 mg/100 g protein), while the lowest was on unblanching treatment (657.65 mg/100 g protein).

Blanching treatment significantly ($P<0.05$) affects the amino acid content of Moringa leaves. Blanching treatment can increase the essential amino acids Moringa leaves except the amino acid leucyne, and lysine. The amino acid leucine amino acids become limiting at un blanching and boil blanching treatment, while the amino acids lysine becomes limiting amino acid in the treatment un blanching compared to reference pattern FAO/WHO/UNU (1985).

For a non-essential amino acids, or a non-essential amino acids, the highest levels found in the amino acid glutamate (178.32 mg/100g protein), the amount of the treatment and the lowest un blanching contained in the amino acid histidine (13.54 mg/100 g protein) was also found in the treatment un blanching.

In general, there has been an increase in the essential amino acids and non esessensial as blanching treatment effects in moringa leaves are sufficient and relatively high.

4. Conclusion

Blanching pretreatment was used to improve digestibility of *Moringa oleifera*. Blanching leafy vegetable samples were higher in compositional attributes than unblanched vegetables. Blanched is one of the most possible strategies for preservation of *Moringa oleifera* leaves, which are highly seasonal and perishable too. The abundantly available inexpensive leaves of *M. oleifera* can serve as a pool house of nutrients and can be used in the developing countries to combat micronutrient deficiencies.

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