

Full Length Research Paper

A study of *Lactobacillus sp.* with *Moringa oleifera* leaves extract for food supplement

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A study was carried out to find out the best combination treatment between *Moringa oleifera* leaf concentration and filler to produce encapsulated *Lactobacillus* food supplement. Inoculation of *Lactobacillus* on to media containing different types of amino acid and mannitol to promote the growth of organism showed a promising result. The use of proline (0.45% w/v), lysine (0.30% w/v) and mannitol (0.75% w/v) reduced the lag phase from 12 hours to 8 hours and reached the stationer phase after 13 hours of incubation. Encapsulation of *Lactobacillus* with liquid medium containing 8% *Moringa oleifera* leaf and 15% maltodextrin then dried with spray dryer producing a promising product containing protein 27.37%; vitamin A 159.60 mg/g vitamin C 50.95 mg/100g; calcium 773.45 mg/100g. On allowing standing the encapsulated organism in nutrient broth at 30°C showed that the viable cell count only reduced by 5% and the growth rate was similar with that of fresh culture.

Keywords: *Lactobacillus sp.*, *Moringa oleifera*, probiotics, coconut milk.

INTRODUCTION

Moringa olifeira (oleifera) has great potential to become the most economically important tree crops for either tropics or subtropics due to its properties which offer nutrition food, clean water, medicine, and income. The commodity contain very strong concentration of Vitamin A, C, B complex, iron, Ca, protein and high essential amino acid which are very important for releasing the extent of malnutrition problem (Elkhalifa et al., 2007).

The nutritional benefits of *Moringa olifeira (Moringa oleifera* Leaves Extract) become well known throughout south western Senegal, and have been socialized through training, guidance and counseling to mothers and local communities. This program was initiated in 1999 and had a good future to be developed (Fuglie, 2001).

In Indonesia *Moringa oleifera* leaf (*Moringa oleifera* Leaves Extract) and pods are used for making traditional dishes and has not been exploited as source for the production of healthy food. The socialization of *Moringa oleifera* leaf to women at rural region should be carried out and urgently required. However the preparation man-

agement practices for the formulation of healthy food using *Moringa oleifera* leaf carried out in developing countries such as Indonesia is not always possible. The low level in handling technology of the people at rural region become the main hindrance the extension service for using *Moringa oleifera* leaf as food supplement in the fresh form. Under such circumstances, the use of *Moringa oleifera* as raw material for making probiotic product containing *Lactobacillus* is expected to provide beneficial effect.

Lactobacillus isolated from fresh coconut milk has been examined to have high protease and lipase enzyme activity (Padaga et al., 2007) and could attach with carbohydrate such as tapioca. This evidence indicated that this organism has the potential to be used as probiotic or functional food. The encapsulation of these organisms is expected to have greater flexibility and control during distribution to rural region.

Encapsulation is defined as the technology of packaging solid or liquid or gas in miniature sealed capsules that can release their contents at controlled rate under specific conditions. Vidhyalakshmi et al. (2009) stated that encapsulation is a technique to immobilize cell which gained several food industries acceptance. Kailasapathy (2002) reported that the materials used for encapsulation

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Table 1. Amino acid Composition of *M. oleifera* food supplement

Amino acid (mg)	Amino acid composition (8 g leaves extract)
Arginine	98
Histidine	35
Isoleucine	60
Methionine	22
Phenylalanine	105
Leucine	149
Tryptophan	28
Threonine	86
Lysine	96
Valine	74

of viable bacterial cell should be gentle and Non-toxic. By far the most commonly used as bio-gum for microencapsulation are alginat, and also dextrin (Luz, 2008) and Maltodextrin (Tamizhari et al., 2009). The addition of *Moringa oleifera* leaf extract to dextrin or maltodextrin is expected to provide an excellent material having several amino acid, vitamin and mineral which is beneficial for women and also their children.

The encapsulation of *Lactobacillus* or LAB with *Moringa oleifera* leaf extract and carbohydrate may provide healthy probiotic food supplement. Spray drying is the most commonly used for drying in food industry and involves: 1) Formulation of an emulsion of coating and core material and 2) nebulization of the emulsion into a chamber containing dry air (Jackson et al., 1991).

The objective of this study was to find out the best combination treatment between *Moringa oleifera* leaf concentration and filler to produce encapsulated LAB food supplement.

MATERIALS AND METHODS

Encapsulation of *Moringa oleifera* leaves extract

Preparation of MRS Broth was conducted by diluting 0.52 g powder with 10 ml aquadest and than sterilized cooled and stored at 4°C. Pure culture of *Lactic acid bacteria* (LAB) was inoculated on to the broth and incubated at 30°C for 24 hour, harvested by centrifugation. The sediment cells were washed three times in a 0.1% peptone solution, resuspended to the desired concentration and then used immediately in the experiments.

Moringa oleifera leaves were obtained from Pasuruan region, East Java, Indonesia, stored at 4°C. The juice was obtained by crushing the leaves using mortar, then filtered and centrifuged at 3500 rpm for 15 minutes. The supernatant was heated on the water bath for 1 minute.

Pure culture of *Lactobacillus sp.* (LAB) was obtained from Agricultural Technology Division, Brawijaya University, Malang, Indonesia. This *Lactobacillus* was cultured on MRS broth for 48 hr at 30°C which harvested by centrifugation. The sediment cells were washed three times with 0.1% buffer peptone and used immediately in the experiment.

A factorial randomized block design was used to carry out this study. The *Moringa oleifera* Leaves Extract was used as the first factor (8%, 10% and 12% v/v) and type of filler (dextrin and maltodextrin) at the concentration of 15% (w/v) as the second factor and repeated three times.

The fresh culture (10 ml) was mixed with 90 ml aquadest and then was added with MRS Broth (4.68 g), prolyne (0.45% w/v), lysine (0.30% w/v), mannitol (0.75% w/v) and Virgin Coconut Oil/VCO (6% v/v) The solution media, was then mixed with *Moringa oleifera* leaf juice at different level (8%, 10%, 12%). Each sample was centrifuged at 3000 rpm for 15 minutes. The pellet was homogenate (homogenized) with 200 ml aquadest then was added with 15% (w/v) filler (dextrin or maltodextrin), and pasteurized at 80 °C for 15 min.

Spray dryer used in this study has a capacity of 200 ml/hours. The slurry was inserted into the nozzle with an inlet temperature of 120°C and outlet 60°C for 4 hours. The encapsulated products were collected for protein content, minerals, vitamin A, and vitamin C analyses (Table 2).

Chemical analyses

The chemical composition analysis was determined by AOAC series method (Horwitz et al., 2010): vitamin A by AOAC official method number 992.04, vitamin C (AOAC number 984.26), Ca (AOAC ,1980) protein content (AOAC number 967.12), vitamin A (AOAC Official Method 992.04). Amino acid was determined with the method described by Babu et al. (2002).

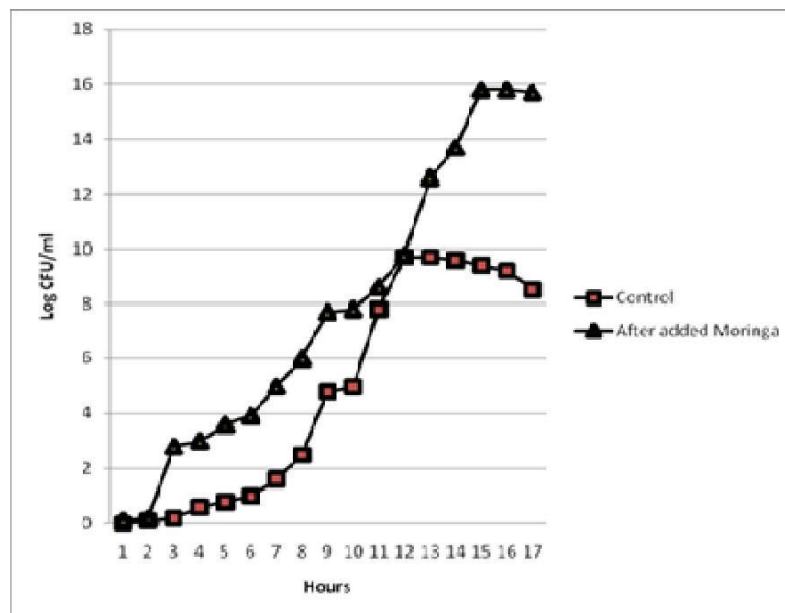


Figure 1. Growth curve of Lactic acid bacillus (LAB)

Feeding trial

The albino rats with weight varied from 200 to 900 g were used as the feeding trial. These rats were obtained from the Department of Research, East Java Province, Indonesia.

The selected rats were divided into two groups:

Group 1: The rats fed with standard feed (BRI) without *Moringa* leaf supplement.

Group 2: The rats fed with standard feed and added with the encapsulated or food supplement obtained from the best treatment.

The standard feed (BRI) is a mixture of rice bran and concentrate purchased at local animal feed shop. The feeding trial was conducted for 15 days. Each group was fed 20 g feed twice a day.

RESULTS

Characteristic of the food supplement

All of the essential amino acids can be found in *Moringa* leaves extract. There are 18 amino acid types found in the encapsulated product and 9 of which are essential. In this study only the essential amino acids were determined, the other non essential amino acids were not mentioned due to the body is able to made this amino acids except arginine, because is very important for infant who are suffering the valuable protein for their growth.

Growth of LAB on the basal medium and on the *M.oleifera* extract

The experimental result given in Figure 1, shows that in *Moringa oleifera* leaf extract, the LAB cells began to grow after an initial lag of 3 hours. The cell counts had increased after 10 hours from 10^3 cells to 10^8 cells/ml and the extract began to be viscous. In the basal medium the initial lag was about 5 hours and reached the stationer phase after 12 hours which then decreased. In the presence of *Moringa oleifera*, the organisms still grow after 12 hours and reached the stationer phase after 15 hours. The presence of essential amino acid in the *Moringa oleifera* leaf improved the growth of the organisms.

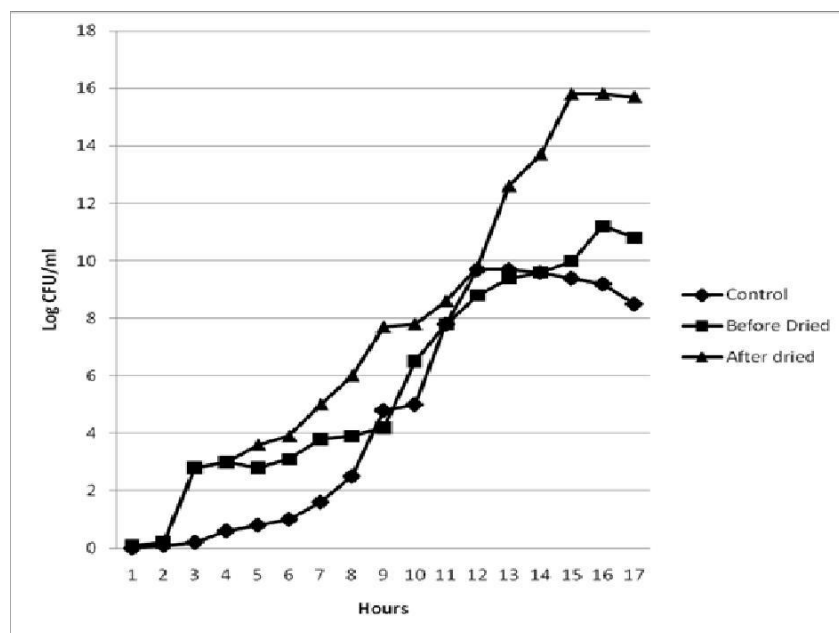
Effect of *Moringa* leaf concentration and types of filler

The *Moringa oleifera* leaves extract had total counts of about 1.6×10^7 cells/ml was used. There was a significant interaction between treatments on the chemical composition of the encapsulated product, and the result in (Table 2), shows that the highest protein, calcium, vitamin A and vitamin C content was obtained in the treatment of 8% (w/v) *Moringa oleifera* leaf and the filler was with maltodextrin filler. Prior to drying the slurry found in the dextrin filler was more viscous than that in maltodextrin. The decreased of the chemical composition

Table 2. Chemical composition of the encapsulated product

<i>Moringa oleifera</i> leaf (%)	Filler	Protein (%)	Calcium (mg/100g)	Vit.A (µg/g)	Vit.C (mg/100g)
8	Dextrin	26.52 d	758.21 b	143.16 b	43.89 a
10	Dextrin	21.24 b	734.82 b	133.54 a	36.89 a
12	Dextrin	17.76 a	708.99 a	129.96 a	36.84 a
8	Maltodextrin	27.37 e	773.45 c	159.60 d	50.95 a
10	Maltodextrin	24.60 c	745.90 b	150.12 c	45.56 a
12	Maltodextrin	21.18 b	725.12 b	136.68 a	38.61 a

*) means followed by the same letters at the same column are not significantly different (p=0.05)

**Figures 2.** The growth curve of LAB before and after dried

tested may be due to the increased of soluble in the slurry (revise to be understood by readear). The viable cell count of the treatment containing 8% (w/v) *Moringa oleifera* leaf and maltodextrin filler was higher than that of dextrin filler.

The growth curve of Lactic acid bacteria in the control broth: 1) without *Moringa oleifera* leaves extract; 2) with treatment containing 8% (w/v) *Moringa oleifera* leaves and maltodextrin without drying and 3) after spray drying was presented in Figure 2. High number of surviving cells was noted after the dissolution of the encapsulated. The cell of all treatments had increased about 10^5 cells/ml. The highest viable count 0.3×10^7 was obtained with treatment of D3F2 (8% *Moringa oleifera* leaves extract and the filler was maltodextrin filler). The spray drying of the slurry has affected the growth curve of the organisms,

but the lag phase were similar to with the treatment prior to dry and the stationary phase after 16 hours of incubation too. The dry encapsulated product has finely structure and the color was whitish.

Effect of food supplement on the weight of rats

The result given in Figure 3, shows that the addition of *Moringa oleifera* powder (the ground encapsulated *Moringa oleifera* Leaves) in the feed significantly increased the rat weight gain of the rats. After 15 days, feeding rats with *Moringga* powder increased the rat weight from 150 g to about 230 g. If the rats were fed with standard feed (control), after 15 days of feeding, the weight was only about 180 g.

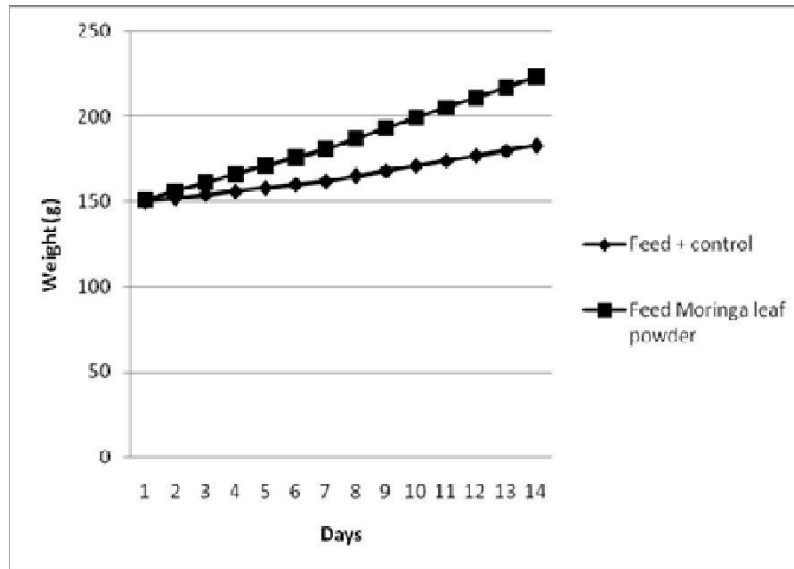


Figure 3. The weight of rats during the trial

DISCUSSIONS

Amino acid content found in *M. oleifera* leaves extract was similar with the finding obtained by Fuglie (2001). The high content of amino acid such as histidine, arginine, leucine, isoleucine, phenylalanine in *M. oleifera* leaves extract has a potential role in promoting the organism to grow (Ritcher, 2003). As indicated in Figure 1, the organism only adapted for 24 h and began to multiply and still continue to be in the exponential phase within 14 days. The addition of *M. oleifera* leaves extract for encapsulation of LAB producing promising results. The growth curve of the Lab after being encapsulated in compare with Lab prior to drying was significant different. Apparently, the amino acid presence in the basal medium interacts with the amino acid in the encapsulated material. Maltodextrin is a promising material to protect the organisms during drying Loret (2006) stated that maltodextrin is different with other typical carbohydrate due to it is very soft and easy to be enzymatically altered by the organisms to increase the moisture content surrounded the organism which supported improved the cell to grow. This evidence also reported by Kailashapty (2002).

From the results discussed above, it can be concluded that the addition of *M.oleifera* leaf extract increased the growth of LAB. The use of 8% *M.oleifera* leaves extract and 15% Maltodextrin is the best treatment for making encapsulated food supplement. The feeding trial showed that the food supplement increased the weight of rats approximate by 30%.

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