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SURAT KETERANGAN

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Lembaga Penelitian dan Pengabdian Kepada Masyarakat (LPPM) Universitas Nahdlatul Ulama Surabaya menerangkan telah selesai melakukan pemeriksaan duplikasi dengan membandingkan artikel-artikel lain menggunakan perangkat lunak **Turnitin** pada tanggal 13 Juli 2020.

Judul	: *The Effect of Drying Methods on Hygienic and Quality Level of Indsutrial Moringa oleifera Leaves*
Penulis	: Nidatul Hikmah Mavrianingtyas, Maharani Pertiwi Koentjoro, Isdiantoni, Ida Ekawati, dan Endry Nugroho Prasetyo
No. Pemeriksaan	: 2020.07.14.356

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Submission date: 13-Jul-2020 07:52PM (UTC+0700)
Submission ID: 1356977527
File name: enic_and_Quality_Level_of_Indsutrial_Moringa_oleifera_Leaves.pdf (1.26M)
Word count: 372
Character count: 21798

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Volume 2215

The 3rd International Conference on Mathematics and Science Education (ICoMSE) 2019

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The effect of drying methods on hygienic and quality level of industrial *Moringa oleifera* leaves

Cite as: AIP Conference Proceedings **2215**, 070007 (2020); https://doi.org/10.1063/5.0000862
Published Online: 01 April 2020

Nidatul Hikmah Mavrianingtyas, Maharani Pertiwi Koentjoro, Isdiantoni, Ida Ekawati, and Endry Nugroho Prasetyo

The Effect of Drying Methods on Hygienic and Quality Level of Industrial *Moringa oleifera* Leaves

Nidatul Hikmah Mavrianingtyas[1)], Maharani Pertiwi Koentjoro[2)], Isdiantoni[3)], Ida Ekawati[3)], and Endry Nugroho Prasetyo[1, a)]

[1]*Department of Biology, Faculty of Science, Institut Teknologi Sepuluh Nopember (ITS), Gedung H Kampus ITS Keputih, Sukolilo Surabaya 60111 Indonesia*
[2]*Study Program of Medical Laboratory Technology, University of Nahdlatul Ulama Surabaya, Indonesia*
[3]*Department of Agribusiness, Faculty of Agronomy, Universitas Wiraraja, Jalan Raya Sumenep–Pamekasan Km. 5 Patean, Sumenep 69451, Indonesia.*

[a)]Corresponding author : endry@bio.its.ac.id

Abstract. *Moringa oleifera* leaf is widely used as a food and medical purposes since it contains plenty amino acid, anti-inflammatory compound and high antioxidant capacity. There are several steps to process *Moringa oleifera* leaf before using as food and medicine. Drying is a part of the processing step to reduce water in order to inhibit microorganism growth and to extend shelf life. The purpose of this research was to determine the effect of drying methods (oven, sun drying, room temperature and roasting) on hygienic *Moringa oleifera* leaf. The parameter of hygienic level was carried by measuring MPN Coliform level, TPC (Total Plate Count), the presence of *Salmonella* sp. and *Staphylococcus aureus.* Furthermore, the *Moringa oleifera* quality was measured by the level of total flavonoid and antioxidant capacity. All experimental group of hygienic were classified to the standard of SNI (National Standard Indonesia) number 7388. The best experimental group was achieved by oven drying resulting in total flavonoids and antioxidant capacity of 14.29 mgQE/g and 69% respectively.

INTRODUCTION

Moringa oleifera is a plant that easily grows in tropical such as Indonesia [1] In Madura, *Moringa oleifera* is used as food and medicine [2] *Moringa oleifera* leaf contains amino acids, antioxidant, and anti-inflammatory so it is used for medicinal materials and addition of nutrients [3]. There are several of *Moringa oleifera* leaf processing in which one of them is the drying process [4]. Drying is a process of decreasing the moisture content of the material, in order to slow the rate of product damage, due to biological and chemical activities [5]. Basically, drying is divided into two types, natural drying and artificial drying. Natural drying uses sunlight while artificial drying uses the instrument and can be manipulated by human [5].

Drying process affects the content of chemical compounds contained in a medicinal plant, especially as antioxidants. The total phenolic and flavonoids in a plant that has antioxidant activity of its stability can be influenced by the drying process [6]. The quality of the *Moringa oleifera* leaves should be paid attention to during processing because the product will be consumed [7]. Therefore, this research conducted testing on each process of *Moringa oleifera* that have been dried to find out the most appropriate drying method. To find out the quality of *Moringa oleifera* leaf processing based on Indonesian national standard on vegetable powder, this research conducted the test of hygiene and quality *Moringa oleifera* leaves through Most Probable Number (MPN) analysis, Total Plate Count (TPC), *Salmonella* sp., *Staphylococcus aureus*, total flavonoids and antioxidant capacity.

The 3rd International Conference on Mathematics and Sciences Education (ICoMSE) 2019
AIP Conf. Proc. 2215, 070007-1–070007-7; https://doi.org/10.1063/5.0000862
Published by AIP Publishing. 978-0-7354-1968-1/$30.00

MATERIALS AND METHODS

Drying Method on *Moringa oleifera* leaves

Moringa oleifera leaves were washed using several types of water which are bottled drinking water, cartridge filter drinking water, government water treatment, and well water. Then soaked in 1% salt solution, the salt used were industrial salt and raw salt. The drying process the started with several methods, the leaves that have been washed using bottled water, government water treatment, cartridge filter drinking water and well water were drained and dried using an oven, while for sunlight drying, room drying and roasted drying leaves used were only that were washed using bottled water. Drying with oven method was done at 40 ° C for 24 hours. Drying was also done by roasting using a heated frying pan until the leaves were dry (can be kneaded). Drying with sunlight were done for 3 days from 08.00 until 14.00 until the leaves were dry and fragile. For room drying, *Moringa oleifera* was placed on trays in a room with good ventilation conditions, this drying for 4 days.

Most Probable Number

Dried *Moringa oleifera* leaf samples were then weighed as much as 10 g, then mashed and added to 100 ml physiological solution which was 10^{-1} dilution. MPN test used 3 series of tubes. Homogeneous samples were inoculated as much as 10 ml of each dilution into a test tube containing 5 ml medium double strength lactose broth, 1 ml sample into a test tube containing 5 ml medium single strength lactose broth, and 0.1 ml sample into a test tube containing 5 ml medium single strength lactose broth. Test tubes wereinoculated in incubation for 24 hours to 48 hours and observed [8].

Total Plate Count

Moringa oleifera were weighed as much as 10 g then mashed and added to 100 ml physiological solution, then diluted 10^{-1}, 10^{-2} and 10^{-3}. 1 ml of each dilution were taken and inoculated into a petri dish containing sterile NA medium and flattered. Then incubated at room temperature for 24 to 48 hours and observation. The growing colony amounted to approximately 30-300 calculated using a colony counter. The number of growing bacterial colonies can be calculated using the calculation formula Based on PerKa BPOM number HK. 03.1.23.08.11.07331 year 2011

Salmonella sp and *Staphylococcus aureus* Test

Method of testing *Salmonella* sp. based (SNI 012332.2 -2006). Salmonella test was done with stages of pre-enrichment, enrichment and planting on selective media. In the pre-enrichment stage, 10 g of sample *Moringa oleifera* leaves were prepared, and diluted until 10^{-3}. 1 ml samples were taken and inoculated into 10 ml lactose broth sterile and then incubated for 24 hours. Then 1 ml of the sample were taken with a pipette and inserted into the 10 ml tetrathionate brilliant green broth, then incubated for 24 hours. Then from the enrichment phase inoculated in a selective medium Salmonella Shigella Agar (SSA) as an affirmation test (biochemistry test), then incubated for 24 hours. A suspected positive colony of Salmonella sp. on this medium are-pink with or without a black core in the middle.

Staphylococcus aureus test based on [9] were conducted. 10 g of sample *Moringa oleifera* leaves were prepared, and diluted until 10^{-3} samples were taken as much as 0.1 ml and inoculated into the petri dish which contained the medium of the Manitol Salt Agar (MSA). The further inoculated samples were incubated for 24 to 48 hours at room temperature. *Staphylococcus aureus* on Medium mannitol salts (MSA) showed the growth of a yellowish white colony and is surrounded by yellow zones due to the ability to ferment mannitol. Bacteria that are unable to ferment mannitol appeared to be red or pink zones. The yellow zone indicates the fermentation of mannitol, which is the acid produced, causing the change of phenol red in order to change from red to yellow [10]. The growing colony numbered between 30-300 and was calculated using the colony counter.

Total Flavonoid

Measurement of total flavonoids based on Ahmad et al., 2015. Weighed 100 mg of extract, then dissolved in 10 ml ethanol, then 1ml extract was taken and added 3 ml of ethanol on the measuring flask. Then, 0.2 ml $AlCl_3$ 10% was added into 0.2 ml of potassium acetate, chopped with aquades up to 10 ml. The solution was stored for 30 minutes in a dark place at room temperature. The Absorbance was determined using the Uv-Vis spectrophotometry method at a wavelength of 435 nm. Determination of flavonoids values was done based on formula[11]:

$$\text{Flavonoid Total} = \frac{C \times V}{W}$$

C = Flavonoid concentrations that calculated using standard curve equations
V = volume of solvent (ml)
W = weight of powder (g)

Antioxidant Capacity

Determination of antioxidant capacity based on Shimamura et al., (2014) [12]. and modified. *Moringa oleifera* Leaf extract was taken as much as 300 µl and then added 900 µl of DPPH solution (2.2-diphenyl-1-Picrylhydrazil) was then homogenized using a vortex. Furthermore, the solution was incubated for 30 minutes at room temperature in the dark place. Then absorption of mixture was measured at 517 nm wavelength with UV-vis spectrophotometer. 300 µl Ethanol 70% plus 900 µl DPPH is used as Blanko. The percentage of antioxidant activity can be calculated using the following formulas:

$$\%\ \text{inhibisi} = \frac{\text{A blanko} - \text{A sample}}{\text{A blanko}} \text{ x } 100\ \%$$

RESULTS AND DISCUSSION

Total Microbial Analysis on the leaf samples of Industrial *Moringa oleifera*

Analysis of microbiology of Total plate count (Fig. 4.1) shows that on drying oven with washed well water and sunlight drying over the limit of Indonesian national standard (SNI: 7388 year 2009), the maximum amount of total plate count is 5 x 10^4 CFU/g. This can be caused by well water that is derived from the soil layer relatively close to the ground surface, so it is easily contaminated through seepage derived from human impurities, animals, or domestic household waste [13]. In addition, the drying of the sun can cause discoloration, the material can be contaminated with dust, difficulty controlling the temperature and the occurrence of microbial contamination [14].

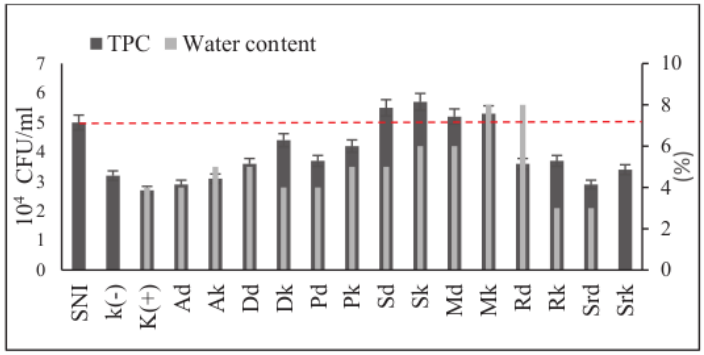


FIGURE 1. Analysis of Total Plate Count and Water Content in Several Drying Method

Information: K(-): Control without dried; K(+): Control BPOM; Ad: oven + bottled water + industrial salt; Ak: oven + bottled water + raw salt; Dd: oven + catridge filter drinking water + industrial salt; Dk: oven + catridge filter drinking water + raw salt; Pd: oven + government water treatment+ industrial salt; Pk: oven + government water treatment+ raw salt; Sd: oven + well water + industrial salt, Sk: oven + well water + raw salt; Md: sun drying + bottled water + industrial salt; Mk: sun drying + bottled water + raw salt Rd: room drying + bottled water + industrial salt; Rk: room drying + bottled water + raw salt; Srd room drying + bottled water + industrial salt; Srk: roasting + bottled water + raw salt.

The lowest number of colonies was the oven method washed using bottled water and roasting method. This can be due to that drying with high temperatures can prevent the growth of bacteria [14]. So bacteria that can grow after drying are *Bacillus cereus, Staphylococcus aureus, E coli*, and Salmonella which can grow at temperatures > 40ºC [15]. Based on Fig. 4.1 the water content of *Moringa oleifera* leaves in each drying method is less than 10%, it shows that *Moringa oleifera* leaves that have dried have a longer shelf life because a moisture content below 10% can inhibit the growth of microorganism [16].

Most Probable Number analysis of Industrial *Moringa oleifera* Leaves

Analysis of MPN (Fig.2) the highest Coliform contamination is in the sample dried using oven method washed using well water and dried sun drying method that is equal to 2.1 / g. This can be caused by well water used for washing that has low hygiene because well water is most likely caused by seepage of household wastewater through the ground [18]. Sun drying is also vulnerable to dust and microbial contamination [14]. However, the lowest results in the MPN test were samples washed using bottled water and oven drying. This is caused by the oven method of contaminants [19].

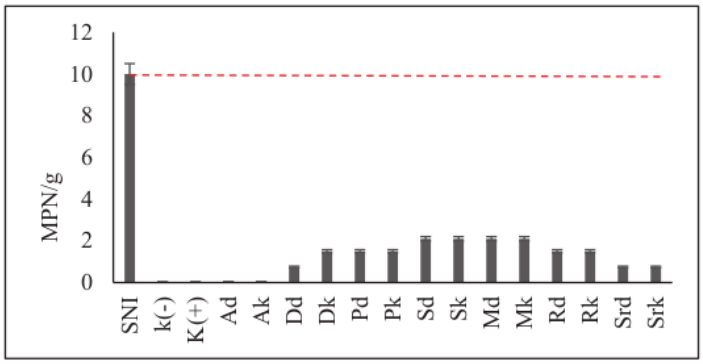
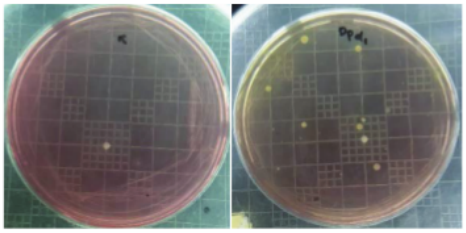


FIGURE 2. Most Probable Number Analysis in some Drying Method

Information: K(-): Control without dried; K(+): Control BPOM; Ad: oven + bottled water + industrial salt; Ak: oven + bottled water + raw salt; Dd: oven + catridge filter drinking water + industrial salt; Dk: oven + catridge filter drinking water + raw salt; Pd: oven + government water treatment+ industrial salt; Pk: oven + government water treatment+ raw salt; Sd: oven + well water + industrial salt, Sk: oven + well water + raw salt; Md: sun drying + bottled water + industrial salt; Mk: sun drying + bottled water + raw salt Rd: room drying + bottled water + industrial salt; Rk: room drying + bottled water + raw salt; Srd room drying + bottled water + industrial salt; Srk: roasting + bottled water + raw salt. However, each type of drying is still within the safe limits of Indonesian National Standard number 7388. The presence of Coliform is an indication of inadequate sanitation conditions [20].

Analysis of Enteric Bacteria Contamination in Industrial *Moringa oleifera* Leaves

Salmonella sp. and *Staphylococcus aureus* performed to find out the contamination of enteric bacteria on some of the drying methods. Analysis results of *Staphylococcus aureus* is shown in Fig.3 and Fig.4



(a) (b)

FIGURE 3. The test result *Staphylococcus aureus* (a) Negative *Staphylococcus aureus* (b) Positive *Staphylococcus aureus*

Based on Fig. 3, it is known that the positive results of *Staphylococcus aureus* are shown in Fig.3b. Characterized by colonies of yellow growing in the MSA medium. MSA medium contains salt up to 7.5% to inhibit bacterial growth. Yellow in the colony because of the fermentation of mannitol by bacteria so that it becomes acidic, the indicator of phenol red on the medium can detect the production of acid by bacteria, thereby converting the red phenol indicator to yellow [21].

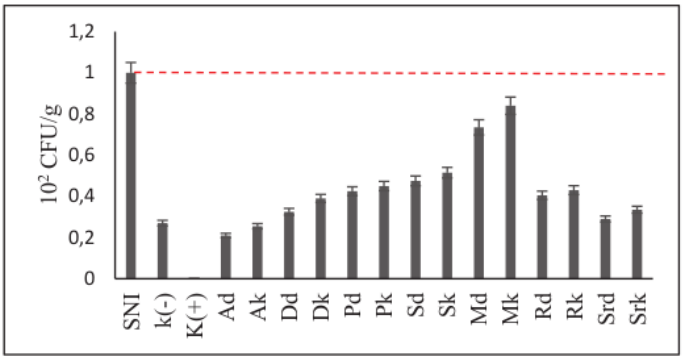
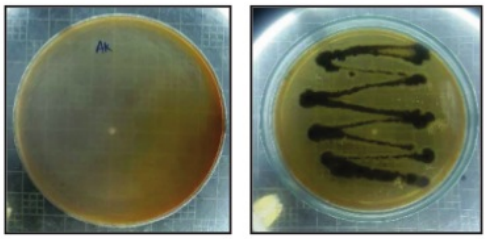


FIGURE 4. *Staphylococcus aureus* test in some Drying Method

Information: K(-): Control without dried; K(+): Control BPOM; Ad: oven + bottled water + industrial salt; Ak: oven + bottled water + raw salt; Dd: oven + catridge filter drinking water + industrial salt; Dk: oven + catridge filter drinking water + raw salt; Pd: oven + government water treatment+ industrial salt; Pk: oven + government water treatment+ raw salt; Sd: oven + well water + industrial salt, Sk: oven + well water + raw salt; Md: sun drying + bottled water + industrial salt; Mk: sun drying + bottled water + raw salt Rd: room drying + bottled water + industrial salt; Rk: room drying + bottled water + raw salt; Srd room drying + bottled water + industrial salt; Srk: roasting + bottled water + raw salt.

Based on Figure 4 is known that the highest contamination is the sun-drying method, this can be caused by the drying of easily contaminated so that it can be contaminated by *Staphylococcus aureus* in the air [22]. However, each type of drying method is still within the limits determined by SNI: 7388, 2009 in which the maximum amount of 1 X 10^2 CFU/g. *Staphylococcus aureus* can grow at 6.5-46 ° C and at pH 4.2-9.3 [10]. When the number of *Staphylococcus aureus* bacteria exceeds the maximum limit, it can cause enterotoxin in food products and cause food poisoning [22]. The result of *Salmonella* sp.test shown in Fig.5 that analysis using Salmonella Shigella Agar (SSA).



(a) (b)

FIGURE 5. Test results of *Salmonella* sp. (a) negative *Salmonella* sp. (b) Positive *Salmonella* sp.

The positive result of *Salmonella* sp. is shown in Fig. 5.B, characterized by a black colony that grows on the SSA medium. Black in the colony caused of H_2S formed from sodium thiosulfate that is reduced to sulfite gases and H_2S forms an insoluble black deposits of sulfide iron and reacts with ferric ions in the SSA medium so that the colonies are black [21].In this research sun drying and room drying method is positive *Salmonella* sp. Positive results on the *Salmonella* sp. Room drying with a temperature of about 28-35 ° C, in the case of Salmonella bacteria can still grow. *Salmonella* sp. Bacteria can grow at a temperature of 5 – 47 ° C and salinity 0.4-4% [23]. So that the drying of the high temperature roasting obtained negative results that there is salmonella.

Effect of Drying Method on Quality of Industrial *Moringa oleifera* Leaves

The effect of drying up to the quality of the leaves of *Moringa oleifera* can be known based on total flavonoids and the percentage of antioxidant capacity presented in Fig. 6

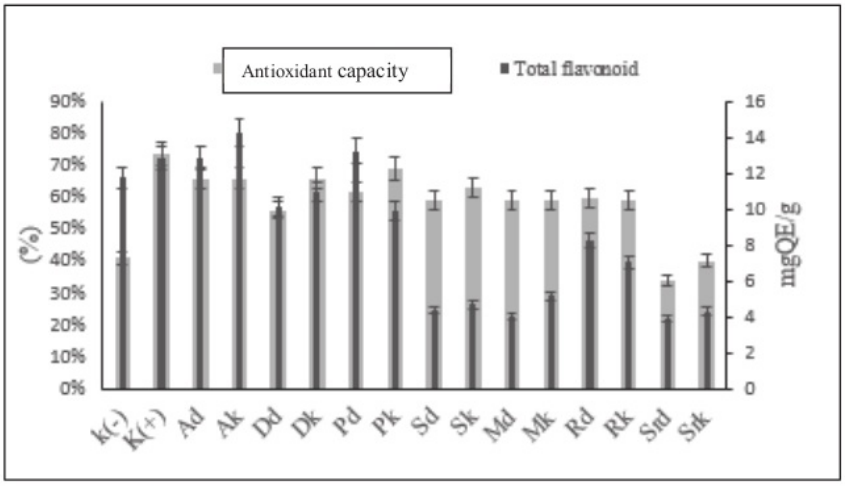


FIGURE 6. Analysis of Total Flavonoid and Antioxidant Capacity

Information: K(-): Control without dried; K(+): Control BPOM; Ad: oven + bottled water + industrial salt; Ak: oven + bottled water + raw salt; Dd: oven + catridge filter drinking water + industrial salt; Dk: oven + catridge filter drinking water + raw salt; Pd: oven + government water treatment+ industrial salt; Pk: oven + government water treatment+ raw salt; Sd: oven + well water + industrial salt, Sk: oven + well water + raw salt; Md: sun drying + bottled water + industrial salt; Mk: sun drying + bottled water + raw salt Rd: room drying + bottled water + industrial salt; Rk: room drying + bottled water + raw salt; Srd room drying + bottled water + industrial salt; Srk: roasting + bottled water + raw salt.

Based on the Figure 6 it can be seen that the highest total flavonoids achieved by oven method with total flavonoids of 14.29 mgQE/g and antioxidant capacity of 69%, while the lowest total flavonoids and antioxidant capacity obtained Roasted method of 2.89 mgQE/g and of 34%. Oven method has a higher total of flavonoids and antioxidant capacity thatcan control temperatures resulting in the stability of the flavonoids compounds [24]. Instead, drying with the roasted method obtained the lowest a total of flavonoids and antioxidant capacity which were caused by excessively high and unstable temperatures [25].

Drying can damage the phytochemical substances by affecting the integrity of the cell walls so that it causes displacement of some flavonoids components.In addition, the reduced flavonoids can be caused by chemical reactions i.e. oxygen and enzymes [26]. In control (-) has relatively low antioxidant capacity compared to the dried leaves of *Moringa oleifera*, it is in accordance with the statement Huriawati et al (2016) [5]. Increased antioxidant capacity in the drying process can be caused by low water content because the drying process will cause substances contained in food ingredients to become more concentrated.

CONCLUSIONS

Based on research that has been done it can be concluded that the drying method affects the level of hygiene and quality of *Moringa oleifera* leaves through microbial analysis and antioxidant capacity. Drying by oven method has the best level of hygiene and quality compared to other drying methods, with a total of flavonoids 14.29 mgQE/g and antioxidant capacity of 69%. While the roasted drying method has the lowest quality with a total of 2.89 mgQE/g flavonoids and antioxidant capacity 34%. Further research needs to be done using more controlled temperatures. In addition, it needs to be associated with the influence of water type in *Moringa oleifera* washing process.

ACKNOWLEDGEMENT

The authors are grateful for the grant provided by Biomaterial and Enzyme Technology Research Group.

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