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EVALUATION OF ANTIBACTERIAL ACTIVITY, NUTRIENTS, AND TOTAL BACTERIAL COUNT OF MORINGA LEAF POWDER WITH VARIOUS DRYING METHODS

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ABSTRACT
Moringa leaves were widely used as raw material for food product. However, unsuitable drying method might cost the loss of antibacterial activity and nutrients content but still leave a high total bacterial count. The aim of this study was to determine the best drying method for moringa leaves, in terms of antibacterial activity, nutrients, and total bacterial count, in order to obtain valuable and safe product. Fresh leaves were dried by various drying methods, namely sun drying, room temperature (air) drying, oven drying, and freeze drying, and followed by grinding and sieving. Antibacterial actvity of moringa leaf powder was analysed by Kirby-Bauer method with agar disk diffusion, total bacterial count by Total Plate Count, bioactive compound by colorimetric method, β-caroten by Thin Layer Chromatography, and some minerals by Atomic Absorption Spectrophotometry. The results showed that moringa with all drying methods could not inhibit the growth of *Vibrio cholerae* and *Bacillus cereus*, but freeze-dried, oven-dried, and air-dried moringa leaf could inhibit the growth of *Escherichia coli*. Freeze-drying also showed the higher of bioactive compound than the other methods. Furthermore, total bacterial count of freeze-dried moringa leaf powder was $<2.50 \times 10^2$ cfu/g while that of other drying methods were significantly higher ($>10^6$ cfu/g). However, freeze-drying also caused greater decrease in mineral content compared to the other drying methods. In short, freeze drying could be the best choice to obtain safe and valuable moringa leaf powder but with compromised mineral content.

1.Introduction

Moringa (*Moringa oleifera*) is a tropical plant of Moringaceae family that all parts of the plant (especially the leaves) have pharmacological values for anti-diabetic, anti-inflammatory, anti-tumor, and anti-hypertension (Foline *et al*., 2011; Qureshi *et al.,* 2015). Besides, moringa contains a lot of macro and micro nutrients such as β-carotene, protein, sodium, phosphorus, iron, potassium, calcium, magnesium, vitamin C, vitamin B3, vitamin E, and vitamin A (Mulyaningsih *et al*., 2018; El Sohaimy *et al.,* 2015). Moringa contains 7 times higher of vitamin C than oranges, 10 times higher of vitamin A than carrots, 17 times higher of calcium than milk, 9 times higher of protein than yoghurt, 15 times higher of potassium than bananas, and 25 times higher of iron than spinach (Rockwood *et al.,* 2013). Therefore, moringa is widely used as a basic ingredient for food products, supplements, and medicines. Even in some developing countries, moringa is a primary source to cure malnutrition. In addition to nutrition, moringa leaves also contain various phytochemical compounds such as tannins, phenols, alkaloids, flavonoids, oxalates and saponins that can function as antibacterials against several Gram-positive pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus sp*., *Bacillus subtilis*), and Gram-negative *(Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*) (Ajayi *et al.,* 2015; Devendra *et al.,* 2011; Vieira *et al.,* 2010; Kalpana *et al.,* 2013).

Moringa leaves can be diversified into moringa tea, moringa milk, moringa biscuits, moringa cereals, moringa nuggets, etc. Generally, firstly, moringa leaves are made into powder by drying and grinding them. After that, they are added to the dough for further process to become food products. This drying process is also useful for extending the shelf life but it could decrease its nutritional and phytochemical content. Hussein *et al.* (2015) said that improper drying temperature can reduce the antibacterial activity in moringa leaf extract. In addition, it has also been reported that improper drying will leave a high number of bacterial total plates in food products. Adu-Gyamfi *et al.* (2014) reports that retail food products based on dried moringa leaves have a number of bacterial plate count of 5-8 x 10^{10} cfu/g, a million times higher than the limit, that is 10^4 cfu/g according to international standards and 10^2 cfu/g based on domestic standards. Such conditions will cause the quality decrease of food products in terms of nutrition and microbiological safety.

Optimization of drying techniques needs to be done to maintain the stability of mineral content and make safe foods based on microbiological criteria. J *et al*. (2015) stated that drying with various temperatures can affect phosphorus, potassium, calcium, magnesium, iron, and zinc found in moringa leaves. Foline *et al*. (2011) claimed that the total plate count (TPC) in moringa powder dried by sun drying technique was three times greater than air drying. Adu-Gyamfi *et al.* (2014) also said that the TPC of moringa leaves which were dried by air drying technique was higher than mechanical drying and solar drying. This drying optimization is useful for getting nutritious and safe food products.

The purpose of this study was to determine the appropriate drying method so that the antibacterial activity, bioactive compound, mineral content of moringa leaves would not be decreased and to evaluate the effect of drying techniques on microbiological safety through the determination of the total bacterial count (TBC). Hopefully, moringa leaves are processed with the right drying technique can be used as a basic ingredient in making various of food products quality in terms of nutrition and microbiological safety

2. Materials and methods

2.1.Sample preparation and collection

Moringa leaves were obtained from plantations located in South Tangerang, Banten. Moringa leaves were collected and washed in running water. Then, the leaves' surfaces were sterilized by using NaCl, ethanol, and distilled water (Mishra *et al.,* 2012). Next, moringa leaves were dried using various techniques. In sun drying, 300 g of moringa leaves were dried under the sun with 33^{0}C for 8 hours. Within air drying, moringa leaves were dried at room temperature (24^{0}C) for 5 x 24 hours. Using drying oven, moringa leaves were dried in the oven at a temperature of 45^{0}C for 24 hours. In freeze drying, moringa leaves were dried at -34° C for 6 hours 30 minutes. The dried leaves were then mashed with mortar. Then, they were sifted into a size of 0.5 mm - 1 mm.

2.2.Evaluation of Antibacterial Activity

The crude ethanolic extracts of moringa leaf powder were screened for their antimicrobial activity againts some Gram-positive and Gram-negative bacterial caused food-borne disease, namely *Escherichia coli* (ATCC 25922), *Vibrio cholerae* (NCTC 11348), and *Bacillus cereus (*ATCC 13061) by Kirby-Bauer method with agar disk diffusion.

The susceptibility of the bacteria were estimated by measuring the diameter of zone inhibition (Al_husnan *et al.,* 2016). Chloramphenicol was tested as a positive control.

2.3.Determination of moisture content, bioactive compound, β-carotene

The moisture of each sample of moringa leaf powder from those four drying techniques was measured using the AOAC method (1995) by placing the powder in air circulating oven with a temperature of 105^{0}C for 6 hours. β-carotene was analysed by adding chloroform into each sample of moringa leaf powder, then the filtrate was spotted into TLC layer and eluted by dicholoromethane : ethyl acetate as well as measured by TLC scanner at wavelength of 289 nm. Bioactive compounds (tannin and flavonoid) were also determined by AOAC method (1984).

2.4.Determination of Mineral Content

Determination of Ca, Mg and K were determined by percolation using ammonium acetate 1 M (pH 7) while Fe were determined by destructing the powder and drying with a gradual temperature from 100^{0}C until 200^{0}C. Then, they were measured using the Automatic Absorption Spectrophotometry (AAS) based on the AOAC method (2005).

2.5.Determination of Total Bacterial Count

Total Bacterial Count (TBC) was determined using the Total Plate Count (TPC) with spread plate technique. One g of moringa leaf powder was diluted serially in physiological NaCl solution 6 times. The last three dilutions were plated using the Nutrient Agar media (Merck) and incubated for 24 hours at 37°C.

The total bacteria was calculated and compared with Indonesian Standard (BPOM's Head Regulation Number 13 of 2019) concerning microbiological criteria in processed food.

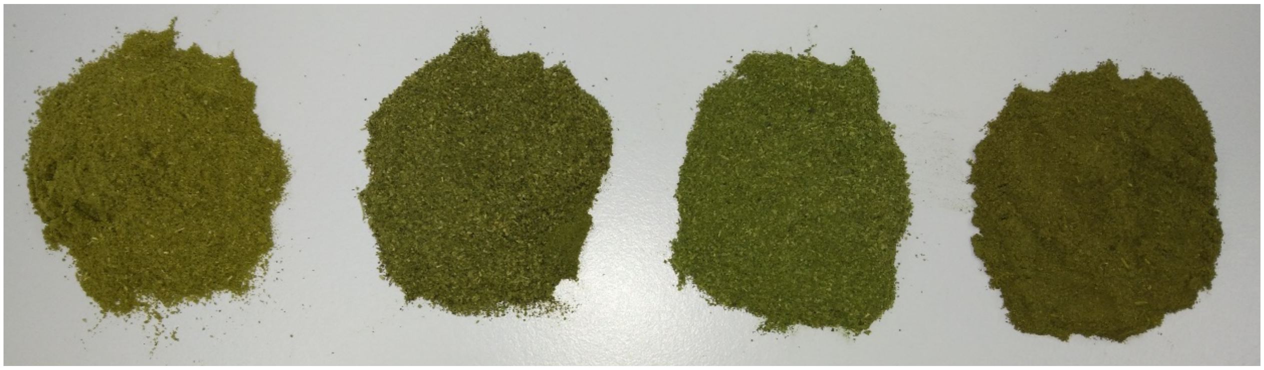
3.Results and discussions

3.1. Evaluation of Antibacterial Activity

Drying of foodstuffs aims to reduce moisture content so that it has longer shelf life (Garba *et al.,* 2019). Moringa leaves which dried with various techniques showing color differences (table 1 and figure 1). In spite of the difference between the four is not too significant, freeze-dried moringa powder was greener and brighter than sun, oven, air-drying techniques' results. These results were in line with Setyowati *et al.* (2017) which shows that the green colour of pandan leaves in freeze drying is brighter than oven drying. This is due to the low temperature which prohibit the release of magnesium ions in green pigments (chlorophyll) so that no brownish pheophytin compounds are formed. These pheoophytin compounds are formed when magnesium ions are released from chlorophyll and replaced with hydrogen ions due to an increase in temperature. Therefore, the higher the drying temperature of the leaves, the darker the colour will be.

Table 1. Colours character of moringa powder in various drying techniques

Drying Techniques	Colour
Freeze drying	Light green
Oven drying	Brownish green
Sun drying	Green
Air drying	Dark green



A B C D

Figure 1. Colours of moringa powder in various drying techniques: A. Freeze drying, B. Oven drying, C. Sun drying, D. air drying

The difference in the color characteristics of moringa leaves represents differences in quality of moringa, in term of antibacterial activity or phytochemical and nutrient content. Antibacterial activity of ethanolic extract of moringa leaf powder with various drying method are shown in table 2. Based on table 2, known that ethanolic extract of moringa leaf with all various drying method (freeze drying, sun drying, oven drying, and air drying) could not inhibit the growth of *Vibrio cholerae* and *Bacillus cereus*. However, three of drying method (freeze drying, sun drying, and air drying) could inhibit the growth of *Escherischia coli* with the diameter of zone inhibition is 2.75 mm, 4.5 mm, dan 1.25 mm, respectively. *V. cholerae, E. coli,* and *B. cereus* are bacteria that cause food-borne disease. *V. cholerae and E.* coli are Gram negative bacteria, while *B. cereus* is Gram positive bacteria. These three bacteria have different levels of sensitivity to antibacterial compounds (Valarmathy *et al.,* 2010; Vieira *et al.,* 2010; Moyo, 2012).

Table 2. Antibacterial activity of moringa leaf with various drying method to *E. coli*, *V. cholerae*, and *B. cereus*

Ethanolic extract of moringa leaf with various drying method	**Diameter zona hambat (mm)**		
	E. coli	***V. cholerae***	***B.cereus***
Freeze drying	2.75	-	-
Sun drying	-	-	-
Oven drying	4.5	-	-
Air drying	1.25	-	-
Fresh moringa leaf (control)	-	-	-

Legend: the sign (-) showed there is no inhibition activity

Table 3. Antibacterial activity of moringa leaf powder to *E. coli*

Type of extraction	**Diameter of zone inhibition (mm)**	**References**
Water	1(*)	Meshram *et al*., (2014)
Ethanolic	4(*)	Ojiako (2014)
Ethanolic	8(*)	Gebregiorgis Amabye *et al.,* (2016)

Legend : The sign of (*) is based on agar well diffusion technique

Some studies said that moringa leaves could not inhibit the growth of *V. cholerae* and *B. cereus* (Valarmathy *et al.,* 2010; Moyo, 2012), but could inhibit the growth of *E. coli* (Valarmathy *et al.,* 2010) even though the inhibitory value showed inconsistent results (Patel *et al.,* 2014; Ojiako, 2014; Gebregiorgis Amabye *et al.,* 2016) (table 3). These can be caused by several factors, namely: 1. Extract type, and 2. Antibacterial activity testing techniques. Altemimi *et al*., (2017) said that the type of solvent used to extract active compounds is very influential on the results obtained. According to him, methanol is the best solvent for extracting bioactive compounds of plant extract. The active compound extracted will certainly also affect its antibacterial activity. In addition, testing for antibacterial activity using agar well diffusion technique provide a high value of antibacterial activity compared to the method for agar disk diffusion. Valgas *et al.* (2007) said that the use of agar disk diffusion made bioactive compound which has many free hydroxyl group will be adsorbed to the surface of the disk and not diffuse into the agar, so that the antibacterial activity might be undetectable. In otherwise, agar well diffusion made that bioactive compound will diffuse directly into agar, so that the antibacterial activity might be higher than agar disk diffusion

3.2.Determination of moisture content, bioactive compound, β-carotene

Flavonoids can be antibacterial because they contain phenols which can denaturate lipoproteins in bacterial cell walls by forming protein-phenol complexes through hydrogen bonds (Rahmawati *et al.,* 2014; Sapara *et al.* 2016). Tannins are antibacterial by activating metabolic enzymes in bacteria and interfering with protein transport in the inner layer of cells (Sapara *et al.* 2016; Rahman *et al,* 2017). Based on inhibitory zone against *E. coli*, moringa leaves with oven drying have greater antibacterial activity, followed by freeze drying, and air drying. This can be related to the resistance of antibacterial compounds (tannins and flavonoids) to the drying temperature. Flavonoid and tannin levels will decrease along with increasing temperature (Obiang-Obounou *et al.*, 2013).

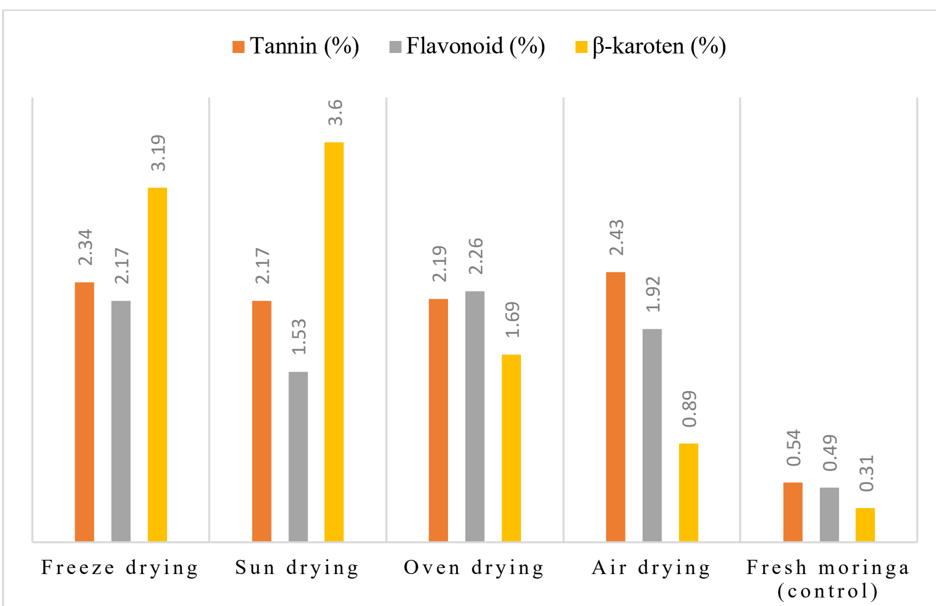


Figure 2. Tannin, flavonoid, and β-karoten of ethanolic extract of moringa leaf on various drying method

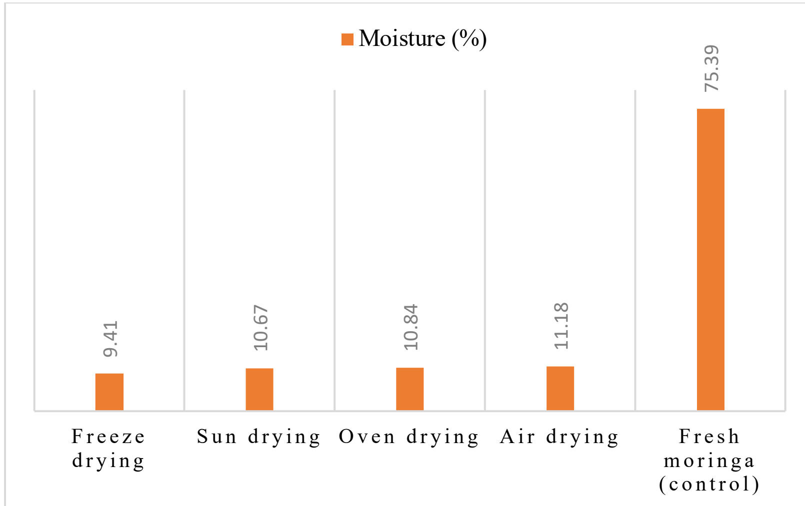


Figure 3. Moisture content (%) of ethanolic extract of moringa leaf on various drying method

Figure 2 showed that the tannin content of moringa leaves dried at air drying is greater than other drying methods, while the flavonoid levels in oven drying is greater than other drying methods. This showed inconsistent results, presumably non-uniform drying time contributes to the inconsistency of the results. However, total tannins and flavonoids in oven drying and freeze drying are greater than air drying. These results are consistent with their antibacterial activity (table 2). Fresh Moringa leaf extract does not show any antibacterial activity against the three bacteria and has the smallest level of antibacterial compounds. This can occur due to the presence of moisture content which gives relativity to leaf mass.

Figure 3 showed that the moisture content in moringa leaf powder with freeze dried is the lowest compared to other drying techniques so that the weight is lighter, although the difference between the four is not too significant. If used as raw material in food processing, then moringa leaf powder with freeze dried is needed in large quantities per mixture of the dough.

Although oven drying showed the best results on antibacterial activity, it turns out that β-carotene levels are not as good as the antibacterial activity. Figure 2 shows that freeze and sun drying techniques provide the best results on β-carotene levels. Mujumdar *et al.* (2016) said that freeze drying is one of the best way to remove water from a product while retaining its bioactive compound. β-carotene is a precursor of vitamin A that serves to sharpen vision at night. Fouad *et al.* (2019) said that β-carotene and flavonoids are natural antioxidants that are mostly obtained from moringa leaves which can be used as antibacterial and can overcome the problem of multidrug resistance.

3.3. Determination of Mineral Content

Reffer to the colour differences of moringa leaf powder, Wijaya *et al.* (2015) said that differences in mineral content in food contribute to differences in physical characteristics such as color, texture, taste, and stability. Minerals are essential constituents in food products even though the body needs a little of it.

Figure 4 showed the effect of drying techniques on the mineral content of moringa leaf powder. The mineral content of air-dried moringa shows the most stable number compared to sun-dried, oven-dried, and freeze-dried moringa. The content is almost equivalent to fresh moringa leaves; even the iron content has not decreased. It caused by the increasing temperatures which will make minerals become destructed. Oni *et al.* (2015) claimed that sun drying and oven drying decreases mineral content of Mg, Zn, Mn, and Fe in edible botanicals because they are destructed by high temperatures. However, it is contradicted to Wijaya *et al.* (2015) who said that minerals cannot be structured due to exposure to heat, light, oxidizing, and extreme pH, but can be eliminated because of their solubility to water. In the same study, Oni *et al.* (2015) also said that the freeze-dried technique is an effective technique for preventing the decrease in mineral content. This is contrary to the results obtained in this study. The mineral content of the freeze dried technique actually decreases significantly, that is as much as 25% Ca, 35% K, 14.81% Mg, and 78% Fe. Referring to Wijaya's statement (2015), perhaps most of the mineral is dissolved with water so that it is eliminated along with the substrate of the water component when freeze drying is carried out. Freeze drying is a method of removing water by sublimating ice crystals from frozen material without passing the liquid phase. Water contained in foodstuffs is sublimated at -34°C for a certain time, then the remaining water is desorbed so that the material becomes completely dry (Gaidhani *et al.* 2015).

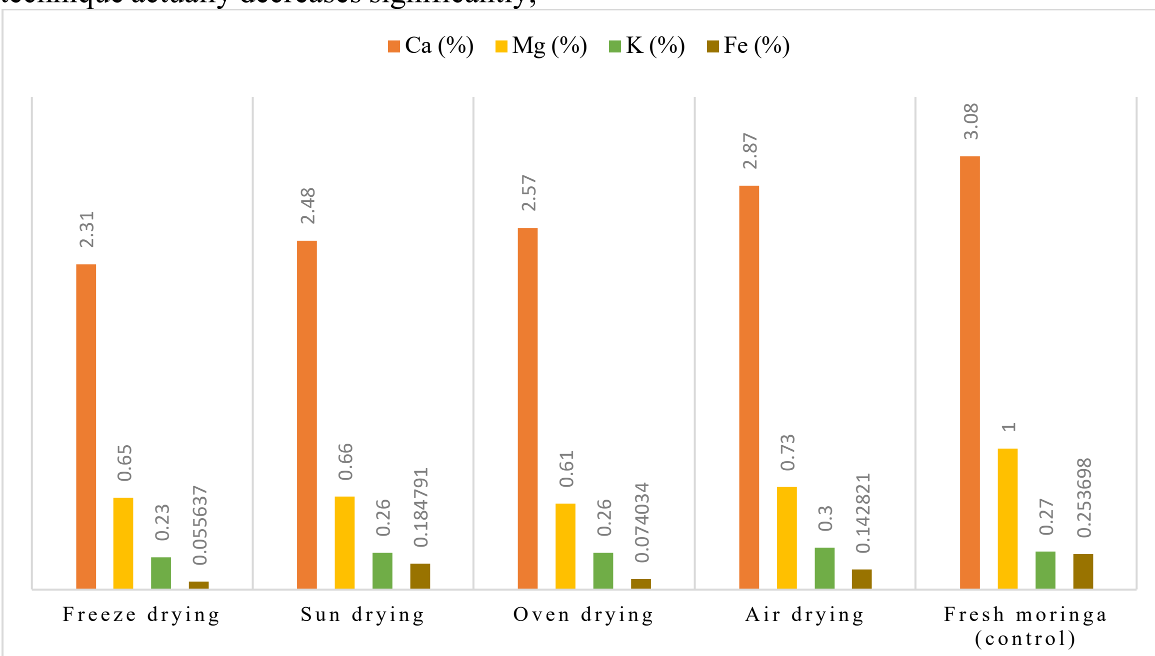


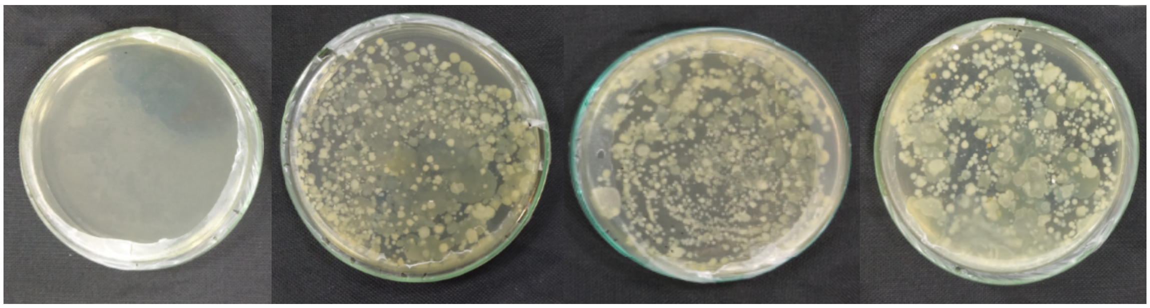
Figure 4. Mineral content of moringa leaf powder on various drying method

3.4. Determination of Total Bacterial Count

Drying technique can reduce moisture content which is useful for slowing decay by microbes and slowing down enzyme activity (Akani *et al.*, 2017; Garba *et al.,* 2019). However, dried-food can still contain high amounts of microbes. Adu-Gyamfi et al (2014) reported that retail food products based on dried moringa leaves have a total bacterial plate count of one million times higher than the international standard allowed. Table 4 and Figure 5 are the result of TBC determination on moringa leaf powder with various drying techniques.

Table 4. Total bacterial count on moringa leaves with various drying techniques

Drying Techniques	Total Bacterial Count (cfu/g)
Freeze drying	< 2.5 x 10^2
Oven drying	4.08 x 10^6
Sun drying	1.95 x 10^7
Air drying	2.46 x 10^8



A B C D

Figure 5. Total Bacterial Count on moringa leaf powder with various drying techniques: A. Freeze drying, B. Sun drying, C. Oven drying, D. Air drying

TBC values on sun-dried, oven-dried, and air-dried showed above 10^6 cfu/g. Only freeze-dried moringa shows no bacterial growth (TBC value < 2.5 x 10^2 cfu/g). This can be caused by the moisture content of freeze-dried moringa leaf powder is lower than the results of other drying techniques. Therefore, it does not allow bacteria to grow in it. Sequentially, the drying technique of moringa leaf powder with TBC values can be ranked from the highest to the lowest bacterial growth, those are air-dried > sun-dried > oven-dried. This is consistent with the moisture content contained in the moringa leaf powder in each technique. According to BPOM's Head Regulation Number 13 of 2019 concerning the maximum limit of microbial contamination in processed food, the TBC value for herbal/spice powder should not be more than 10^4 cfu/g so that the only freeze-dried moringa leaves that meets the requirements of passing BPOM standards.

4. Conclusions

Drying techniques can affect the quality of food products in terms of antibacterial activity, nutrients, and total bacterial count. Based on the results of the study, the air-dried technique is the most effective technique for maintaining the stability of the mineral leaves of moringa leaf powder especially iron, but has a low antibacterial activity. However, from its microbiological safety, air drying still leaves high TBC value. The value is a thousand times higher than the BPOM standard. Moreover, the level of β-carotene has also decreased. In contrast, the freeze-dried technique is the most ineffective technique for maintaining the stability of the mineral, but it is best for microbiological terms. The TBC value is < 2.5 x 10^2 and it meets the BPOM standards. Freeze drying also has a better antibacterial activity especially to *E.coli* and has a higher β-carotene as well as bioactive compound than air-dried technique. Looking at the cost, air drying is recommended as long as the process should be sterile to avoid bacterial contamination. However, if consider the speed of time, freeze drying is recommended. Further research is needed to minimize the risk of nutrient loss in freeze drying.

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