# Food Research 6 (4): 399 - 406 (August 2022)

Journal homepage: https://www.myfoodresearch.com



# Evaluation of stability and quality characteristics of moringa (*Moringa oleifera*) herbal tea during storage

<sup>1</sup>,\*Jayani, N.I.E., <sup>1</sup>Hean, M.R., <sup>1</sup>Krisnayanthi, N.L.A., <sup>2</sup>Islamie, R., <sup>3</sup>Rani, K.C. and <sup>3</sup>Parfati, N.

<sup>1</sup>Department of Pharmaceutical Biology, Faculty of Pharmacy, University of Surabaya, 60293 Kalirungkut, Surabaya, Indonesia

<sup>2</sup>Department of Clinical and Community Pharmacy, Faculty of Pharmacy, University of Surabaya, 60293 Kalirungkut, Surabaya, Indonesia

<sup>3</sup>Department of Pharmaceutics , Faculty of Pharmacy, University of Surabaya, 60293 Kalirungkut, Surabaya, Indonesia

#### Article history:

Received: 10 August 2021 Received in revised form: 25 September 2021 Accepted: 19 January 2022 Available Online: 28 August

#### Keywords:

2022

Moringa leaves, Brewed tea, Teabag, Stability test, Quality control

#### DOI:

https://doi.org/10.26656/fr.2017.6(4).568

#### **Abstract**

Determining the product's shelf life was necessary to ensure its quality, effectiveness, and safety. This study aimed to determine the stability of moringa tea products. The real-time stability test was carried out at room temperature (28.5-33.5°C) with 50-75% relative humidity, and the sampling interval was once every third month (Month-0, Month-3 and Month-6). Physical parameters (organoleptic properties and moisture content), chemical parameters (total phenolic content and total flavonoid content), and microbial parameters (total plate count and total yeast and mould count) were measured using the standard procedures. The organoleptic test showed no changes in colour, taste, smell, and shape of the teabag and brewed tea products up to the sixth month of evaluation. However, there were significant changes in the moisture content (MC), total phenolic content (TPhC), and total plate count (TPC) of the teabag and only in the TPhC of the brewed tea product throughout the quality control. Physical, chemical, and microbial parameters still met the quality requirements set by the Pharmacopoeia Herbal Indonesia and the Indonesian Food and Drug Authority. The qualities of moringa tea bags and brewed moringa teas stored at room temperature (28.5-33.5°C) and 50-75% relative humidity showed good physicochemical properties and stability in the test during six months of observation.

### 1. Introduction

Moringa oleifera is a nutrient-rich plant of the Moringaceae family that contains important phytochemical compounds in its leaves, pods, and seeds. Research shows that moringa has vitamin C seven times higher than oranges, vitamin A ten times higher than carrots, calcium 17 times higher than milk, protein nine times higher than yoghurt, potassium 15 times higher than bananas, and iron 25 times higher than spinach. Moringa's phytochemicals include tannins, sterols, saponins, phenolics, alkaloids, flavonoids (quercetin, isoquercetin, kaemferol, isothiocyanates), and glycosides (Gopalakrishnan et al., 2016; Vergara-Jimenez et al., 2017). Flavonoids and isothiocyanates are responsible for the plant's benefits and bioactivities (Kou et al., 2018). Flavonoids play a role in the antidiabetic and antioxidant activities of moringa, and flavonoids such as quercetin reportedly have antiproliferative and anticancer properties. In addition, moringa is a nutraceutical that

pharmacologically serves as an anti-inflammatory, hepatoprotective, neuroprotective, antidiabetic, and antihyperlipidemic agent. *Moringa oleifera* is an excellent source of macro and micronutrients rich in antioxidant compounds (Sahay *et al.*, 2017). Because of the high nutritional values and pharmacological benefits, it has the potential to be developed into functional food (Saini *et al.*, 2016) like herbal tea. According to Sugahara (2018), moringa can be processed into herbal teas that contain abundant antioxidants to prevent free radical-induced interferences in the body (Sugahara *et al.*, 2018).

Herbal teas are tea made with plant materials other than *Camellia sinensis* leaves. These beverages are made from the simple extraction (e.g., infusion or decoction) of dried leaves, flowers, fruits, or herbs in boiled or heated water (brewing) for a few minutes (Kamiloglu *et al.*, 2016). Herbal teas are widely consumed because of their health-promoting potential and sensory

characteristics (Kinki, 2021) and are thus increasingly popular, especially in developing countries. *Moringa oleifera* has green leaves containing antioxidants, polyphenols, and other phytochemical compounds (Okafor and Ogbobe, 2015; Lalas *et al.*, 2017). Fresh moringa leaves are processed into herbal teas to make them readily used and facilitate storage and preservation, allowing longer shelf life than fresh leaves.

There are many types of moringa herbal tea on the including tea bags and brewed teas. "KELORITA" is a moringa tea product by Sri Rejeki Women Farmer Group in Bogo Village (Bojonegoro, Indonesia) that has received a Home Industry Product certificate from the local health office. It is marketed on a limited scale to the surrounding community but without an expiration date written on the product. Determining the product's shelf life is necessary to ensure its quality, effectiveness, and safety. For this purpose, a traditional product stability test is performed to calculate the shelf life of the final product that has been packaged and stored in appropriate conditions and retains its physical, microbiological, and chemical specifications (Association of South East Asian Nations, 2013).

The most crucial aspect of a product stability study is storage condition. Storage temperature and humidity determine the shelf life of the product. Four climatic zones have been determined for the determination of conditions in the herbal product stability test. These test conditions may vary from country to country. Indonesia itself is included in climatic zone IV, namely countries with a tropical climate. Other countries included in climatic zone IV include Brazil, Ghana, Nicaragua, Nigeria, and the Philippines (Kumadoh and Ofori-Kwakye, 2017).

The purpose of stability testing is to obtain proof of variation in the quality of the herbal products over a specified time under the influence of environmental factors (e.g., temperature, light exposure, oxygen level, and moisture) and dosage form characteristics (i.e., other ingredients or excipients, particle size, microbial contamination, trace metal contamination, and leaching from the container) and to propose recommended storage conditions and shelf life (Höhne et al., 2011). Annex V of the ASEAN Guidelines on Stability Study and Shelf-Life of Traditional Medicines describes that the stability test parameters for traditional preparations like herbal tea bags/brewed teas include organoleptic properties, the concentrations of certain active compounds, moisture contents, and microbial contamination (Association of South East Asian Nations, 2013). The stability test itself can be carried out in real-time or accelerated according to the conditions and sampling frequency specified in the guidelines. This research aimed to evaluate the stability

of "KELORITA" moringa tea bags and brewed teas.

#### 2. Materials and methods

#### 2.1 Materials

The research materials were Peptone Dilution Fluid (PDF) (Merck, Indonesia), Plate Count Agar (Merck, Sabouraud Dextrose Agar Indonesia). (Merck. Indonesia), ethanol p.a. (Merck, Indonesia), methanol p.a. (Merck, Indonesia), 10% aluminium chloride solution (Merck, Indonesia), aquadem (Faculty of Pharmacy, University of Surabaya, Indonesia), Folin-Ciocalteu reagent (Merck, Indonesia), 1 M sodium acetate solution (Merck, Indonesia), 1% sodium hydroxide solution (Merck, Indonesia), gallic acid (Sigma-Aldrich, Singapore), and quercetin (Sigma-Aldrich, Singapore).

## 2.2 Equipment

The research tools included Mettler Toledo moisture analyzer, Scout pro digital scale, Ohaus analytical balance, thermo hygrometer, Laminar Air Flow (LAF) SPEG AIR TECH VF-100-B, spirit lamp, All American autoclave sterilizer, BD-115 incubator binder, Shimadzu UV-Vis spectrophotometer, Socorex micropipettes, IKA magnetic stirrer with a heating plate, and laboratory glassware (beaker, test tube, Erlenmeyer flask, measuring cup, volume pipette, measuring pipette, drop pipette, glass funnel, Petri dish, stirring rod, vial, and lid).

## 2.3 Research sample

The moringa tea products tested were "KELORITA" tea bags and brewed teas produced by Sri Rejeki Women Farmers Group in Bogo Village, Kapas District, Bojonegoro Regency, Indonesia. One cardboard box contained 25 tea bags, each weighing 2 g, while the brewed tea was packaged in a 100 g pouch. The testing was conducted in real-time with a sampling interval of three months (Month-0, Month-3 and Month-6). The products were stored at room temperature of 28.5–33.5° C with 50–75% relative humidity.

#### 2.4 Organoleptic test

The organoleptic test involves the senses of sight, smell, taste, and touch to observe the macroscopic appearance, texture, colour, smell, and taste of a crude sample (Ulhas, 2015). In this stage, the organoleptic properties of the moringa powdered crude sample (tea bag) and chopped crude sample (brewed tea) were examined and compared with the 2017 Indonesian Herbal Pharmacopoeia II monograph (Ministry of Health of the Republic of Indonesia, 2017). In addition, this stage compared the sample's organoleptic properties in

Months 3 and 6 with the initial appearance of the product (Month 0).

# 2.5 Total phenolic content

The total phenolic content (TPhC) analysis refers to the 2017 Indonesian Herbal Pharmacopeia II monograph (Ministry of Health of the Republic of Indonesia, 2017).

#### 2.5.1 Test solution for the crude sample

Approximately 1 g of the crude sample—i.e., powdered (moringa tea bags) or chopped (moringa brewed tea) was weighed and placed in an Erlenmeyer flask, added with 25 mL of methanol p.a., then extracted for 1 hr with a magnetic stirrer. Afterwards, through a filter paper, the extract was poured into a 25-mL volumetric flask and added with methanol p.a. up to the marking.

#### 2.5.2 Test procedure

Gallic acid as the calibration standard was prepared in a series of dilutions, i.e., 100, 70, 50, 30, 15, and 5  $\mu$ g/mL. Each diluted calibration standard was placed in a suitable container and mixed with 1 mL of the test solution and 5.0 mL of the reagent Folin-Ciocalteu dilute (7.5% in water). This mixture was allowed to stand for 8 mins, added with 4.0 mL of 1% NaOH, and then incubated for 1 hr. For each series, the absorbance of the mixture was read at a maximum absorption wavelength of approximately 730 nm. The blank measurement was prepared and conducted in the same way, without adding the test solution. Afterwards, a calibration curve was created, and the total phenolic content of the test solution was calculated and expressed as mg/100 g GAE (gallic acid equivalent).

#### 2.6 Total flavonoid content

The total flavonoid content (TFC) analysis refers to the 2017 Indonesian Herbal Pharmacopeia II monograph (Ministry of Health of the Republic of Indonesia, 2017).

## 2.6.1 Test solution for the crude sample

Approximately 1 g of the powdered crude sample was weighed and placed in an Erlenmeyer flask, added with 25 mL of ethanol p.a. and extracted for 1 hr with a magnetic stirrer. The extract was poured into a 25-mL volumetric flask through a filter paper, and then the filter paper was rinsed with 70% ethanol. The mixed solution was added with 70% ethanol up to the marking.

#### 2.6.2 Test procedure

Quercetin as the calibration standard was prepared in a serial dilution, i.e., 100, 75, 50, and 25  $\mu g/mL$ . A total of 0.5 mL of the test solution and each quercetin solution

were pipetted separately into a suitable container. Then, each container was added with 1.5 mL of ethanol p.a., 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of water shaken gently, and allowed to stand for 30 mins at room temperature. The absorbance of this mixture was measured at the maximum absorption wavelength. The blank measurement was prepared and conducted in the same way, without adding aluminium chloride. Afterwards, a calibration curve was created, and the total flavonoid content of the test solution was calculated and expressed as mg/100 g QE (quercetin equivalent).

## 2.7 Total plate count

The total plate count (TPC) test procedure follows Agyeman-Duah (2017). First, 1 g of the sample was weighed and dissolved in 9 mL of sterile Peptone Dilution Fluid (PDF) in a test tube to obtain 10<sup>-1</sup> dilution. Then, 1 mL was pipetted into the first test tube containing 9 mL of sterile PDF diluent to obtain a 10<sup>-2</sup> dilution then shaken homogeneously. This dilution technique aimed to obtain  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$  dilution levels. Afterwards, 0.1 mL of each diluted solution was pipetted into a Petri dish and duplicated for each serial dilution. Furthermore, 15-20 mL of Plate Count Agar (PCA) was liquefied at 45±1°C and poured into each Petri dish. Immediately, the Petri dish was shaken gently, allowing the sample to mix with the culture media evenly. After the media was solidified, the Petri dish was incubated at 35°C for 24 hrs in an inverted position (Agyeman-Duah et al., 2017). The TPC is expressed as Colony Forming Unit per gram (CFU/g).

# 2.8 Total yeast and mould count

The total yeast and mould count (TYMC) test procedure follows Agyeman-Duah (2017). In this test, the volume of the sample solution pipetted into a Petri dish containing Sabouraud Dextrose Agar (SDA) for each serial dilution was 0.1 mL. This procedure was duplicated for each serial dilution. Then, 15–20 mL of the SDA was liquefied at 45±1°C and poured into each Petri dish. Immediately, the Petri dish was shaken gently to ensure the sample was evenly mixed with the culture media, incubated at 25°C, and observed on the third day (Agyeman-Duah *et al.*, 2017). An examination was performed during the duplicate testing (including the blank media). The TYMC is expressed as Colony Forming Unit per gram (CFU/g).

## 2.9 Statistic analysis

Each test result (% MC, TPhC, TFC, TPC, TYMC) was analyzed using a non-parametric test (Mann Whitney) on the Statistic Packaging for Social Sciences (SPSS) program. The level of significance used was

0.05% to identify differences between samples.

#### 3. Results and discussion

The crude samples were green to brownish-green, odourless, tasteless moringa leaflets (Ministry of Health of the Republic of Indonesia, 2017). Observations in Month-0 (Day 0), Month-3, and Month-6 showed that the tea bag and brewed tea samples organoleptically met the standards issued by the Indonesian Herbal Pharmacopoeia and that there was no physical change in the colour, shape, smell, and taste during storage. The organoleptic test results are presented in Figure 1 and Table 1. The moisture content (% MC) of the product is related to the effectiveness of the drying process of herbal tea. Drying is a critical parameter in maintaining product quality throughout its shelf life because, if conducted incorrectly, several enzymes can be reactivated and cause the decomposition of active compounds in plant ingredients, increasing the risk of microbial growth (bacteria and fungi) (Builders et al. 2020). A proper drying process is at a temperature of approximately 55°C (Castillo et al., 2020). In the Indonesian Herbal Pharmacopoeia, crude drug drying is carried out at no more than 60°C and until the moisture content reaches <10% (Ministry of Health of the Republic of Indonesia, 2017). At the same time, it should not be excessive because drying at high temperatures (>130°C) can damage the crude drug's antioxidant compounds (Razak et al., 2018).

Table 2 shows that the moisture contents (%MC) of the teabag and brewed tea products in Months 0, 3, and 6 were  $6.48\pm0.0029\%$ ,  $7.09\pm0.0007\%$ ,  $7.23\pm0.0005\%$  and  $8.44\pm0.0017\%$ ,  $8.52\pm0.0010\%$ ,  $8.60\pm0.0007\%$ , respectively. The statistical test results indicate that the moisture contents of the tea bags vary significantly

during six-month storage (P = 0.027), while those of the brewed teas vary insignificantly (P = 0.148). Herbal products of Moringa teabag and brewed tea showed an increase in %MC. Storage temperature increases over time and is followed by an increase in the %MC of the product (Razak et al., 2018; Kim et al., 2019). The herbal product absorbs moisture from the surrounding environment; temperature and relative humidity are the two most influencing factors. The degree of fines of herbal tea powder during storage also affects its stability. Herbal tea powders stored in airtight containers can last up to a year, while teas stored in tea bags can last longer. Crude drug and extract of Nauclea latifolia were stored at room temperature in a glass container and the tropical climate (Nigeria) was stable for more than one year (Ameh et al., 2010).

Table 2. Moisture contents of the moringa herbal tea samples

Observation time	Moisture Content (%)		
(month)	Tea bags	Brewed tea	
0	6.48±0.0029 <sup>a</sup>	$8.44\pm0.0017^{d}$	
3	$7.09\pm0.0007^{b}$	$8.52\pm0.0010^{d}$	
6	$7.23\pm0.0005^{c}$	$8.60\pm0.0007^{d}$	

Values are presented as mean $\pm$ SD (n = 3). Values with different superscript are significantly different (p>0.05).

Moringa tea bags are commonly packaged in semipermeable cardboard boxes, allowing moisture transfer through the container's surface. The plastic pouch used for packaging moringa brewed teas is watertight, and this will also affect storage temperature and humidity (Indonesian Food and Drug Authority, 2009). In this study, the products were stored at room temperature (28.5–33.5°C) with 50–75% humidity (adequately high). Recommended storage conditions are 30±2°C and 75±5% relative humidity (Association of South East Asian Nation, 2013). However, the increase in the %MC



Figure 1. Visual, physical characteristics of the tea bag (a-c) and brewed tea samples (d-f) at different observation times: (a) and (d) Month 0 (Day 0), (b) and (e) Month 3, (c) and (f) Month 6.

Table 1. Organoleptic test results of the moringa herbal tea samples

Observation time (month)	Sample	Colour	Odour	Shape	Taste
0	Tea bags	Green	Odourless	Fine powder	Tasteless
	Brewed teas	Brownish-green	Odourless	Chopped crude leaves (coarse-sized)	Tasteless
3	Tea bags	Green	Odourless	Fine powder	Tasteless
	Brewed teas	Brownish-green	Odourless	Chopped crude leaves (coarse-sized)	Tasteless
6	Tea bags Brewed teas	Green Brownish-green	Odourless Odourless	Fine powder Chopped crude leaves	Tasteless Tasteless
				(coarse-sized)	

Table 3. Total phenolic contents and total flavonoid contents of the moringa herbal tea samples

Observation time	Total Phenolic Content (mg/100 g GAE)		Total Flavonoid Content (mg/100 g QE)	
(month)	Moringa tea bags	Brewed Moringa tea	Moringa tea bags	Brewed Moringa tea
0	$12.04\pm0.07^{a}$	$14.20\pm0.00^{d}$	$7.86 \pm 0.79^{g}$	$5.39 \pm 0.10^{h}$
3	$11.87 \pm 0.13^{b}$	$14.06\pm0.05^{e}$	$7.17\pm0.15^{g}$	$5.25\pm0.04^{h}$
6	$11.40\pm0.40^{c}$	$13.87 \pm 0.05^{\rm f}$	$7.06\pm0.14^{g}$	$5.19\pm0.06^{h}$

Values are presented as mean $\pm$ SD (n = 3). Values with different superscript are significantly different (p>0.05).

of the products is still below the requirement, which is <10%.

TPhC and TFC tests aim to ensure that the active compounds acting as antioxidants remain stable during storage (Association of South East Asian Nation, 2013). Table 3 shows the test results. The total phenolic contents of moringa tea bags after 0, 3 and 6 months of storage were 12.04±0.07, 11.87±0.13, and 11.40±0.40 mg/100 g GAE, while those of the brewed teas were 14.20±0.00, 14.06±0.05, and 13.87±0.05 mg/100 g GAE, respectively. The statistical test results indicate that the TPhC of both products varies significantly (P = 0.039and P = 0.023) after 0, 3, and 6 months of storage. Scholars have found that the TPhC of herbal teas positively correlates with antioxidant effectiveness, which is expressed in Trolox Equivalent Antioxidant Capacity (TEAC) (Vyas et al., 2015; Yanakieva et al., 2015; Fotakis et al., 2016). The antioxidant capacity refers to the roles of phenolic compounds as reducing agents, hydrogen donators, and singlet oxygen quenchers (Srivastava et al., 2012; Yamin et al., 2021). The total flavonoid contents of the tea bags and brewed teas in Months 0, 3, and 6 were  $7.86\pm0.79$ ,  $7.17\pm0.15$ ,  $7.06\pm0.14$  mg/100 g QE, and  $5.39\pm0.10$ ,  $5.25\pm0.04$ , 5.19±0.06 mg/100 g, respectively. The statistical test results indicate no significant difference (P = 0.063 and P = 0.061) during 0, 3, and 6 months of storage. Previous research by Jayani et al. (2020) shows that the total flavonoid content of powdered moringa tea produced in Bogo Village is 13.08±0.08 mg/100 mg QE. The strong antioxidant properties of M. oleifera are attributed to its high phenolic and flavonoid contents, which are the main compounds responsible for antioxidant activities (Sulastri et al., 2018). The tests also showed that TPhC and TFC tests of the tea bags and brewed teas decreased during storage. These results correspond to a previous stability study of Bacopa monnieri that found that the marker compounds (bacopaside I and bacoside A) significantly decrease during storage and that the plant ingredients are only stable for three months under appropriate storage conditions (Srivastava et al., 2012).

European Medicines Agency (EMA) (2011) requires that if the level of active herbal compounds associated with their therapeutic activity is known, any variation in this level during storage should not exceed  $\pm 5\%$ , and if it is unknown, the range of variation should not exceed

±10% of the initial value (Tournas and Katsoudas, 2008). The level of marker compounds, such as essential oil in herbal tea preparation, generally decreases by more than 20% of the initial level. In this case, the stability can be assessed by looking at the standard marker value required by the Herbal Pharmacopoeia in the country. Indonesian Herbal Pharmacopoeia requires that the flavonoid content of crude drugs from moringa leaves is not lower than 0.5% and is calculated as quercetin equivalent (Ministry of Health of the Republic of Indonesia, 2017). The results indicate that the TFC of the tea bags and brewed tea is stable for six-month storage, which meets the requirement set in the Indonesian Herbal Pharmacopoeia.

In the country, herbal tea consumption shows an increasing trend because of the product's effectiveness for health. In addition to being effective, herbal tea products must be safe for consumption and free from microbes. Pathogenic pathogenic microbial contamination in herbal tea preparations has been widely reported, including Aspergillus niger, A. flavus, Penicillium spp., Eurotium rubrum, E. chevalieri, Fusarium spp., Alternaria alternata, and yeasts in several herbal tea samples on the market (Tournas and Katsoudas, 2008). Apart from fungi, many cases of aerobic bacterial contamination have also been reported, although it does exceed the predefined limit. A crosssectional study was carried out in the city of Macapa, Brazil. A total of 31.8% of the herbal medicine samples exceeded the safety limits for Bacterial growth (Acceptable limits of bacteria  $\leq 10^5$  colonies/g). It was also found that 31.0% of the samples exceeded the safety limit for fungal growth. The microorganisms most isolated from the herbal medicines were S. aureus (49.2%), Salmonella spp. (34.8%), E. coli (25.8%), and P. aeruginosa (14.4%) (Sousa Lima et al., 2020). Table and Figures 2–5 show the results of the microbiological testing for total plate count (TPC) and total yeast and mold count (TYMC).

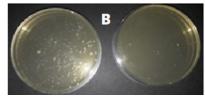
The results showed that the total plate counts of the tea bags and brewed teas after 0, 3, and 6 months of storage were  $2.1-3.1\times10^3$ ,  $3.0-4.8\times10^3$ ,  $8.3-19\times10^3$  CFU/g and  $4.95-22.80\times10^2$ ,  $5.55-38.50\times10^2$ ,  $7.25-66.20\times10^2$  CFU/g, respectively. The statistical test results indicate significant differences in the total plate counts of the moringa tea bags (P = 0.039) and

Table 4. Total plate count and total yeast and mould count of Moringa herbal tea

Observation time	Total Plate Count (CFU/g)		Total Yeast and Mold Count (CFU/g)	
(month)	Moringa tea bag	Brewed Moringa tea	Moringa tea bag	Brewed Moringa tea
0	$2.1-3.1\times10^{3a}$	$4.95-22.80\times10^{2d}$	$3.1-4.1\times10^{3e}$	<100 <sup>f</sup>
3	$3.0 - 4.8 \times 10^{3b}$	$5.55 - 38.50 \times 10^{2d}$	$3.6 - 4.6 \times 10^{3e}$	$<100-2.3\times10^{2f}$
6	$8.3 - 19 \times 10^{3c}$	$7.25-66.20\times10^{2d}$	$4.1-5.9\times10^{3e}$	$1.85 \text{-} 3.80 \times 10^{2} \text{f}$

Values with different superscript are significantly different (p>0.05).





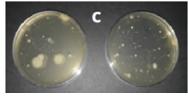
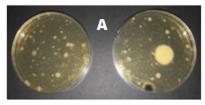
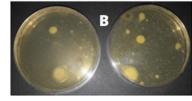


Figure 2. Microbial growth in plate count agar containing ten-fold diluted moringa tea bag samples collected after (A) 0 month (B) 3 months and (C) 6 months of storage.





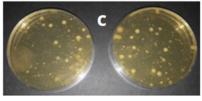
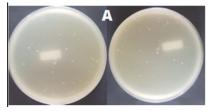
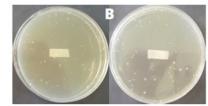


Figure 3. Microbial growth in Sabouraud dextrose agar containing ten-fold diluted moringa tea bag samples collected after (A) 0 month (B) 3 months and (C) 6 months of storage.





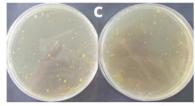
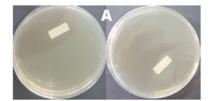
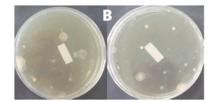


Figure 4. Microbial growth on plate count agar containing ten-fold diluted brewed moringa tea samples collected after (A) 0 month (B) 3 months and (C) 6 months of storage.





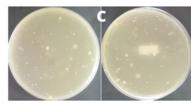


Figure 5. Microbial growth in Sabouraud dextrose agar containing ten-fold diluted brewed moringa tea samples collected after (A) 0 month (B) 3 months and (C) 6 months of storage.

insignificant TPC differences for the brewed teas during storage (P = 0.393). Meanwhile, the total yeast and mould count of the tea bags and brewed teas in Months 0, 3, and 6 were  $3.1-4.1\times10^3$ ,  $3.6-4.6\times10^3$ ,  $4.1-5.9\times10^3$ CFU/g and <100,  $<100-2.3\times10^2$ ,  $1.85-3.80\times10^2$  CFU/g, respectively. The statistical test results indicate that the total yeast and moulds count of both products do not vary significantly (P = 0.191 and P = 0.073) during sixmonth storage. The Indonesian Food and Drug Authority has set the acceptable bacterial of  $\leq 5 \times 10^7$  and fungal contamination of ≤5×10<sup>5</sup> colonies/g (Indonesian Food and Drug Authority, 2019). Limit for microbial contaminant based on World Health Organization standard for herbal medicines to which boiling water is added before use: aerobic bacteria  $\leq 10^7$  colonies/g, yeasts, and moulds  $\leq 10^4$  colonies/g, Escherichia coli  $\leq 10$ colonies/g, other enterobacteria  $\leq 10^3$ colonies/g,

clostridia, salmonellae, and shigella must be absence (World Health Organization, 2007). Based on the test results, both TPC and TYMC of the moringa tea bags and brewed moringa teas fulfil the requirements for sixmonth storage. Another study about herbal tea stability was conducted in Nigeria. A stability test has been carried out on three poly-herbal tea variants containing Hibiscus sabdariffa, Moringa oleifera, Citrus limon, and Zingiber officinale. The herbal ingredients were brewed using 250 mL of hot water (100°C), then allowed to cool to room temperature (27°C) and stored in the refrigerator (5°C). Overall, herbal showed good physicochemical properties and stability in the test during one week of observation (Builders et al., 2020).

#### 4. Conclusion

The qualities of moringa tea bags and brewed moringa teas stored at room temperature (28.5–33.5°C) and 50–75% relative humidity showed good physicochemical properties and stability in the test during six months of observation.

#### **Conflict of interest**

The authors declare no conflict of interest.

## Acknowledgements

The authors would like to express their gratitude to the Faculty of Pharmacy and Institute for Research and Community Service, University of Surabaya, for funding this research through the internal grant scheme with a contract number: 050/ST-Lit/LPPM-01/FF/V/2020.

#### References

- Agyeman-Duah, E., Quaidoo, E.Y. and Mills-Robertson, F.C. (2017). Microbial quality of herbal powders in Ghana. *Edorium Journal of Microbiology*, 3(2), 10-17. https://doi.org/10.5348/M08-2017-7-OA-2
- Ameh, S., Obodozie, O., Gamaniel, S., Abubakar, M. and Garba, M. (2010). Physicochemical Variables and Real Time Stability of the Herbal Substance of Niprd-AM1® an Antimalarial Developed from the Root of *Nauclea latifolia* S.M. (Rubiaceae). *International Journal of Phytomedicine*, 2(3), 332-340.
- Association of South East Asian Nations (ASEAN). (2013). Annex V. ASEAN Guidelines on Stability Study and Shelf-Life of Traditional Medicines, p. 1-20. Jakarta, Indonesia: ASEAN.
- Builders, P.F., Mohammed, B.B. and Sule, Y.Z. (2020). Preparation and Evaluation of the Physicochemical and Stability Properties of Three Herbal Tea Blends Derived from Four Native Herbs. *Journal of Phytomedicine and Therapeutics*, 19(2), 448-465. https://dx.doi.org/10.4314/jopat.v19i2.2.
- Castillo, L., Baltodano, E., Ramírez, N., Vargas, R. and Hanley, G. (2020). Design of Experiments assessment for the determination of moisture content in five herbal raw materials contained in tea products. *Borneo Journal of Pharmacy*, 3(1), 1-14. https://doi.org/10.33084/bjop.v3i1
- Fotakis, C., Tsigrimani, D., Tsiaka, T., Lantzouraki, D.Z., Strati, I.F., Makris, C., Tagkouli, D., Proestos, C., Sinanoglou, V.J. and Zoumpoulakis, P. (2016). Metabolic and antioxidant profiles of herbal infusions and decoctions. *Food Chemistry*, 211, 963–971. https://doi.org/10.1016/j.foodchem.2016.05.124

- Gopalakrishnan, L., Doriya, K. and Kumar, D.S. (2016). *Moringa oleifera*: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, 5(2), 49–56. https://doi.org/10.1016/j.fshw.2016.04.001
- Höhne, C., Kruse, S.O., Dammertz, W., Kroll, U., Tegtmeier, M. and Steinhof, B. (2011). On-going Stability Testing of Herbal Medicinal Products. *Pharmazeutische Industrie*, 73(8), 1401-1412.
- Indonesian Food and Drug Authority. (2009). Peraturan Kepala Badan Pengawas Obat Dan Makanan Republik Indonesia Nomor Hk.00.06.1.52.4011 Tentang Penetapan Batas Maksimum Cemaran Mikroba Dan Kimia Dalam Makanan. Indonesia: Indonesian Food and Drug Authority [In Bahasa Indonesia].
- Indonesian Food and Drug Authority. (2019). Peraturan Badan Pengawas Obat Dan Makanan Nomor 32 Tahun 2019 Tentang Persyaratan Keamanan Dan Mutu Obat Tradisional. Indonesia: Indonesian Food and Drug Authority [In Bahasa Indonesia].
- Jayani, N.I.E., Kartini and Putri, L.K. (2020). Standardization of a crude drug *Moringa oleifera* leaf from Africa, India, and local (Indonesian) which cultivated in Bojonegoro Indonesia. *International Journal of Pharmaceutical Research*, 12(1), 660-668. https://doi.org/10.31838/ijpr/2020.12.01.134
- Kamiloglu, S., Toydemir, G., Boyacioglu, D. and Capanoglu, E. (2016). Health Perspectives on Herbal Tea Infusions. in Govil, J.N. and Pathak, M. (Eds). Recent progress in Medicinal Plants Phytoterapeutics II, p. 353–368. USA: Studium Press LLC.
- Kim, J.H., Lee, K., Jerng, U.M. and Choi, G. (2019). Global Comparison of Stability Testing Parameters and Testing Methods for Finished Herbal Products. *Evidence-based Complementary and Alternative Medicine*, 2019, 7348929. https://doi.org/10.1155/2019/7348929
- Kinki, A. (2021). A Review on the Production and Uses of Herbal Teas. *Nutrition and Food Processing*, 4(2), 1–3. https://doi.org/10.31579/2637-8914/044.
- Kou, X., Li, B., Olayanju, J.B., Drake, J.M. and Chen, N. (2018). Nutraceutical or pharmacological potential of *Moringa oleifera* Lam. *Nutrients*, 10(3), 343. https://doi.org/10.3390/nu10030343.
- Kumadoh, D. and Ofori-Kwakye K. (2017). Dosage Forms of Herbal Medicine Products and their Stability Considerations-an Overview. *Journal of Critical Reviews*, 4(4), 1-8. http://dx.doi.org/10.22159/jcr.2017v4i4.16077
- Lalas, S., Athanasiadisa, V., Karageorgoua, I., Batra, G., Nanosc, G.D. and Makris, D.P. (2017). Nutritional

- Characterization of Leaves and Herbal Tea of *Moringa oleifera* Cultivated in Greece. *Journal of Herbs, Spices and Medicinal Plants*, 23(4), 320–333. https://doi.org/10.1080/10496475.2017.1334163
- Ministry of Health of the Republic of Indonesia. (2017). Farmakope Herbal Indonesia Edisi II Indonesia: Departemen Kesehatan Republik Indonesia.
- Okafor, G.I. and Ogbobe, N.M. (2015). Production and Quality Evaluation of Green and Black Herbal Teas from *Moringa oleifera* Leaf, *Journal of Food Resource Science*, 4(3), 62–72. https://doi.org/10.3923/jfrs.2015.62.72.
- Razak, N.A., Hamid, N.A. and Shaari, A.R. (2018). Effect of storage temperature on moisture content of encapsulated *Orthosiphon stamineus* spray-dried powder. *AIP Conference Proceedings*, 2030, 020194. https://doi.org/10.1063/1.5066835.
- Sahay, S., Yadav, U. and Srinivasamurthy, S. (2017). Potential of *Moringa oleifera* as a functional food ingredient: A review. *International Journal of Food Science and Nutrition*, 2(5), 31-37.
- Saini, R.K., Sivanesan, I. and Keum, Y.S. (2016). Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *Biotech*, 6, 203. https://doi.org/10.1007/s13205-016-0526-3
- Sousa Lima, C.M.D., Fujishima, M.A.T., Paula Lima, B.d., Mastroianni, P.C., Sousa, F.F.O.d. and Silva, J.O.D. (2020). Microbial Contamination in Herbal Medicines: a Serious Health Hazard to Elderly Consumers. *BMC Complementary Medicine and Therapies*, 20, 17. https://doi.org/10.1186/s12906-019-2723-1
- Srivastava, P., Raut, H.N., Puntambekar, H.M. and Desai, A.C. (2012). Stability studies of crude plant material of *Bacopa monnieri* and quantitative determination of bacopaside i and bacoside A by HPLC. *Phytochemical Analysis*, 23(5), 502–507. https://doi.org/10.1002/pca.2347
- Sugahara, S., Chiyo, A., Fukuoka, K., Ueda, Y., Tokunaga, Y., Nishida, Y., Kinoshita, H., Matsuda, Y., Igoshi, K., Ono, M. and Yasuda, S. (2018). Unique antioxidant effects of herbal leaf tea and stem tea from *Moringa oleifera* L. Especially on superoxide anion radical generation systems. *Bioscience, Biotechnology and Biochemistry*, 82(11), 1973–1984. https://doi.org/10.1080/09168451.2018.1495552
- Sulastri, E., Zubair, M.S., Anas, N.I., Abidin, S., Hardani, R., Yulianti, R. and Aliyah. (2018). Total phenolic, total flavonoid, quercetin content and antioxidant activity of standardized extract of

- Moringa oleifera leaf from regions with different elevation. *Pharmacognosy Journal*, 10(6), 104–S108. https://doi.org/10.5530/pj.2018.6s.20
- Tournas, V.H. and Katsoudas, E.J. (2008). Microbiological Quality of Various Medicinal Herbal Teas and Coffee Substitutes. *Microbiology Insights*, 1(6), 47-55. https://doi.org/10.4137/MBI.S943
- Ulhas, A.S. (2015). Storage, Packaging And Labeling Concept With Perspective Of Raw Herbs And Their Formulation. *Unique Journal of Ayurvedic and Herbal Medicines*, 3(4), 42-46.
- Vergara-Jimenez, M., Almatrafi, M.M. and Fernandez, M.L. (2017). Bioactive components in *Moringa oleifera* leaves protect against chronic disease. *Antioxidants*, 6(4), 91. https://doi.org/10.3390/antiox6040091
- Vyas, S., Kachhwaha, S. and Kothari, S.L. (2015). Comparative analysis of phenolic contents and total antioxidant capacity of *Moringa oleifera* Lam. *Pharmacognosy Journal*, 7(1), 44–51. https://doi.org/10.5530/pj.2015.7.5
- World Health Organization (WHO). (2007). WHO Guidelines for Assesing Quality of Herbal Medicines with Reference to Contaminants and Residues. Geneva: WHO.
- Yamin, Ruslin, Mistriyani, Sabarudin, Ihsan, S., Armadany, F.I., Sahumena, M.H. and Fatimah, W.O.N. (2021). Determination of total phenolic and flavonoid contents of Jackfruit peel and in vitro antiradical test. *Food Research*, 5(1), 84–90. https://doi.org/10.26656/fr.2017.5(1).350
- Yanakieva, K., Petkova, N. and Ivanov, I. (2015). In vitro antioxidant activity of herbal infusions, presented at Conference: Ecology and environment, Shumen, 2015. Shumen, Bulgaria: Konstantin Preslavsky University Press.