

Effect of Initial Leaf Moisture Content on the Herbal Quality Parameter of *Orthosiphon stamineus* Dried Leaf During Storage

Norawanis Abdul Razak, Abdul Razak Shaari, Aldrin Felix Anak Nat @ Simbas, Sriyana Abdullah

Abstract – The experiment was conducted to determine the effect of short term storage of *Orthosiphon stamineus* herbal leaf, dried at different level of moisture content on its moisture, color property, antioxidant capacity and total phenolic content. The experimental treatments, that consisted of initial sample moisture content (7%, 10% and 13%) and storage period (10, 20, 30 and 40 days), were arranged in a random completed design (RCD) manner. The changes of moisture content and color property were analyzed by using moisture analyzer chroma meter. The measurement of total phenolic compounds was conducted according to Folin-Ciocalteu method. The study of antioxidant activity was analyzed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity assay. The results showed that the initial moisture content of leaf samples significantly affected the sample moisture content of the *O. stamineus* dried herbal leaf during storage. Meanwhile, the storage time induces the increase of antioxidant capacity and slightly affecting the total phenolic content of stored leaves. However, the color properties such as lightness (L^*) and hue (h°) were not significantly influenced by the treatment during storage. The results revealed that both storage parameters, which are initial moisture content of the stored leaves and storage time, positively affect the quality of dried herbs, although the experiment was conducted for only 40 days of storage time. It is proposed that further experiment need to be performed for longer storage periods with an extra focus to the effect on antioxidant activity.

Keywords – Leaf Moisture Content, Storage Period, Herbal Quality, *Orthosiphon Stamineus*, Bioactive Compounds.

I. INTRODUCTION

Storage is among the important operations in production and supply of raw herbal materials to herbal and pharmaceutical industry. The effective storage system can ensure the continuous supply of raw material as well as maintaining the physical and chemical quality of the herb. There are numerous of internal and external factors that can influence the storability of herbal raw material. These factors included storage temperature and humidity, light intensity, sample moisture content and packing material used. Temperature regimes during storage affect the quality of storage products. Padda and Picha (2008) [1] reported that antioxidant and bioactive compound of sweet potatoes increased during a 4 weeks of exposure to low temperature. Similarly, Singh and Sagar (2010) [2] reported that β -carotene retention in dehydrated leafy vegetables was better under low temperature storage if compared to room temperature storage during 3 months of storage period. A group of researchers also found that the levels of polyphenolic compound in freeze-dried potato

peels exceeded the maximum loss at room temperature (25°C) during 8 weeks of storage period [3]. Humidity level is another factor that should be taken into consideration to maintain the effectiveness of storage product. Moraga et al. (2012) [4] discovered that the bioactive compounds and functional properties of grapefruit powder had a great loss when the relative humidity of storage increased. On the contrary, Shin et al. (2008) [5] reported relative humidity had little effect on quality and antioxidant of strawberry fruit. Beside of temperature and relative humidity, light intensity included as an important factor in storage treatment. A study which conducted by Wang et al. (2009) [6] revealed that fruit quality and flavonoid content of red raspberries had a little effect when exposed to the light. Light slightly increased the speed and coloration of red raspberries. Asraf et al. (2012) [7] also described similar outcomes in their work. The light exposure during storage exhibited the minimal effect on the total phenolic compounds, ascorbic acid and antioxidant activity of fresh-cut Carambola fruit. Ferrante et al. (2004) [8] found that the color of fresh-cut leafy vegetables turned into yellowish color during storage under light condition.

It is important to note that the initial sample moisture content is also vital in determining the quality of herbal raw material during storage. For instance, it was found that the initial seed moisture content status prior to storage had significantly affected the germination rate of red lentil [9]. In addition, it was also reported that the seed quality of the hullless barley stored over certain time was also affected by the seed initial moisture content [10].

Orthosiphon stamineus is one of the important and popular herbal species in Malaysia, Indonesia, Thailand and Myanmar. This herbal species are widely used as the traditional medicine and health supplements. *O. stamineus* scientifically proven that its bioactive compound has pharmacological several benefits to human health such as terpenoids (diterpenes and triterpenes), polyphenols (lipophilic flavonoids and phenolic acids), and sterols [11]. The therapeutic effects which given by this species have been reported due to the presence of polyphenol compound [12]. This group of compound has been demonstrated its ability to reduce oxidative stress by suppressing the formation of lipid peroxidation products in biological systems. Research results also indicated that the flavones, sinensetin and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone isolated from *O. stamineus* leaves exhibited a diuretic activity in rats [13]–[15]. Caffeic acid derivatives, including rosmarinic and 2,3-dicaffeoyltartaric acids, are the most abundant polyphenols in an aqueous

methanol extract of *O. stamineus* leaf, which predominate over polymethoxylated flavones[16].

O. stamineus leaves are rested, dried and stored for few months before processing into different herbal products. As compared to the internationally well-established herbal species, information on the association of storage conditions, such as initial moisture content with the bioactive compound constituents of our popular local herbs is very much lacking. As a result, thus far a standardized storage procedure is not fully established. Hence, the general aim of this proposed work is to investigate alterations of these compounds when the *O. stamineus* dried herbal leaves are stored under different initial moisture content.

II. MATERIALS AND METHODS

A. Plant Material

Orthosiphon stamineus fresh leaves were obtained from the herb farm of the Sustainable Agrotechnology Institute, Universiti Malaysia Perlis (UniMAP), Campus Sungai Chuchuh, Padang Besar, Perlis. Leaf samples were cleaned with clean water to get rid of dirt, small worms and dirt. The leaves were air dried in trays for 5 days and subsequently sorted out for uniformity in size. The sorted and air-dried samples were dried further under thin layer condition at 40°C using an oven. The moisture content of the leaves was monitored at the interval moisture analyzer until desired moisture of 7%, 10% and 13% obtained.

B. Chemicals and Reagents

Methanol (analytical grade) and Follin-Ciocalteu's Phenol reagent were purchased from Merck (Merck KgAa Chemical, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was supplied by Aldrich (Sigma-Aldrich Chemie, Germany). Sodium carbonate was purchased from HmbG (Hamburg Indus Inc, USA)

C. Storage Treatment

1gram of oven-dried sample of 7%, 10% and 13 % of sample moisture content was stored at room temperature for 40 days. The experiment was designed randomized completed design (RCD) using three different rooms. The samples were analyzed at 10 days interval samples moisture content, color characteristics, antioxidant capacity and total phenolic content.

D. Moisture Content Analysis

The MS-70 moisture analyzer (A&D, Japan) was used to measure moisture content value of the experimental samples at different storage interval. 1 g of leaves was randomly sampled and subjected to direct moisture measurement by moisture analyzer. The information obtained was then calibrated with standard curve based on an oven method.

E. Color Property

Color property of all stored samples were performed using a Minolta CR-400 chroma meter. The D65 illuminant and CIE 1964 Supplementary Standard Observer was assumed for the calculation of calorimetric data in the CIELAB system (1986). The color properties of samples are defined by the calorimetric or chromaticity coordinates; clarity (L^*), red/green color component (a^*)

and blue/yellow color component (b^*). Coordinate L^* represents clarity ($L^*= 0$ black and $L^* = 100$ colorless), a red/green color component ($a^*>0$ red and $a^*<0$ green) and blue/yellow color component ($b^*>0$ yellow and $b^*<0$ blue).

$$\text{Hue angle (h}^\circ\text{)} = \arctan\left(\frac{b^*}{a^*}\right) \quad (1)$$

F. Sample Extraction

1 g of dried leaves was extracted in 100ml of distilled water that was incubated at 40°C for 3 hours in the shaking water bath. The extract was filtered using filter paper and capped in a sealed bottle before it was stored in a freezer below -20°C for antioxidant capacity and total phenolic content analysis.

G. Total Phenolic Content Analysis

200 μ l of Follin-Ciocalteu reagent (FCR) and 200 μ l of the sample was mixed with 1.58 ml distilled water. After 4 minutes, 1 ml of 20% sodium carbonate was mixed together. The mixed solution was allowed to react for 2 hours in a dark place. After 2 hours, the absorbance was calculated using Shimadzu UV/VIS spectrophotometer ($\lambda=760\text{nm}$) and the reading was recorded. The caffeic acid was used as a standard and the concentration of total phenolic content was expressed in caffeic acid equivalent (CAE).

H. Antioxidant Capacity Analysis

2 ml of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was mixed with 200 μ l of extract sample. Methanol was used to mark up the mixture to 3 ml. The mixed solution was allowed to react in a room temperature for 1 hour. The control was also prepared. After 1 hour, the absorbance value was calculated using a Shimadzu spectrophotometer ($\lambda=517\text{nm}$) and the reading was recorded.

$$\text{Antioxidant capacity} = \left[\frac{Ac - As}{As} \right] \times 100 \quad (2)$$

Where;

Ac = Absorbance of the control.

As = Absorbance of the sample.

I. Statistical Analysis

All of the data obtained from the experiment undergo statistical analysis that conducted using Microsoft excel and JMP pro 11 package software. Differences at $P_{\text{value}}<0.05$ were considered as a significant value. Student's t test was used to analyze the multiple comparisons of means.

III. RESULT AND DISCUSSION

A. Moisture Content

Moisture content is an important parameter that needs to be considered during storage of dried raw herbal material. The moisture content changes of *Orthosiphon stamineus* dried herbal leaves stored for 40 days at low, medium and high initial sample moisture content, 7%, 10% and 13% respectively, are shown in Fig. 1. In general, the changes of sample moisture content during storage were significantly affected ($P_{\text{value}}<0.0001$) by initial sample moisture content before storage irrespective of the levels.

The samples with high initial moisture content (13%) before storage gradually declined ($P_{\text{value}}=0.0015$) in moisture content throughout the storage period and stabilized at 11.73%. On the other hand, the samples with low initial moisture content (7%) had significantly increased ($P_{\text{value}}=0.0243$) in moisture content during storage and attained the moisture level of around 10.29% at the end of the storage period. The sample with medium initial moisture content exhibited the increasing trend similar to low initial moisture content samples and stabilized at 10.83%. The moisture content of samples with medium and low initial moisture content remained the same ($P_{\text{value}}=0.2011$) after 40 days in storage condition. The results clearly indicated that moisture adsorption and desorption had occurred during the storage period and the levels stabilized approximately at 11% for all treatments. The increment and reduction of the sample's moisture content may be associated with the level of storage humidity in the sealed storage plastic bags used in this experiment. This result somewhat differs from the research finding which on the storage of red lentil seeds [9]. They claimed that the storage of red lentil seeds at higher initial moisture content levels (15% and 17.5%) did not indicate much change in sample moisture content during 16 weeks of storage. Withal, the samples with 10% and 12.5% initial moisture content had slightly absorbed moisture after 8 and 11 weeks of storage, respectively. The delay moisture in the absorption of moisture of red lentil seeds as compared to *O. stamineus* dried leaves may be ascribable to the physical attributes of this fabric.

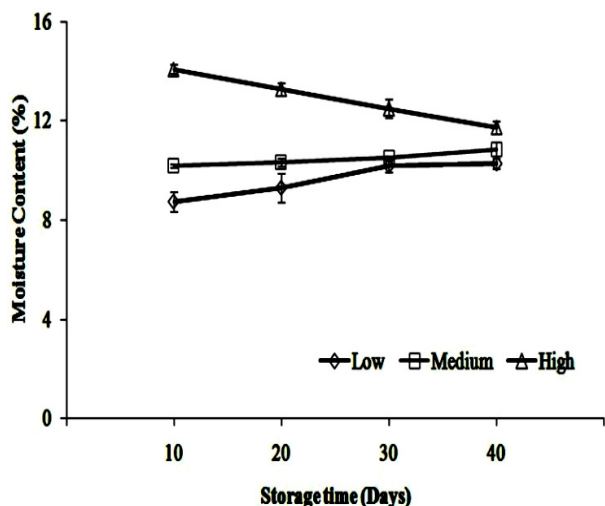


Fig.1. The changes of sample's moisture during 40 days of storage time for low, medium and high initial moisture content.

The red lentil seed is rounded in form which less surface area if compared to *O. stamineus* dried leaves with larger surface area that can enhance and early absorption or desorption of moisture from the environment. In spite of this, our results was in agreement with the results observed by Singh and Sagar (2010) [2]. They reported the changes of moisture content for the studies of dehydrated curry (*Murraya koenigii*) leaves and dehydrated drumstick (*Moringa oleifera*) leaves with the initial moisture content

of 2.5% and 5.5%, respectively. Both samples exhibited the increasing value of moisture content of 4% and 7.5%, respectively, after 3 months of storage under room temperature. The absorption process in both samples occurred more slowly compared to *O. stamineus* dried leaves even they bore a similar physical properties such as leafy, thin and dry. This may be due to the difference of packaging material and sample quantity used in each experiment. The moisture migration increased when less of sample quantity were used.

B. Color Property

The color property and appearance are important herbal quality parameters that associated with consumer demand. The changes in the lightness (L^*) and hue angle (h°) of *O. stamineus* dried herbal leaves with 7% (low), 10% (medium) and 13% (high) stored for the duration of 40 days are shown in Table 1. When the color became darker, a decrease in L^* and hue angle (h°) was observed. The hue angle value showed true color which is effective for visualizing the color of food products [17]. The sample clarity or lightness (L^*) before storage were 44.84, 47.06 and 44.25 for low, medium and high initial sample moisture content, respectively. The hue angle (h°) of samples before initiation of storage were recorded as 85.47°, 79.96° and 83.29° for low, medium and high initial sample moisture content, respectively. From the result, it revealed that the lightness (L^*) and hue angle (h°) values for low, medium and high initial moisture content of the samples remained ($P_{\text{value}}>0.05$) almost constant throughout the storage period. The L^* values were varied from 44.68 to 46.65. Meanwhile, h° values observed in this study were around 78.89° and 83.83°. The maintaining in color values during storage may be attributed by the short storage duration that applied in this experiment. The colour of the sample may turn to brown if the storage time is prolonged and this change may occur non-enzymatically.

Table 1: The changes of sample colour during 40 days of storage time for low, medium and high initial moisture content.

Initial Moisture Content	Storage Time (Days)			
	10	20	30	40
Lightness (L^*)				
Low	46.07a	45.46a	45.22a	46.49a
Medium	46.65a	44.99a	44.68a	45.70a
High	45.98a	45.10a	45.98a	45.59a
Hue Angle (h°)				
Low	82.98a	81.64a	82.25a	83.83a
Medium	82.71a	81.42a	78.89a	83.73a
High	81.12a	81.17a	81.10a	82.55a

Note: Means with the same letter in a column are not significantly different at $P_{\text{value}}<0.05$ (Student's t test).

C. Antioxidant Capacity

Antioxidant capacity and total phenolic content are two main quality parameters of *Orthosiphon stamineus* dried herbal leaves. The changes of antioxidant capacity and total phenolic content during 40 days of storage time are

tabulated in Table 2. The antioxidant capacity that analyzed at different initial moisture content (low, medium and high) were determined as 77.32%, 83.20% and 86.71%, respectively. The result showed that the antioxidant capacity of all samples with different initial moisture content were not significantly different throughout the storage period ($P_{\text{value}}=0.6698$).

Table 2: The changes of sample antioxidant capacity during 40 days of storage time for low, medium and high initial moisture content

Initial Moisture Content	Storage Time (Days)			
	10	20	30	40
Low	74.63a	77.33a	79.09a	82.30a
Medium	72.25a	75.38a	79.01a	81.41a
High	74.78a	73.23a	77.01a	80.65a

Note: Means (n=3) with the same letter in a column are not significantly different at $P_{\text{value}} < 0.05$ (Student's t test). Data expressed in percentage (%) for antioxidant capacity

Fig.2 shows the changes in the mean of antioxidant capacity over storage time. It was found that antioxidant capacity significantly increased from 73.9% at day 10 to 81.5% at day 40 of storage time ($P_{\text{value}}=0.0062$). Al-Weshahy et.al. (2013) [3] reported that the increment of antioxidant capacity on freeze-dried potato peels during storage mainly caused by the production of glycoalkaloids due to enzymatic reactivation during storage. Nevertheless, Leo et al. (2008) [18] reported that the synergistic interactions between individual components, resulting in elevated expression of antioxidant potency.

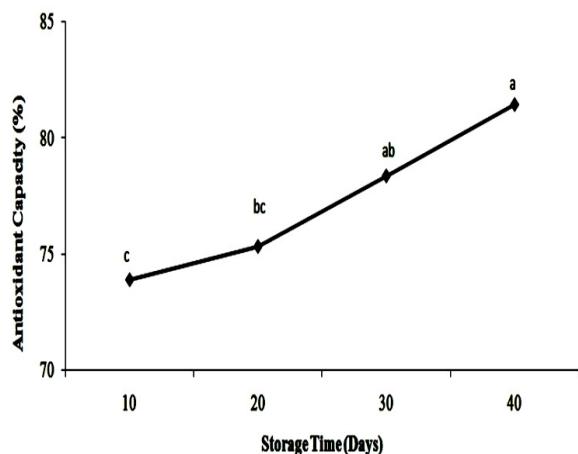


Fig.2. Change over storage time in mean of antioxidant capacity (%). Means with the same letter are not significantly different at $P < 0.05$.

D. Total Phenolic Content

Table 3 shows the effect of initial leaf moisture content on the mean value of total phenolic compound concentration on low, medium and high initial moisture content during storage. The initial phenolic compound concentration before the initiation of storage treatment were 139.53 mg/g, 110.41 mg/g and 94.44 mg/g for low, medium and high sample initial moisture content, respectively. As shown in the result, at 10 days of storage

period, the leaf samples with high initial moisture content significantly contained lower concentrations of phenolic compound (122.49 mg/g) as compared to low and medium initial moisture content level ($P_{\text{value}}=0.0215$). However, the total phenolic compound concentration of low, medium and high initial moisture content samples were not significantly different at day 20 ($P_{\text{value}}=0.5573$), day 30 ($P_{\text{value}}=0.5758$) and day 40 ($P_{\text{value}}=0.6044$) of storage time. At day 20 the phenolic compound concentration of low, medium and high were 158.04 mg/g, 157.96 mg/g and 147.41 mg/g respectively which indicated insignificant differences of the treatments. The similar result pattern was observed for the samples that stored until day 30 and day 40 of storage period.

Table 3: The changes of sample total phenolic content during 40 days of storage time for low, medium and high initial moisture content.

Initial Moisture Content	Storage Time (Days)			
	10	20	30	40
Low	166.58a	158.04a	120.93a	137.86a
Medium	149.78ab	157.96a	130.46a	141.07a
High	122.49b	147.41a	124.35a	130.28a

Note: Means (n=3) with the same letter in a column are not significantly different at $P_{\text{value}} < 0.05$ (Student's t test). Data expressed mg/g for total phenolic content.

Fig. 3 shows the total phenolic content mean from three (low, medium and high) initial moisture content sample during short storage. The combine value of the moisture treatments slow, medium and high slightly fluctuated ($P_{\text{value}}=0.0031$) over time (10, 20, 30 and 40 days). This result seems to be contradicting of the finding by Al-Weshahy (2013) [3] that the total polyphenolic compounds in freeze-dried potato peels increased after 8 weeks of storage time. They suggested that might be due to the stimulating effect of this temperature on the biosynthesis of polyphenolic compounds. In our work, we used *O. stamineus* dried leaves by which the most of the water was removed and the enzyme was considered not activated after drying process. Nevertheless, the enzyme might be reactive with the presence of moisture and residual chlorophyll in the samples.

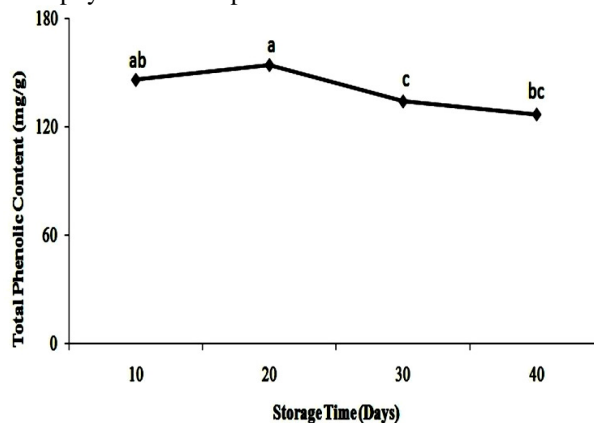


Fig.3. Change over storage time in the mean of total phenolic content (mg/g). Means with the same letter are not significantly different at $P < 0.05$

IV. CONCLUSION

It is concluded that the initial leaves the moisture content of dried *O. stamineus* leaves can positively affect the overall quality of *O. stamineus* raw herbal leaves during storage. Although the leaf color and total phenolic content insignificantly affected by the treatments, but the results revealed that antioxidant capacity increased significantly over the storage period. Additionally, irrespective of the initial moisture levels, the content of water in the stored leaves stabilized at almost similar levels. The stability in the sample moisture content may contribute to extending the storage life of the *O. stamineus* herbal leaves.

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