Quality Changes of Orthosiphon Stamineus Dried Herbal Leaves Under Extremely Low Relative Humidity Storage Condition

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ABSTRACT

The experiment was conducted to determine the effects on the physical appearance and bioactive compounds content when Orthosiphon stamineus dried herbal leaves were stored under the extremely low condition at 10% relative humidity for 180 days. The moisture content changes were examined using a moisture analyzer and the color changes were analyzed using colorimeter. The total phenolic compounds and antioxidant activity were measured using the Folin-Ciocalteu method and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay respectively, and analyzed using UV/VIS Spectrophotometer. The biomarkers were determined using high performance liquid chromatography (HPLC). The results showed that storing O. stamineus dried herbal leaves under very low relative humidity had largely affected the herbal leaves quality. After 180 days of storage time, the color of samples turned to dark-yellow and the moisture content declined to 7.38% wet basis. Antioxidant capacity exhibited an increase in value. Meanwhile, total phenolic content did not change during storage. Rosmarinic acid significantly increased during storage time from 2.89 to 6.58 mg/g. The TMF, sinensetin and eupatorin increased to 48.31, 29.04 and 7.92 ug/g, respectively, at day 90. On day 180, TMF decreased to 11.01ug/g and sinensetin maintained at 19.48 ug/g. However, eupatorin was not detected at day 180 of storage time. The optimum storage condition should be monitored in order to maintain the quality of raw herbal materials for further processing.

INTRODUCTION

The usage of herbs in daily practice is rapidly increasing nowadays. Some of them are claimed to be a good method of treatment for chronic diseases such as kidney problem, diabetes, hypertension and cancer [1]–[4]. Usually, these processed herbal plants are produced in tablets, capsules, sachets, concentrated liquid and so on. The quality of the final products should be prioritised before they are distributed to the consumers. Generally, the quality of herbal products disappears during the production processes especially when heat is involved [5]. Production chain of herbal products commonly included drying, storage of herbal raw materials, extraction, granulation, compression, packaging, final product storage, transportation and marketing. Each process may affect the consistency and quality of herbal products. Thus, the optimum quality should be monitored at each level of the production process. A lot of studies had been done on the processes mentioned earlier but researches on storage are limited. The entrepreneurs have a correct indication to control the quality of the final products. However, the effect of storage towards the plant’s quality is not fully explored yet by the researchers, especially for local herbs. Therefore, this study was conducted to investigate the effect of extremely low relative humidity storage condition on the physical appearance and chemical quality of O. stamineus dried herbal leaves. This condition was selected based on one of our country’s climate, the drought season. During this season, the storage room temperature may increase and becomes extremely dry. Not all entrepreneurs can afford to use air conditioner in their storage room. This study was performed on selected potential herbs, O. stamineus or locally known as ‘misai kucing’, which is believed to be able to treat chronic diseases such as diabetes and cancer [6].

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Additionally, this herb is also believed to be capable of curing gout, kidney stones, high blood pressure, fever, and more [7]. In this study, the main factors were extremely low relative humidity storage condition at 10%RH and storage time. This study was expected to be a useful reference especially for entrepreneurs who are currently active and interested in natural herbal food industry.

METHODS AND MATERIALS

Raw Material Preparation. *O. stamineus* plants were obtained from Sustainable Agrotechnology Institute, Universiti Malaysia Perlis (UniMAP) crop field. The cleaned plants were air dried at ambient temperature for 7 days. Only part of leaves was utilized in this experiment to control the consistency of further analysis.

Storage Treatment. The extremely low relative humidity (±10%RH) storage condition was set up in desiccator using saturated salt, lithium chloride as described by Greenspan [8]. The consistency of relative humidity was monitored using a digital hygrometer which was located in each desiccator. 10 g of *O. stamineus* dried herbal leaves were stored for treatment in each desiccator. Three different rooms with the temperature of ±25°C were utilized as a treatment replication. The readings were taken at the 0, 90 and 180 day of storage time. Each sample was considered as independent samples.

Sample Preparation. 1 g of *O. stamineus* dried herbal leaves was extracted by 100ml of distilled water for 3 hours at 40°C using a shaker water bath (Thermolab, Germany). The extracted solution was filtered using Whatman No.1 filter paper and then were sealed in the bottles and stored in a freezer (-20°C) for chemical quality analysis.

Determination of Color. An average of six readings was taken from individual sample of *O. stamineus* dried herbal leaves using colorimeter CR-400 (Konica Minolta, Japan). The collected data were available in the form of L*, a* and b* color space (CIELAB).

Determination of Moisture Content. The MS-70 moisture analyzer (A&D, Japan) was used to read the 1g sample’s moisture.

Determination of Total Phenolic Content. 200µl of Follin-Ciocalteu reagent (FCR) and 200µl of extract solution were mixed with 1.58 ml distilled water and shook rigorously for 4 minutes before adding 1 ml of 20% sodium carbonate. The mixed solution was allowed to react for 2 hours in a dark place. The concentration of total phenolic content was quantified using UV/VIS spectrophotometer (Shimadzu, Japan) and the absorbance was read at λ=760nm. The caffeic acid was used as standard and the concentration of total phenolic content was expressed in caffeic acid equivalent (CAE).

Determination of Antioxidant Capacity. The antioxidant capacity of the extracts was determined using the modified DPPH method as described by Akowuah [9]. About 2 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was mixed with 200 µl aliquots of samples. 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 UV/VIS spectrophotometer (Shimadzu, Japan) at λ=517nm. The antioxidant capacity of samples was estimated by utilizing the following equation:

\[
\text{Antioxidant capacity} = \left( \frac{A - B}{A} \right) \times 100;
\]

Where A = Control absorbance, B = Sample absorbance

HPLC Analysis. High performance liquid chromatography, HPLC (Shimadzu, Japan), was used equipped with degasser, an auto sampler, a column heater, quaternary pump and UV detector. The column (A LiChrosorb RP-18, 250mm x 4.6mm, 5µm) was maintained at 30°C and the injected sample, about 20µl, was eluted with isocratic mobile phase comprising of methanol: tetrahydrofuran: acidic water (pH3) mixture in the volume ratio 45:5:50. The flow rate was 1 ml/min, 40 min separation time and detection at 340nm. Standard calibration curves were made by plotting the peak area against concentration. The quality reference compounds used were rosmarinic acid (Sigma-Aldrich, U.S.A), 3'-hydroxy-5,6,7,4'-tetramethoxy flavone, TMF (Indofine, U.S.A), sinensetin (Indofine, U.S.A) and eupatorin (Indofine, U.S.A).

Statistical Analysis. All measurements were carried out in triplicate and the results are statistically analyzed using JMP pro 11 package to determine the average value and standard error.

RESULTS AND DISCUSSIONS

**Effect of Extremely Low Relative Storage Condition on Appearance Quality:**

**Moisture Content Changes:**

The changes of moisture content on *O. stamineus* dried herbal leaves were plotted against storage time in days under extremely low relative humidity as shown in Fig. 1. The results obtained from the ANOVA analysis showed that there was a significant change of moisture content by storage time ($p_{value}=0.0007$). The initial moisture content at 0 days starting from 10% (wet basis) significantly declined at day 90 ($p_{value}=0.0008$). This phenomenon could be labeled as desorption process where the environment absorbed the molecules of water from the samples [10]. After 90 days of storage time, the changes of moisture content seem to be maintained ($p_{value}=0.1756$). At this stage, equilibrium moisture content was achieved[11].
Fig. 1: The changes of moisture content of samples during 180 days of storage time under extremely low relative humidity storage condition. Means (n=3) with different letter are significantly different at 95% confidence level.

**Colour Parameters:**

The changes of color parameters by storage time are represented in Table 1. The positive value of L* and b* were referred to lightness and yellowness. The negative value of a* was referred to greenness of sample’s colour [12]. The results obtained from ANOVA analysis showed that there were significant changes of L*, a* and b* by storage time (p-value=0.0101, p-value=0.0007 and p-value=0.0088, respectively). The L* value significantly maintained around 47.18 to 48.11 between the initial and day 90 of storage time (p-value=0.2578). However at day 180, the L* value reading significantly decreased to 44.73 (p-value=0.0040). The a* value significantly decreased to -0.63 at day 90 of storage time (p-value=0.0019) and maintained around -0.39 at day 180 of storage time (p-value=0.0537). The b* value significantly increased from 5.40 to 7.19 at day 90 of storage time (p-value=0.0089) and then the b* value maintained at day 180 of storage time around 7.49 (p-value=0.5431).

**Table 1:** The changes of colour parameters on samples of *O. stamineus* dried herbal leaves during the 180 days of storage time under extremely low relative humidity storage condition.

<table>
<thead>
<tr>
<th>Storage time (Days)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>47.18a</td>
<td>-1.16b</td>
<td>5.40b</td>
</tr>
<tr>
<td>90</td>
<td>48.11a</td>
<td>-0.63a</td>
<td>7.19a</td>
</tr>
<tr>
<td>180</td>
<td>44.73b</td>
<td>-0.39a</td>
<td>7.49a</td>
</tr>
</tbody>
</table>

*Means (n=3) with different letters in a single column are significantly different at the 95% confidence level.

**Effect of Extremely Low Relative Storage Condition on Chemical Quality: Antioxidant and Total Phenolic Content:**

Fig. 2 shows the changes of antioxidant capacity (%) and total phenolic content on samples of *O. stamineus* dried herbal leaves stored for 180 days under extremely low relative humidity storage condition. From the ANOVA analysis result, antioxidant capacity significantly increased by storage time (p-value <0.0001). The antioxidant capacity significantly increased from 72.79 to 79.30% at day 90 of storage time (p-value=0.0007) and slightly increased to 82.45% at day 180 of storage time (p-value=0.0030). This finding was similar to previous research done by Toor and Savage [13]. They claimed that antioxidant capacity of tomatoes increased during storage. Leja and friends also claimed that antioxidant capacity of broccoli flower bud increased during storage [14]. However, the quantity of total phenolic content in the samples significantly was not affected by extremely low relative humidity storage condition and storage time (p-value=0.5729).

**Biomarker Compounds Content:**

Table 2 shows the changes of biomarker compounds on samples of *O. stamineus* dried herbal leaves during the 180 days storage time under extremely low relative humidity storage condition. In this study, four important biomarker compounds of *O. stamineus* were selected; rosmarinic acid, 3’-hydroxy-5,6,7,4’-tetramethoxyflavone (TMF), sinensetin and eupatorin. The results obtained from the ANOVA analysis showed that there were a
significant change on rosmarinic acid (p_value=0.0065), TMF (p_value=0.0030), sinensetin (p_value=0.0031) and eupatorin (p_value=0.0061). The mean value of rosmarinic acid significantly increased during 180 days of storage time from 2.89 to 6.58 mg/g (p_value=0.0058). The mean value of TMF, sinensetin and eupatorin increased to 48.31 (p_value=0.0015), 29.04 (p_value=0.0011) and 7.92 ug/g (p_value=0.0155), respectively, at the 90 day of storage time and significantly maintained at 19.48 ug/g until day 180 for sinensetin (p_value=0.0801) but significantly dropped to 11.01ug/g for TMF (p_value=0.0083). The eupatorin decreased (p_value=0.0022) and was considered as not detected at day 180 of storage time.

Table 2: The changes of biomarker compounds on samples of O. stamineus dried herbal leaves during the 180 days of storage time under extremely low relative humidity storage condition.

<table>
<thead>
<tr>
<th>Storage time (Days)</th>
<th>Rosmarinic acid (mg/g)</th>
<th>TMF (ug/g)</th>
<th>Sinensetin (ug/g)</th>
<th>Eupatorin (ug/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.89b</td>
<td>4.62b</td>
<td>2.51b</td>
<td>2.72b</td>
</tr>
<tr>
<td>90</td>
<td>2.58b</td>
<td>48.31a</td>
<td>29.04a</td>
<td>7.92a</td>
</tr>
<tr>
<td>180</td>
<td>6.58a</td>
<td>11.01b</td>
<td>19.48a</td>
<td>0.00b</td>
</tr>
</tbody>
</table>

*Means (n=3) with different letter in a single column are significantly different at 95 % confidence level.

Conclusion:
As a conclusion, the data collected in this paper showed that extremely low relative humidity significantly affected the quality of O. stamineus dried herbal leaves during 180 days of storage time. The samples became drier and crunchy. The sample's color changed to dark-yellow. Antioxidant increased by storage time. Total phenolic content maintained along the storage time. The chemical quality of the sample increased when the moisture content decreased under extremely low relative humidity storage condition for O. stamineus dried herbal leaves. These finding might be used as an indicator for similar local dried herbal leaves to control the quality of raw herbal material in producing a great quality of herbal finished products.

ACKNOWLEDGEMENT
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REFERENCES