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Effect of storage on phytochemical contents of *misai kucing* (*Orthosiphon stamineus* Benth) leaves

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Abstract

The effect of storage on the phytochemical contents of fresh and dried *misai kucing* leaves was studied. The fresh leaves were packed in perforated polyethylene bags and stored at 10 °C for 4 weeks. The leaves were removed from the cold room at weekly intervals, oven-dried and packed in sealed packages and stored for another 6 months at ambient condition. Analyses of the phytochemical changes were done on both fresh and dried leaves. Results indicated that long term storage of dried leaves for 6 months was found to enhance the amount of total phenolic and rosmarinic acid contents of *misai kucing* especially after they had been stored for 2 weeks at 10 °C prior to the long term storage. However, the amount of total flavonoid seemed to decrease when the dried leaves were subjected to prolonged storage. The total flavonoid content of fresh *misai kucing* leaves also rapidly declined during storage. Factors such as storage condition and storage period may affect the phytochemical content of *misai kucing*.

Keywords: Lamiaceae, total phenolic, total flavonoid, rosmarinic acid

Introduction

Misai kucing, the local name for *Orthosiphon stamineus* Benth, is a potential herb with health benefits as it contains high phytochemicals including the phenolic group such as rosmarinic acid (Tezuka et al. 2000; Olah et al. 2004; Akowuah et al. 2004), antioxidants (Akowuah et al. 2005; Ho et al. 2010) and total flavonoids (Yit at al. 2005; Amzad and Mizanur 2013). These phytochemicals may help to prevent diseases such as high fever, abdominal pain, gout, hypertension, hepatitis, jaundice and diabetes (Wiart 2002; Chin et al. 2008). It is also widely applied to treat bladder and kidney diseases (Hegnauer 1966; Maheswari and Venkatnarayanan 2013).

History of postharvest handling and primary processing such as condition of storage and the drying process may have a significant effect on the preservation of phytochemicals in processed herbs (Mrkic et al. 2006). Storage studies on a few leafy herbs have been reported. Clement and Sankat (1996) revealed that storage at 10 °C in LDPE packaging was effective in extending the shelf life of *Eryngium foetidum* L. for 2 weeks as compared to only 4 days under the traditional (ambient) handling. Negi and Roy (2001) also reported that storage of dehydrated green vegetable of sovoy beet (*Beta vulgaris* var *bengalensis*, cv. *Pusa Jyoti*) and amaranth (*Amaranthus tricolor* cv. *Pusa Kiran*) at low

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temperature effectively reduced degradation of phytochemical (β-carotene, ascorbic acid and chlorophyll) and helped in reducing browning. Shiow (2012) also showed that the level of phytochemical contents may increase or decrease depending on the storage environment.

Besides storage at low temperatures, drying process is also one of the critical activities in postharvest handling of herbs as it helps to prolong their storage life before further processing into products. Drying also helps to constrain microbial growth and inhibits enzymatic degradation as well as maintains the quality of the harvested plant. Total phenolic and flavonoid contents were higher in *misai kucing* when dried in oven compared to drying in barn, solar and low humidity dryers. Hossain et al. (2010) reported that drying has been found to be a useful technique for increasing the amount of phenolic content and antioxidant capacity in the extracts of six Lamiaceae herbs, and in fresh and dried rosemary, oregano, marjoram, sage, basil and thyme.

Very few studies had been conducted on the storage of *misai kucing* leaves and information on the phytochemical changes during storage is still lacking. Therefore, this study was conducted to determine the phytochemical changes of fresh and dried *misai kucing* leaves during storage.

Materials and methods

Leaves of *misai kucing* at commercial maturity (about 8 – 9 weeks after planting) were taken from a commercial farm in Melaka. The leaves were washed, tossed to remove excess water and packed in perforated polyethylene (PE) bags with each bag containing 450 g leaves. The fresh leaves were then stored for 4 weeks at 10 °C with three replications. Samples were withdrawn from cold storage at weekly interval and were subjected to oven drying at 50 °C. The dried leaves were then vacuum-packed in nylon bags and further stored at ambient temperature for another 6 months. The dried samples were analysed for phytochemical contents immediately after drying (fresh) and after 6 months storage at ambient.

About 5 g of dried leaves was ground into powder with a grinder (IKA MF10 micro fine grinder, UK) and mixed with 150 ml of 70% methanol. The mixture was continuously shaken for 24 h at room temperature using an orbital shaker (Protech Model 720, Malaysia). The extracts were filtered under suction and evaporated using a rotary evaporator (Buchi R-215, Switzerland). The extract was dissolved and diluted to a final volume of 10 ml with 70% methanol and stored at 4 °C for further analysis.

The total phenolic content of the extracts was determined using the Folin-Ciocalteu assay (Singleton et al. 1999). Samples of extract (500 µl) were mixed with 8 ml deionised water, followed by 500 µl of Folin-Ciocalteu reagent and (1 ml) 200 g/litre sodium carbonate. The tubes were allowed to stand for 5 min before absorbance at 750 nm was measured using UV visible spectrophotometer (UV 1601 Shimadzu, Japan). The calibration equation for gallic acid was $y = 0.005x + 0.014$ ($R^2 = 0.999$) where y is the absorbance and x is concentration of gallic acid. Total phenolic content was expressed as mg gallic acid equivalent (GAE)/100 g.

Total flavonoid content was determined by colorimetric method (Zhishen et al. 1999; Zou et al. 2004). Briefly, 1 ml of the extract was diluted with 4 ml deionised water followed by the addition of 0.3 ml of 5% sodium nitric solution. The mixture was allowed to stand for 5 min and 0.3 ml of 10% aluminium chloride solution was added. The mixture was allowed to stand for another 1 min before being added with 2 ml of 1 M sodium hydroxide solution. The mixture was made up to 10 ml with deionised water. After being vortexed for 5 s, the absorbance was measured at 510 nm using UV spectrometer (UV 1601 Shimadzu, Japan). The calibration equation for catechin was $y = 0.0032x + 0.00093$ ($R^2 = 0.9991$).

The results were expressed as mg catechin equivalent per 100 g of sample dry weight (mg catechin/100 g DW).

To determine rosmarinic acid, extracts (1 ml) were filtered through a 0.45 µm membrane filter prior to HPLC analysis for identification and quantification of markers in the sample extract. Mobile phase A with 0.1% trifluoroacetic acid (TFA) in water and mobile phase B with 0.1% TFA in acetonitrile were used to elute sample at 1 ml/min. The temperature was maintained at 25 °C, with injection volume of 20 µl and flow rate of 1 ml/min. The results are reported as mg/ml rosmarinic acid of dry leaf weight. HPLC (Agilent Technologies Series 1100 system) analysis was performed using an automatic injector, a column oven and UV detector. An Agilent Zorbax RX-C18 column (250 mm x 4.6 mm, 5 µm particle size) was used. Elution was monitored at 330 nm.

Statistical analysis

All the data were statistically analysed using Statistical Analysis System (SAS) software version 9. Least significant difference (LSD) test was employed to determine significant differences (p <0.05) among storage duration.

Results

Effect storage on fresh leaves

The fresh *misai kucing* leaves were stored in 10 °C for 4 weeks and changes in total phenolic, total flavonoid and rosmarinic acid were recorded (*Table 1*). For overall, there were significant changes in total phenolic, total flavonoid and rosmarinic acid during cold storage of fresh *misai kucing* leaves within 4 weeks duration.

Total phenolic was maintained during the first 2 weeks of storage, but the content was significantly reduced on the following weeks. Although, the reduction of total phenolic content from 807 mg GAE/100 g in removal, storage to 704 mg GAE/100 g at 4 weeks storage was considered small.

The total flavonoid had the same trend observed in storage condition. The flavonoid was in average 147 mg catechin/100 g at harvest time and decreased to 22 mg catechin/100 g (80% reduction) at the end of storage period. Meanwhile, the amount of rosmarinic acid increased from the beginning of the experiment (720.2 mg/100 g) until 2 weeks storage period (1741.1 mg/100 g). However, the acid slightly decreased to 1166 mg/100 g after 3 weeks, and reduced further to 322.2 mg/100 g after 4 weeks.

Table 1. Changes in percentage yield of total phenolic, total flavonoid and rosmarinic acid contents of *misai kucing* leaves during fresh storage at 10 °C and after 6 months dried storage at ambient

	Total phenolic (mg GAE/100 g)	Total flavonoid (mg catechin/100 g)	Rosmarinic acid (mg/100 g)
Fresh storage (week)	*	**	**
0	807.9a	147.1a	720.2b
1	795.5a	115.2b	1845.7a
2	801.1a	54.9d	1741.1ab
3	734.2b	78.3c	1166.6c
4	704.6b	22.1e	322.2d
Dried storage (month)	*	*	*
0	605.7b	90.1a	1152.1b
6	931.6a	77.5b	1566.4a
Fresh storage x Dried storage	ns	*	*

*Significantly different at p <0.05; **Significantly different at p <0.001

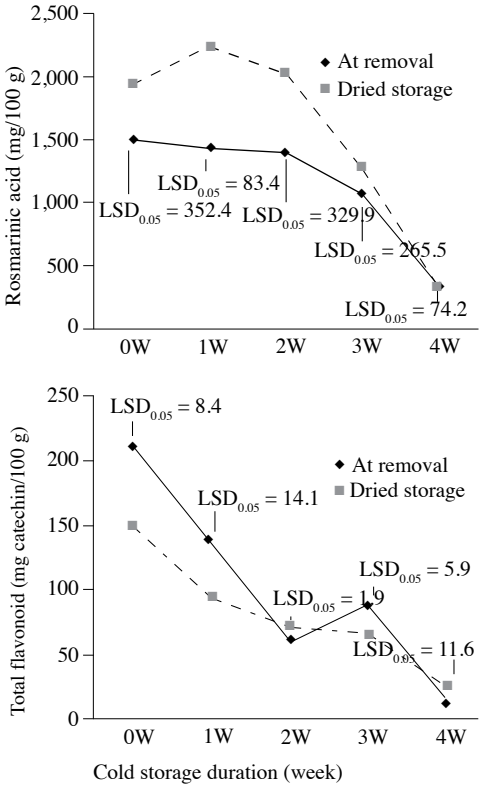

Figure 1. Changes in rosmarinic acid and total flavonoid contents in misai kucing leaves at removal (dried sample without storage) and dried storage (dried sample stored for 6 months)

Effect storage on dried leaves

The changes of phytochemical content were immediately analysed after 6 months of storage. There were significant changes with stored and unstored dried leaves. *Misai kucing* leaves stored for 6 months had higher content (931.6 mg GAE/100 g) of total phenolic compared to *misai kucing* leaves at initial (605.7 mg GAE/100 g). Rosmarinic acid showed the same trend (*Figure 1*) and did not show any degradation. In fact, the amount of rosmarinic acid increased by 26% during storage from 1152.1 mg/100 g at initial. However, the amount of total flavonoid (*Figure 1*) significantly decreased from 90.1 to 77.5 mg catechin/100 g.

Discussion

Total phenolic was not stable in fresh form during storage and declined with time. The reduction of total phenolic corresponds to decrease in the total flavonoid of the produce. Our result suggests that fresh *misai kucing* can be stored at 10 °C within 2 weeks without affecting the benefit of rosmarinic acid. Storage at 10 °C for more than 2 weeks significantly reduced the rosmarinic acid both in the fresh and dried forms. These results indicated that rosmarinic acid in *misai kucing* leaves stored at 10 °C for 2 weeks could be increased by 28 – 57% when these leaves were further stored in dried form at ambient condition. Therefore, storage of fresh *misai kucing* leaves at low temperature followed by drying and prolonged storage at ambient is very important to preserve or increase the rosmarinic acid content. This phytochemical possesses the antioxidant, anti-inflammatory and also antimicrobial activities (Hassan et al. 2012).

This finding suggests that prolonged storage of *misai kucing* in dried form at ambient condition could enhance the total phenolic content although after the leaves were subjected to fresh storage for several weeks at low temperature. Similar outcomes reported by Mulokozi and Svanberg (2003) convinced that leafy vegetables kept in dried form can retain more phytochemicals (pro-vitamin A carotenoids). These results confirmed the documentation of Kevers et al. (2007) and also Jasenka et al. (2009) that determined the total phenolic content of many fruits and vegetables remained stable and increased during storage. At longer storage time, the total flavonoid contents in *misai kucing* leaves both with and without prolonging dried storage did not differ significantly. According to Mohd Zainal et al. (2009), the loss of flavonoid in leafy herbs such as *Centella asiatica* occurred due to drying. Schieber et al. (2001) also reported that losses of phytochemical content such as flavonoid during heat treatment might be due to the harsh drying

conditions, in particular, the temperature and duration of heat exposure. Prolonged storage of *misai kucing* for 6 months in the dried form significantly increased the rosmarinic acid content.

Conclusion

The phytochemicals in *misai kucing* leaves such as total phenolic, total flavonoid, rosmarinic acid content and the percentage of crude extract were significantly different during fresh storage at 10 °C and after 6 months of storage at ambient temperature. An interesting finding was that after 6 months storage in the dried form at ambient conditions, total phenolic and rosmarinic acid contents were higher, especially after the herb had been stored for 2 weeks at 10 °C in the fresh form. These results provided an advantage to the herbal producers as they can delay the processing of *misai kucing* after harvest without losing total phenolic and rosmarinic acid values. But if the product requires high total flavonoid content, storing the leaves in fresh or dried form is not recommended.

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Abstrak

Kesan penyimpanan pada kandungan fitokimia daun misai kucing segar dan kering telah dikaji. Daun segar telah dibungkus dalam polietilena beg (PE) dan disimpan pada suhu 10 °C selama 4 minggu. Daun dikeluarkan dari bilik sejuk setiap minggu, dikeringkan di dalam ketuhar dan dibungkus dalam bungkusan yang divakum dan disimpan selama 6 bulan pada keadaan ambien. Analisis terhadap perubahan fitokimia telah dijalankan ke atas kedua-dua daun segar dan kering. Keputusan menunjukkan bahawa simpanan jangka panjang daun kering misai kucing selama 6 bulan meningkatkan jumlah fenolik dan asid rosmarinic terutamanya selepas disimpan selama 2 minggu pada 10 °C sebelum penyimpanan jangka masa panjang. Walau bagaimanapun, jumlah flavonoid berkurangan apabila daun kering disimpan dalam penyimpanan berpanjangan. Jumlah kandungan flavonoid daun segar misai kucing juga cepat merosot semasa penyimpanan. Data daripada kajian ini jelas menunjukkan bahawa faktor seperti keadaan penyimpanan dan tempoh simpanan boleh mempengaruhi kandungan fitokimia daun misai kucing.