2011 International Conference on Food Engineering and Biotechnology
IPCBEE vol.9 (2011) © (2011)IACSIT Press, Singapore

Drying characteristics and herbal metabolites composition of misai kucing (*Orthosiphon staminiues Benth.*) leaves

Sriyana Abdullah[1], Muhammad Syarhabil Ahmad[2], Abdul Razak Shaari[3], Hafizah Mohd Johar[4], Nor Fashihah Mohd Noor[5]

[1-4]School of Bioprocess Engineering, University Malaysia Perlis, 02600 Arau Perlis, Malaysia

[5]Institute of Engineering Mathematics, Universiti Malaysia Perlis, Malaysia

Abstract. In this research the drying characteristics and relationship between drying temperature and marker compounds constituent of misai kucing (*Orthosiphon staminiues Benth.*) leaves were investigated. The leaves of misai kucing herbal plant were dried by oven method at different temperatures: 40°C, 55°C and 70°C. Drying at higher temperature shortened the drying time and increased the drying rate. Initial moisture content of the leaves was 77.00% (w.b). The drying process was done until the equilibrium moisture content was achieved. The total antioxidant activity of the dried leaves extract increased with the oven temperatures. The total phenolic content (TPC) in the extract was not significantly affected by the oven temperatures ($P>0.05$). The high performance liquid chromatography (HPLC) analysis for main marker compounds concentration which sinensetin (SEN) and rosmarinic acid (RA) were increased and decreased with the temperatures, respectively.

Keywords: misai kucing; oven method; drying characteristics; phenolic content; marker compounds.

1. Introduction

Misai Kucing plant (*Orthosiphon staminues Benth*) is a herbal species which indigenous to South-East Asia regions. This herbal plant is a genus in the family of *Lamiacea.* The plant is grows to a height of 1.5m and can be harvested in 3-4 months after propagating its stem cuttings. It was being a popular herbal tea at beginning of 20th century when it was introduced to Europe [1]. Misai kucing plant is believed to have antiallergic, antihypertensive, anti-inflammatory, antioxidant [2, 3], and diuretic properties [4] . The medicinal properties of this plant species have been proofed in the literature by numbers of active compound isolated and identified from the extract. The most important group of the compounds is the phenolic group such as caffiec acid derivatives, lipophilic flavones, flavonol glycosidase and polymethoxylated flavones [2]. Other chemical consitituents isolated from *O. Stamineus* are norstaminane- and isopimarane-type diterpenes [5]. Four main marker compound in *O. Stamineus* extract are sinensetin (SEN), eupatorin (EUP), and 3-hydroxy-5,6,7,4-tetramethoxyflavone (TMF). Nowadays misai kucing is being commercialized as herbal food supplement such as herbal tea.

The production of misai kucing food supplement starts with a drying process as initial treatment after harvesting process is done and before any other processing. As the drying aspect plays an important role to ensure a high quality product, the study of its characteristics needs to be explored to prevent over-drying and thus it can decrease drying time, energy cost, mass losses and the risk of quality deterioration. Drying characteristics of dill [6], parsley [6], coriander [7], mint [8], rosemary [9] and olive[10] leaves have been studied by several researchers. These works also included study of the effects of different drying methods on the quality of the dried herbs. Herbs quality is being referred to its colour, antioxidant content, total phenolic content, and mineral content. Some researchers also referred the dried herb quality to the specific bioactive compounds constituent. Most of them were used HPLC to determined the concentration of known bioactive

compounds. However there was no literature for drying of misai kucing plant. Thus this attempt to study the drying behaviour of misai kucing leaves will assist researchers to do so for other local potential herbs especially in Malaysia. The objectives of this research were to determine the drying characteristics of misai kucing leaves by oven at different temperatures and to determine the relationship of oven drying at different temperatures on marker compounds constituent of misai kucing leaves.

2. Materials and Methods

2.1. Drying Experiment

The misai kucing plant which was grown at UniMAP Agrotechnology Research Station, Sg. Chucuh, Perlis was randomly harvested in the early morning and was kept in a fridge within 24 hours before processing. The initial moisture content of the leaves was determined by using Sartorious Moisture Analyzer. The samples of 20 g misai kucing leaves were dried by using an oven. Three temperatures were chosen that were 40^{o}C, 55^{o}C and 70^{o}C to obtain the drying characteristics of misai kucing leaves. The sample was dried until the equilibrium moisture content was achieved. At the temperature of 40^{o}C, the samples were weighed every hour to obtain the moisture content. An interval of 15 minutes was chosen for drying temperatures of 55^{o}C and 70^{o}C. The data were recorded and the experiment was repeated three times for each temperature.

The drying rate during drying was calculated by using this equation:

$$\text{Drying rate} = \frac{M_{t+dt} - M_t}{dt} \qquad (1)$$

Where M_t and M_{t+dt} are moisture content at time, t and moisture content at time, t+dt (kg moisture/kg wet matter), respectively.

2.2. Extraction Process

Metabolites from dried misai kucing leaves were extracted by using Sochlet extraction and water was used as a solvent. 5 g of dried misai kucing leaves were extracted for three hours. The oxygen free extracts were frozen at -20^{o}C in a tight plastic bottle for further analysis.

2.3. Total phenolic content (TPC) determination

The concentration of total phenolic compounds in the extracts were determined by using Follin-Ciocalteu (FC) reagent and external calibration curve with gallic acid [11]. 0.2 ml extract was added to 0.2 ml FC reagent and1.58 ml water and the mixture was mixed thoroughly. After 4 min, 1 ml of 20% $CaCo_3$ was added, and the mixture was allowed to stand for two hours at room temperature. The absorbance value was measured at 760nm by using a Shimadzu spectrophotometer. The data was compared with the gallic acid external calibration curve to give the concentration of total phenolic compounds in the extracts.

2.4. Total antioxidant activity determination

Total antioxidant activity was determined by using free radical scavenging activity (FRSA) method [12]. Two ml of the methanolic solution of 1,1-diphenyl-2- picrylhydrazyl (DPPH) was mixed with 200 µl water extract of misai kucing leaves and was added with methanol to make a final volume of 3 ml. The mixtures were made to stand for 60 min, the absorbance value was measured against methanol as a blank at 517nm by using the spectrophotometer. The free radical-scavenging activities (%) of the tested samples were compared with a control (2 ml DPPH solution and 1 ml methanol). The free radical-scavenging activity was measured by using this formula:

$$FRSA = \left[\frac{(A_C - A_S)}{A_C} \right] \times 100 \qquad (2)$$

Where, A_c is absorbance value for control and A_s is absorbance value for sample .

2.5. HPLC Analysis

1.5 ml of of misai kucing extract was filtered through a 0.45µm nylon membrane filter into a HPLC vial prior to HPLC analysis. A Shimadzu HPLC was utilized to perform the analysis which was equipped with autosampler, column oven and UV/VIS detector. A HPLC column used was Merck Licrochart Purospher Start RP 18 column (250mm, 4.6 mm i.d, 5µm pore size). The mobile phase used was a mixture of water: methanol: tetrahydrofuran (50: 45: 5 v/v) [3]. Each sample was analysed at the mobile phase flow rate of 1 ml/min, detector wavelength of 340nm at 30°C for 40 min. For qualification and quantification purpose, calibration curve was made with standard marker compounds purchased from Chromadex for SEN and Sigma Aldrich for RA.

3. Result and Discussion

3.1. Drying Characteristics of misai kucing leaves

Figures 1-3 show the drying curves of misai kucing leaves at different oven temperatures.

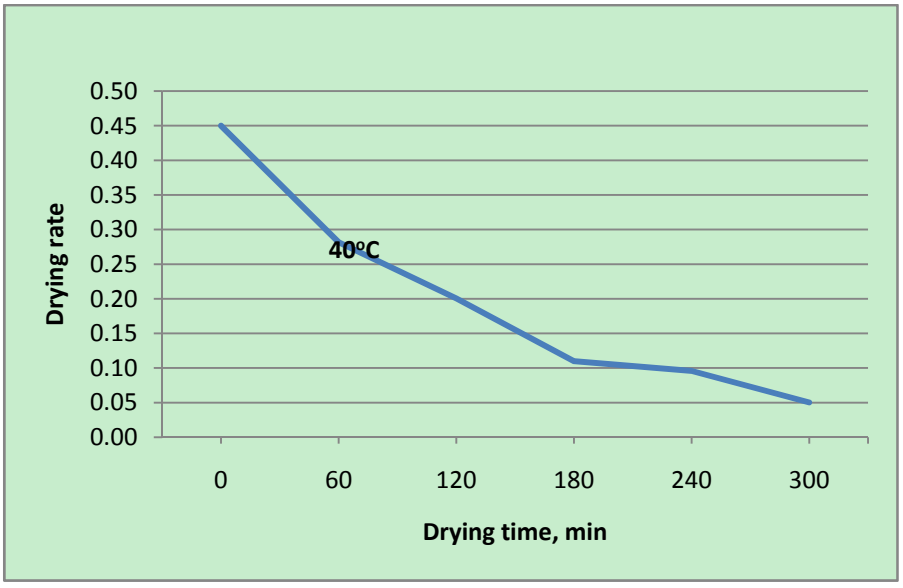

Fig. 1: Drying curve of misai kucing leaves at 40oC

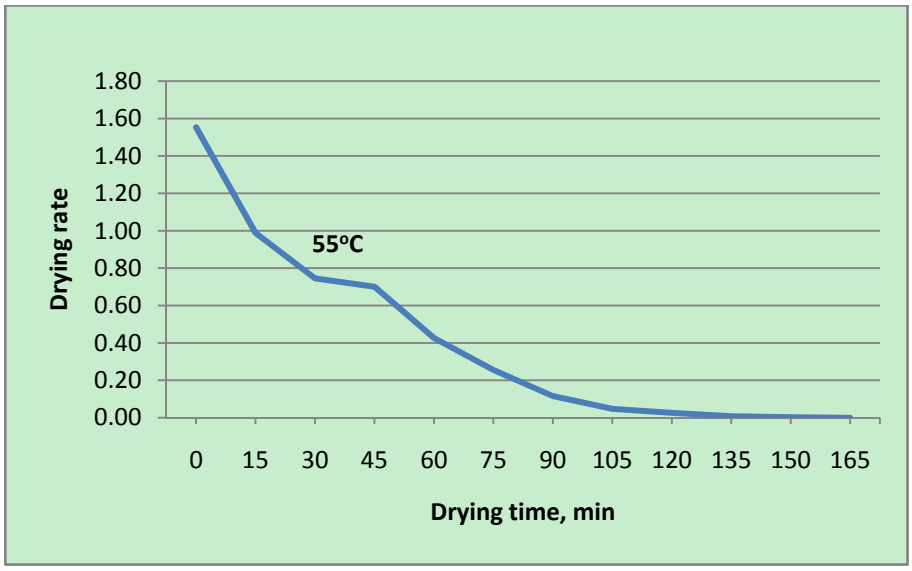

Fig. 2: Drying curve of misai kucing leaves at 55oC

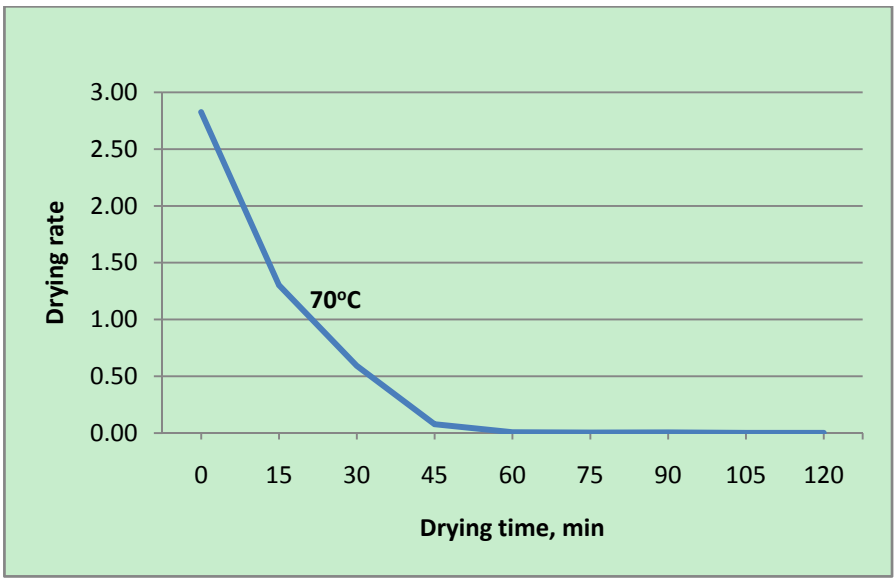

Fig. 3: Drying curve of misai kucing leaves at 70oC

Drying rate was calculated using Eq. (1) and the graphs were plotted. There is no constant rate period in the drying curves which clearly shows that the drying process took place at falling rate period. These results are in agreement with other drying experiments which done earlier [6, 7, 13]. The drying curves also show that the fastest drying rate is occurred when misai kucing leaves are dried at higher temperature. The leaves take only 2 hours to dry at 70°C and almost 3 hours for 55°C while, at 40°C the leaves took 6 hours to dry. The extracts of dried misai kucing leaves were underwent further experiments to evaluate the effects of the drying temperatures on the metabolites concentration of the extracts.

3.2. Metabolites analysis

The quality of dried misai kucing leaves after treatment is represented by its metabolites concentration. Results of the metabolites analysis are shown in Table 1.

Table 1: Metabolites analysis of dried misai kucing leaves extract

Temperatue (°C)	DPPH (%)	TPC (ppm)	Concentration (ppm)	
			RA	SEN
40	35.482ab	58.042	0.0155	1.0770a
55	39.714a	58.000	0.0166	0.7420b
70	50.456b	48.333	0.0655	0.3114c

[a]Means within column with different letters indicate significantly different values (P<0.05)

Metabolites analysis was done and shows no simple relationship between total antioxidant activity and total phenolic compounds to the drying temperatures. It is because antioxidant activity is not limited to phenolic compound only but the presence of other antioxidant components such as sugars and other compounds that function as hydrogen donor also affects the total antioxidant activity in the extracts [2]. The concentration of marker compounds relates to the total phenolic content in the extracts. However, the content of phenolic compounds in the extracts at the various drying temperatures are not significant (P>0.05) because RA concentration and SEN concentration increases and decreases as temperature increases, respectively. HPLC analysis has to be made for more phenolic compounds so that the TPC may give a significant value. The RA concentration increases when the temperature increases which complies with the total antioxidant capacity. RA is an antioxidant because of the presents of phenolic hydroxyl group in its structure. RA concentration in misai kucing leaves shows the highest value when dried at 70°C and it is not

degraded during extraction process at high temperature. Consumption of misai kucing in terms of herbal tea will give good antioxidant benefit to the consumers. The concentration of SEN shows highest value at low drying temperature and significantly affects by the temperature ($P<0.05$).

4. Conclusion

Misai kucing leaves were oven dried at different temperatures in order to study the drying trend by plotting the drying curves. Drying at higher temperature shortened the drying time as shown in Figs 1-3. The drying curves show that the drying process took place at falling rate period. The drying temperatures also affect the metabolites concentration of the dried leaves extracts. But no simple relationship is obtained to represent the effects of the drying temperatures to the metabolites concentration since RA concentration and SEN concentration increases and decreases as temperature increases, respectively.

5. Acknowledgement

The study will be not done successfully if did not get a full support from the University Malaysia Perlis (UniMAP) especially to the School Of Bioprocess Engineering, UniMAP.

6. References

[1] Indu Bala Jaganath, N.L.T., Muthuvelu Chinna, Razali A. Rahman, ed. *Herbs The green Pharmacy of Malaysia*. 2000, Vinpress Sdn. Bhd.: Malaysia. 126.

[2] Akowuah, G.A., et al., Sinensetin, eupatorin, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone and rosmarinic acid contents and antioxidative effect of Orthosiphon stamineus from Malaysia. *Food Chemistry*, 2004. **87**(4): p. 559-566.

[3] Akowuah, G.A., et al., The effects of different extraction solvents of varying polarities on polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging activity. *Food Chemistry*, 2005. **93**(2): p. 311-317.

[4] Adam, Y., et al., Diuretic properties of Orthosiphon stamineus Benth. *Journal of Ethnopharmacology*, 2009. 124(1): p. 154-158.

[5] Awale, S., et al., Norstaminane- and isopimarane-type diterpenes of Orthosiphon stamineus from Okinawa. Tetrahedron, 2002. **58**(27): p. 5503-5512.

[6] Doymaz, I., N. Tugrul, and M. Pala, Drying characteristics of dill and parsley leaves. *Journal of Food* Engineering, 2006. **77**(3): p. 559-565.

[7] Ahmed, J., U.S. Shivhare, and G. Singh, Drying Characteristics and Product Quality of Coriander Leaves. Food *and Bioproducts Processing*, 2001. **79**(2): p. 103-106.

[8] Jin Park, K., Z. Vohnikova, and F. Pedro Reis Brod, Evaluation of drying parameters and desorption isotherms of garden mint leaves (Mentha crispa L.). *Journal of Food Engineering*, 2002. **51**(3): p. 193-199.

[9] Arslan, D. and M. Musa Özcan, Evaluation of drying methods with respect to drying kinetics, mineral content and colour characteristics of rosemary leaves. *Energy Conversion and Management*, 2008. **49**(5): p. 1258-1264.

[10] Erbay, Z. and F. Icier, Optimization of hot air drying of olive leaves using response surface methodology. *Journal of Food Engineering*, 2009. **91**(4): p. 533-541.

[11] Chan, E.W.C., et al., Effects of different drying methods on the antioxidant properties of leaves and tea of ginger species. *Food Chemistry*, 2009. **113**(1): p. 166-172.

[12] Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. , Two new flavonoids and other constituents in licorice root their *relative* astringency and radical scavenging effect. *Chemistry and Pharmacology Bulletin*. **36**: p. 1090-1097.

[13] Kavak Akpinar, E., Y. Bicer, and F. Cetinkaya, Modelling of thin layer drying of parsley leaves in a convective dryer and under open sun. *Journal of Food Engineering*, 2006. **75**(3): p. 308-315.