

PHYSICOCHEMICAL PROPERTIES OF DRIED GUIZHOU AS AFFECTED BY PRETREATMENTS, DRYING TECHNIQUES AND STORAGE PERIOD

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Abstract: The effects of pretreatment methods (hot-water blanching, steam blanching and acetic acid soaking) and drying techniques (hot-air drying, vacuum drying and freeze drying) on the physical properties and antioxidant activities of dried Guizhou are investigated in this study. Results show that blanching improved the drying rate, and was faster than untreated and acetic acid treated samples. Vacuum drying resulted in slower drying than hot air. Samples subject to hot-air drying and vacuum drying exhibited more shrinkage than those subjected to freeze drying. Dried-blanching samples had greener and darker color than acetic acid pretreated and untreated dried samples. Freeze-dried samples had the highest total phenolic compounds and antioxidant activity determined by DPPH assay. Moisture content, water activity, color change (ΔE) of hot air dried, vacuum-dried and freeze-dried samples gradually increased throughout the 6-month storage period, while total phenolic compounds and DPPH radical scavenging activity showed a decreasing trend. Aluminum foil provided better protection against moisture uptake by dried samples than polypropylene containers. Freeze-dried samples kept in aluminum foil at room temperature gave the longest shelf life. Results suggested that pretreatment by blanching, suitable drying methods and aluminum packaging improved physical properties and protected phenolic acid content of dried Guizhou.

Keywords: Pretreatment, Guizhou, drying method, antioxidant, shelf life.

Introduction

Guizhou (*Artemisia lactiflora*) is known as mugwort or Jing-ju-chai in Thailand. The leaves are used as a traditional Chinese medicine to treat menstrual disorders and also have anti-cancer and anti-oxidative properties. Kulprachakarn *et al.* (2018) reported antioxidant activity of Guizhou at 27.20 ± 1.06 mg TEAC/g of dry extract, with total phenolic content of 213.33 ± 117.80 mg GAE/g of dry extract. However, utilization of fresh Guizhou is restricted because it is a perishable plant. Drying is therefore proposed to extend its storage life.

Drying is a popular post harvest process to reduce water content. Reduction of water (a_w) to a low level, whereby growth of microorganisms, enzymatic, and other deteriorative reactions are inhibited, prolongs the shelf life of the products.

Many studies reported significant color changes caused by the browning reaction (Yanyang *et al.*, 2004; Arabhosseini *et al.*, 2007). Different drying methods operate under diverse conditions. For example, in vacuum drying and freeze drying, the drying chamber operates at reduced pressure as an oxygen deficient environment. Quality of vacuum dried and freeze dried products tends to be better than those subjected to conventional hot-air drying.

Normally, pretreatment is applied before drying to reduce contamination by microorganisms (Phungamngoen *et al.*, 2013) and improve the physicochemical properties of the product (Farooq *et al.*, 2020). There are many methods for pretreating fruits and vegetables. Chiewchan & Morakotjinda (2009) reported that soaking sliced cabbage in acetic acid (0.5-

1.5% v/v) reduced the number of *Salmonella anatum* on the cabbage surface. Hot water and steam treatment are widely used to improve product color (Phungamngoen *et al.*, 2013). Hot water and steam are the simplest methods of heat treatment, with fast energy transfer as latent heat to bacterial and fungal spores on the surface of fruits and vegetables.

Many papers have been published concerning the anti-oxidant and medical properties of Guizhou but information detailing the combined effects of pretreatment and drying methods on the quality of dried Guizhou is lacking. Thus, here, the effects of pretreatment and drying techniques on physicochemical properties of dried Guizhou were investigated. Different packaging during storage on physicochemical properties of the dried product was also assessed.

Materials and Methods

Pretreatments

Fresh Guizhou was purchased from an organic farmer in Prachinburi, Thailand. The edible leaves of Guizhou were washed with tap water for 2 minutes. Guizhou leaves were pretreated by three methods, including soaking in acid, blanching and steaming.

Acid pretreatment involves soaking the samples in acetic acid solution at 0.5% v/v (glacial acetic acid, J.T. Baker, Phillipsburgh, USA). The Guizhou leaves to acid solution ratio was 1:10 (w/v). The mixture was shaken in a rotary shaker at 120 rpm for 5 minutes and at 30°C.

The blanching conditions were pre-determined to inhibit peroxidase. For hot water blanching pretreatment, the sample to boiling water ratio was 1:10. The samples were blanched in boiling water for 4 minutes during which the enzyme peroxidase activity was not detected. Steam blanching pretreatment was followed by a water bath (Memmert, Model WNB22, Germany). A single layer of the Guizhou leaves sample was steamed by placing it on a perforated tray suspended over hot water at 97 °C for 2

minutes. After blanching the samples were immediately cooled in a water bath at 4-5°C. Fresh Guizhou leaves were used as control.

Drying Methods

Approximately 300g of pretreated samples were placed on a 50 × 70 cm tray and spread in a single layer. Samples were dried at 60°C with an average air flow velocity of 1.6 m/s in a hot air dryer (Kluaynamthai, 12Tray, Thailand). Samples were taken at regular intervals to measure moisture content using a standard gravimetric method (AOAC, 2000). Drying time required to obtain the products with the final moisture content of less than 8% (w.b.).

Vacuum drying was conducted following the same procedure using different equipment (Memmert, Model VO500, Germany). All experiments were performed under pressure of 10 mbar, with drying temperatures at 60°C. Freeze drying was conducted under pressure of 70 Pa for 24 hours. Before drying, the samples were frozen at -40°C for 20 hours and then dried using a ScanVac Model CoolSafe 4-15L, Germany. All experiments were performed in triplicate.

Storage Period

To evaluate the physicochemical properties, 10g of dried samples were kept at room temperature (~ 30°C) for 6 months with two types of packaging – aluminum foil and polypropylene containers.

Water Activity Determination

3-5g of dried samples were placed in quartz cuvette and water activity (a_w) was measured using a Water Activity Meter (AquaLab Model Series 3TE, Washington, USA) at 25°C after calibration with saturated salt solutions ranging between 0.33 and 0.85.

Color Measurement

The samples were placed in cuvette and then placed in a Hunter Colorimeter (Hunter Lab, Model Colorflex 45/0, Virginia). Reflection

spectra were registered and Hunter Lab color parameters for 10° vision angle and D65 illuminant (L : brightness, a : redness, b : yellowness) were calculated. The color of dried samples were compared with the color value of fresh Guizhou leaves. Total color change (ΔE) was also calculated as:

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (1)$$

$$\Delta L = L - L_0, \quad \Delta a = a - a_0 \quad \text{and} \quad \Delta b = b - b_0$$

where L_0 , a_0 , b_0 are the color values of fresh Guizhou leaves.

Total Phenolic Content

Guizhou tea was prepared for measuring the total phenolic content (TPC). Dried samples (2g) were steeped in 200mL hot water (approximately 97 °C) for 5 min. Total phenolic content of dried Guizhou extracts was determined by the Folin-Ciocalteu method, performed following the method of Johari & Khong (2019). Briefly, 100 μ L of the extract (1 mg/mL) was mixed thoroughly with 0.75mL of Folin-Ciocalteu reagent for 5 minutes, followed by adding of 0.75 mL of sodium carbonate (Na_2CO_3). The test tube was kept in the dark for 30 minutes. The absorbance of the mixture was measured using a UV-Vis Spectrophotometer (Biochrom, Model Libra s12, USA) at 750nm. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight (mg GAE/g).

DPPH Radical Scavenging Assay

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay as previously described by Johari & Khong (2019) with some modifications. About 0.1mM solution of DPPH in ethanol was prepared and 1mL of DPPH solution was added to 3mL of the sample with different concentrations. The mixture was shaken and kept in the dark for 30 minutes at room temperature. Then, the absorbance was measured at 517nm using the UV-Vis

spectrophotometer. The calculation for the percentage of inhibition of the DPPH radical is:

$$\%inhibition = \frac{(A_0 - A_s)}{A_0} \times 100\% \quad (2)$$

where A_0 represents absorbance value of the control reaction (contains all reagents except the sample), and A_s represents absorbance value of the sample.

Experimental Design and Statistical Analysis

The first part of the experiments were conducted at three levels of the pretreatment methods (acetic acid soaking, hot water blanching and steam blanching) and three levels of the drying methods (hot air drying, vacuum drying and freeze drying). The second part was conducted at two levels of the packaging (aluminum foil and polypropylene). A completely randomized design (CRD) was used to schedule the experiments. Differences between mean values were established using Tukey's multiple range tests at a confidence level of 95%.

Results and Discussion

Effect of Pretreatments

Table 1 shows the effect of pretreatments on moisture content and water activity of the samples. Pretreated samples showed no significant difference in water activity (0.979-0.994). Initial moisture content of the untreated sample was 17.26 ± 1.01 g/g (dry basis). Water blanching and steam blanching pretreatment led to higher moisture content than soaking in 0.5% acetic acid pretreatment and the untreated sample because disruption of plant cells allowed the uptake of cool water after blanching (Gonzalez-Fesler, 2008), consistent with the findings of Phungamngoen et al. (2013).

Combined Effect of Pretreatments and Drying Methods

Effects of pretreatment and drying methods on drying time, moisture content and water activity (a_w) of dried Guizhou are presented in Table 2. Hot air drying and vacuum drying

Table 1: Moisture content and water activity (a_w) of pretreated dried Guizhou.

Pretreatment	Moisture content (g/g dry basis)	a_w
Untreated	17.26±1.01 ^b	0.979±0.006 ^b
Water blanching	20.07±2.41 ^{abc}	0.994±0.001 ^a
Steam blanching	19.74±0.67 ^a	0.990±0.003 ^a
Acid pretreatment	17.65±2.77 ^{ab}	0.990±0.004 ^a

Mean values in the same column with different superscripts are significantly different ($P < 0.05$).

Table 2: Drying time, moisture content and water activity (a_w) of dried Guizhou with different pretreatments and drying methods.

Pretreatment	Drying method	Drying time (h)	Moisture content (%d.b.)	a_w
Untreated	Hot air drying	2.40±0.08 ^c	8.04±0.01 ^a	0.429±0.010 ^a
Water blanching		2.00±0.08 ^d	7.93±0.03 ^a	0.268±0.001 ^e
Steam blanching		2.05±0.20 ^d	7.92±0.20 ^a	0.301±0.003 ^d
Acid pretreatment		2.55±0.05 ^c	7.71±0.21 ^{ab}	0.301±0.003 ^d
Untreated	Vacuum drying	8.20±0.16 ^a	8.01±0.01 ^a	0.395±0.009 ^b
Water blanching		7.20±0.08 ^b	7.96±0.04 ^a	0.399±0.011 ^{ab}
Steam blanching		7.00±0.08 ^b	7.80±0.14 ^a	0.369±0.005 ^e
Acid pretreatment		7.90±0.08 ^a	7.94±0.02 ^a	0.423±0.004 ^a
Untreated	Freeze drying	Overnight	7.16±0.11 ^c	0.320±0.060 ^{cd}
Water blanching			7.02±0.07 ^c	0.280±0.017 ^{de}
Steam blanching			7.03±0.06 ^c	0.267±0.015 ^e
Acid pretreatment			7.14±0.23 ^c	0.307±0.021 ^{de}

Mean values in the same column with different superscripts are significantly different ($P < 0.05$).

exhibited similar behavior for all samples (data not shown). Moisture content decreased continuously as drying time increased. Drying time of the vacuum-dried sample was longer than the hot-air dried sample since velocity flow in the hot-air dryer was approximately 2 m/s, while there was no air movement in the vacuum dryer with low mass transfer rate. In the case of freeze drying, the sample was pre frozen overnight before the drying process.

Results showed that blanched samples dried faster than unblanched samples due to structure softening during blanching that accelerated water removal. When plant tissues are blanched or cooked, the cells become more permeable to moisture. Similar observations were reported

for drying vegetable leaves (Lopez *et al.*, 2000; Phungamngoen *et al.*, 2013). Final moisture content and a_w of freeze dried samples were lower than hot-air dried and vacuum-dried samples. According to the Thai Community Product Standard No. 120 (2016), green tea should have a moisture content at less than 8%. Results showed that moisture content of the dried products were all below this standard, with a_w values ranging 0.267 to 0.429.

Dried samples obtained from the different pretreatments using hot-air drying at 60°C are shown in Figure 1. Blanched leaves exhibited more shrinkage than acid pretreated and untreated samples because thermal processing inhibited the enzymatic browning reaction

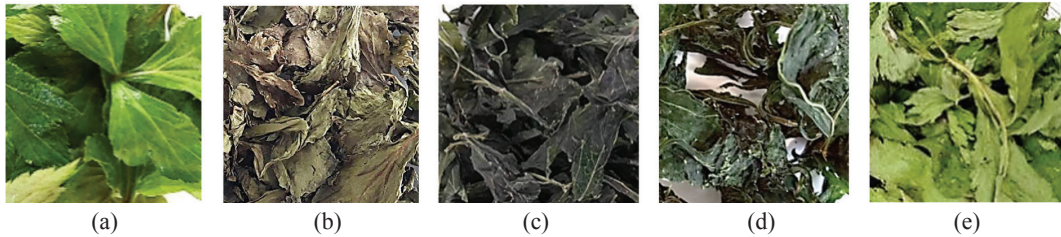


Figure 1: Dried Guizhou with different pretreatments, (a) fresh Guizhou, (b) untreated, (c) water blanching, (d) steam blanching, and (e) soaking in 0.5% acetic acid using hot air drying at 60 °C.

and reduced the drying time due to tissue wall softening (Obob, 2005). The hot water and steam blanched products showed greater shrinkage. However, the applied pretreatment combined with drying methods did not show effect on the shrinkage of the dried Guizhou.

Table 3 shows the color values of dried samples at final moisture content. Untreated samples had a redder color than acid pretreated and blanched samples, respectively. This was due to enzymatic browning reactions that were activated during the drying process for non-thermal treatment methods, as shown in the case of untreated and acid pretreated samples. Blanched samples became darker and greener with slight loss of yellowness due to leaching of the soluble pigments during blanching

(Wolfe & Liu, 2003), while blanching inhibited enzymatic browning reactions leading to less red color. In addition, blanching pretreatment led to intercellular air trapped in the vegetable could obstruct the greenness of chlorophyll (Phungamngoen et al., 2013). Although vacuum drying and freeze drying took longer than hot-air drying, the lightness (*L* value) of vacuum-dried and freeze-dried samples showed a higher value than the hot-air dried sample. This occurred because the drying chambers of vacuum drying and freeze drying are operated at reduced pressure or vacuum in an oxygen-deficient environment. Removal of the air in the chamber during drying resulted in the strong green color of the dried Guizhou. The final moisture content of blanched samples exhibited higher total color change (ΔE) than acid pretreated samples.

Table 3: Effect of pretreatments and drying methods on color properties of dried Guizhou.

Pretreatment	Drying method	<i>L</i>	<i>a</i>	<i>b</i>	Total color change (ΔE)
Untreated		35.72±1.78 ^{cd}	-0.37±0.80 ^b	12.81±0.68 ^a	85.07±9.75 ^d
Water blanching	Hot air drying	28.80±1.23 ^e	-3.95±0.24 ^c	8.77±0.88 ^{bc}	116.35±18.32 ^{ab}
Steam blanching		28.69±0.60 ^e	-3.74±0.25 ^c	8.52±1.55 ^{bcd}	114.01±36.24 ^{abcd}
Acid pretreatment		34.26±1.58 ^d	-0.03±0.20 ^a	12.06±0.35 ^a	99.54±3.35 ^{bc}
Untreated	Vacuum drying	41.08±0.48 ^b	-3.29±0.43 ^c	8.03±0.71 ^d	95.72±0.70 ^c
Water blanching		29.44±0.97 ^e	-4.61±0.32 ^d	8.47±1.12 ^{bcd}	117.62±23.54 ^{ab}
Steam blanching		28.51±1.91 ^e	-4.28±0.31 ^{cd}	8.45±1.29 ^{bcd}	118.81±25.23 ^{ab}
Acid pretreatment		44.10±0.67 ^a	-3.83±0.13 ^c	8.49±0.33 ^b	102.35±0.57 ^b
Untreated	Freeze drying	38.76±0.63 ^{bc}	-3.51±0.11 ^c	8.08±0.44 ^{cd}	30.04±6.70 ^f
Water blanching		33.76±0.58 ^d	-4.43±1.85 ^d	7.88±0.42 ^d	30.34±0.82 ^f
Steam blanching		34.63±0.85 ^d	-3.92±0.31 ^{cd}	7.16±0.88 ^e	30.08±3.41 ^{ef}
Acid pretreatment		38.72±1.42 ^{bc}	-4.07±0.47 ^{cd}	8.92±0.75 ^b	30.38±14.75 ^{ef}

Mean values in the same column with different superscripts are significantly different (P<0.05).

This is probably because the removal of air in the drying chamber could preserve chlorophyll pigment, the ΔE of blanched samples was larger than the acid pretreated sample. However, hot water and steam blanching had little effect on the ΔE of dried Guizhou.

Total phenolic content and DPPH radical scavenging activity of dried Guizhou are indicated in Table 4. Results showed that steam blanching had higher TPC and DPPH than water blanching, soaking in 0.5% acetic acid and the untreated sample. Although blanching applied high heat to the sample, it reduced drying time to reach final moisture content and prevented quality loss during drying. High TPC gave high percentage inhibition of DPPH due to the linear correlation between the two parameters. Freeze drying under lower pressure then removing the ice by sublimation, is used to prolong the shelf life of samples. Freeze drying causes less damage to substances than other drying methods using higher temperature and retains antioxidant activity of the product. These results concurred with Farooq *et al.* (2020). Based on quality with high antioxidant activity of dried Guizhou, steam blanching using hot air drying, vacuum drying and freeze drying were selected as the three conditions to study the effect of packaging

during storage because they gave the highest TPC of each drying method.

Effect of Different Packaging during Storage

Figure 2 shows moisture content (A), water activity (B) and total color change (C) of dried Guizhou during storage. Moisture content of tea stored in aluminum foil and polypropylene (PP) increased as storage time went on. The changes in moisture content of dried Guizhou are caused by the relative humidity of the environment. When the relative humidity of the air was higher than the product, then the material could absorb water.

Aluminum foil and PP packaging showed no difference in moisture content during storage for 6 months. This is because the packaging is able to maintain water vapour within and outside the product, resulting in no water vapour transfer during storage. Results indicated that increasing moisture content was confirmed by the change of a_w during storage (Figure 1B).

In all cases, TPC and DPPH decreased with increasing storage period. For vacuum drying and freeze drying (Figure 3), the destruction of antioxidant parameters was more pronounced during the first period of storage due to a sudden

Table 4: Effect of pretreatments and drying methods on total phenolic content and DPPH of dried Guizhou.

Pretreatment	Drying method	Total phenolic content (mg GAE/g)	DPPH (% inhibition)
Untreated	Hot air drying	184.61±14.89 ⁱ	23.93±0.29 ^f
Water blanching		567.51±18.90 ^f	26.73±0.42 ^e
Steam blanching		593.43±18.47 ^{ef}	27.69±0.55 ^e
Acid pretreatment		494.34±25.72 ⁱ	25.37±0.24 ^e
Untreated	Vacuum drying	496.22±26.80 ⁱ	26.34±0.72 ^e
Water blanching		638.73±17.32 ^e	28.08±1.08 ^e
Steam blanching		858.11±33.47 ^b	28.31±4.28 ^d
Acid pretreatment		542.06±14.07 ^{gh}	26.49±1.09 ^{ed}
Untreated	Freeze drying	834.19±71.89 ^b	31.51±5.26 ^d
Water blanching		784.61±44.89 ^e	53.83±4.88 ^{ab}
Steam blanching		946.27±12.63 ^a	63.86±10.66 ^a
Acid pretreatment		716.13±19.54 ^d	43.98±0.29 ^c

Mean values in the same column with different superscripts are significantly different (P<0.05).

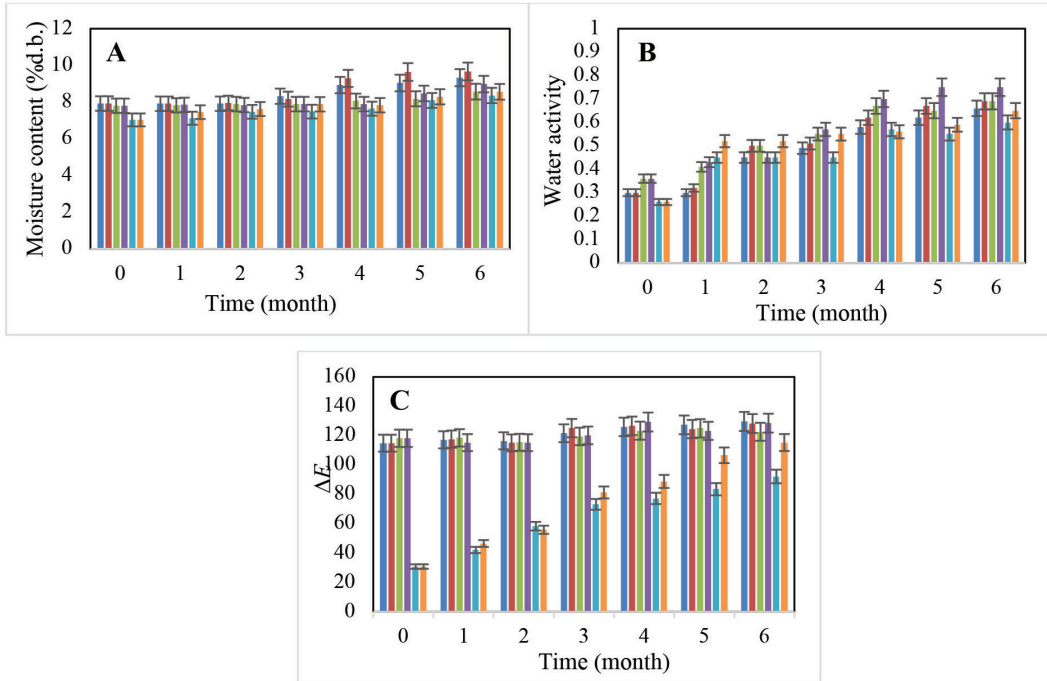


Figure 2: Physiochemical properties change of dried Guizhou during storage by hot air drying kept in foil (■) and polypropylene (■); vacuum drying kept in foil (■) and polypropylene (■) and freeze drying kept in foil (■) and polypropylene (■). A: moisture content, B: water activity and C: ΔE .

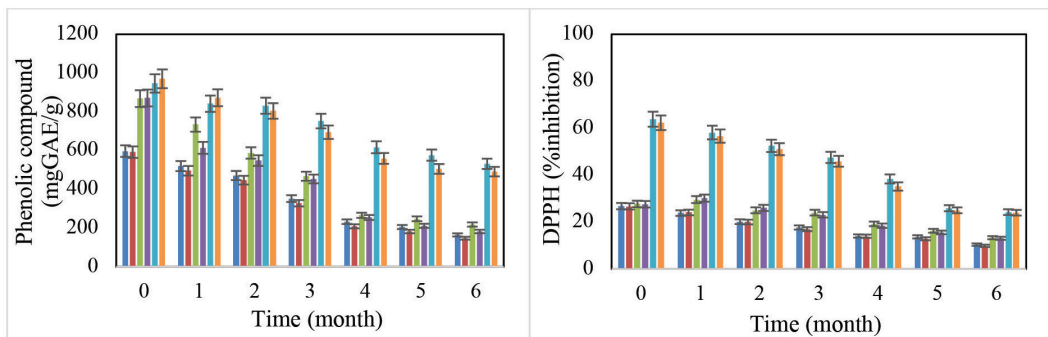


Figure 3: Change in phenolic compounds and antioxidant activity by DPPH radical scavenging assay of dried Guizhou during storage by hot air drying kept in foil (■) and polypropylene (■), vacuum drying kept in foil (■) and polypropylene (■), and freeze drying kept in foil (■) and polypropylene (■).

decrease in the value of TPC and DPPH. At the initial period of storage, DPPH was highest in freeze-dried samples followed by vacuum dried and hot-air dried samples. There was only a slight reduction in DPPH of hot air dried and vacuum dried samples during storage compared to initial values. Freeze dried samples showed a significant reduction in DPPH compared to other drying methods.

The effect of packaging was more pronounced for the change of DPPH during storage in the case of freeze dried samples. Aluminum foil retained DPPH and TPC better than PP packaging. Products stored in aluminum foil were of better quality than those stored in PP packaging.

Shelf Life

Moisture content is essential to determine shelf life of dried products. Changes in moisture content of dried products under different conditions of storage are presented in Figure 4. Moisture content increased with increasing storage time for all conditions. Changes in moisture content fitted to a first order regression model with good correlation ($R^2 = 0.6848-0.9538$). Results in Table 5 showed that freeze-dried products showed the highest correlation (0.9456-0.9538) followed by hot-air dried products (0.8506-0.9042) and vacuum-dried products (0.6848-0.6903), respectively. Based on the standard moisture content of tea at less than 8% (Thai Community Product Standard No. 120, 2016), longer shelf life was observed in products packed in aluminum foil bags compared to products packed in polypropylene bags. The layer of aluminum foil was found to be good for long-term storage of dried product; this observation was consistent with Gogoi *et al.* (2006).

Treatment of Guizhou with 0.5% acetic acid did not affect drying time by hot-air drying and vacuum drying but resulted in a significant increase in total color change, TPC and DPPH. Blanched samples exhibited shorter drying time than untreated and treated in acid samples. Blanching pretreatment with steam and hot water improved antioxidant activity (higher TPC and DPPH) of dried Guizhou. Steam blanched product for each drying method was selected to study the effect of different packaging during storage. Freeze-dried samples showed higher TPC and DPPH (% inhibition) than vacuum dried and hot-air dried samples during the first period of storage (0 month). During storage for 6 months, physical properties of dried products exhibited an increasing trend, while antioxidant activity showed a decreasing trend. First order regression analysis was used to predict change in water activity of all dried products ($R^2 = 0.6848-0.9538$). Freeze-dried samples kept in aluminium foil at room temperature gave the longest shelf life at 4.86 months.

Conclusions

How the physicochemical properties of dried Guizhou are affected by pretreatment, drying method and storage period were investigated.

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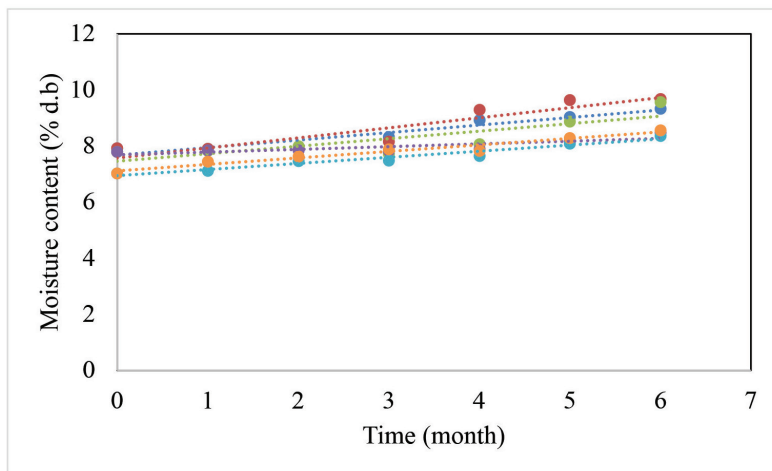


Figure 4: Moisture content change of dried Guizhou during storage by hot air drying kept in foil (■) and polypropylene (■), vacuum drying kept in foil (■) and polypropylene (■) and freeze drying kept in foil (■) and polypropylene (■).

Table 5: Prediction model for shelf life of dried Guizhou kept in different packaging.

Drying method	Packaging	Model*	R ²	Shelf life (month)
Hot air drying	Aluminum foil	$y = 0.2682t + 7.6754$	0.9042	1.21
	Polypropylene	$y = 0.3589t + 7.5718$	0.8506	1.19
Vacuum drying	Aluminum foil	$y = 0.2679t + 7.4593$	0.6903	2.02
	Polypropylene	$y = 0.1575t + 7.6861$	0.6848	1.99
Freeze drying	Aluminum foil	$y = 0.2170t + 6.9450$	0.9453	4.86
	Polypropylene	$y = 0.2314t + 7.1143$	0.9538	3.83

*y = moisture content (% w.b.) and t = time (month)

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