



Full Proceeding Paper

EFFECT OF DRYING ON CRUDE GANODERIC ACIDS AND WATER-SOLUBLE POLYSACCHARIDES CONTENT IN *GANODERMA LUCIDUM*SIEW KIAN CHIN¹, CHUNG LIM LAW^{1,*}, POH GUAT CHENG²

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ABSTRACT

Ganoderic acids and polysaccharides are important pharmaceutical components in *Ganoderma lucidum* which give advantages on human's health such as anti-inflammatory effect, demonstrate strong immunomodulatory and antitumoral activities. However, they are thermolabile and easily degraded during drying. Freeze drying could preserve most of the thermolabile pharmaceutical active ingredients during drying process. Nevertheless, this method is cost intensive and requires long drying time, which indirectly increases the operating cost of drug manufacturing process. Hence, a feasible drying method which could retain relatively high amount of active ingredients in *Ganoderma lucidum* and at lowest operating cost is determined. Four different drying methods were investigated for drying of *Ganoderma lucidum*, namely convective hot air drying, vacuum drying, freeze drying and heat pump drying. The results show that heat pump dried *Ganoderma* retained most of the active ingredients with the shortest total drying time required as compared to other drying methods. It could retain 94% of crude ganoderic acids and 88.5% of water soluble polysaccharides. Although vacuum dried *Ganoderma lucidum* could minimize the loss of these active ingredients but it required longer drying time to achieve its equilibrium moisture content. On the other hand, convective hot air drying of *Ganoderma lucidum* showed significant loss of the active ingredients during the drying process. It could only retained 72% of crude ganoderic acids and 82% of water-soluble polysaccharides.

Keywords: Ganoderic acids, Polysaccharides, Convective hot air drying, Vacuum drying, Freeze drying, Heat pump drying

INTRODUCTION

Ganoderma lucidum is a traditional Chinese medicinal mushroom which contains bioactive ingredients such as ganoderic acids and water-soluble polysaccharides. Water soluble polysaccharide is the main bioactive ingredient in the *Ganoderma* fruiting body which has been found to be medically active in several therapeutic effects such as anti-tumor, anti-inflammatory and anti-viral¹. Whereas crude ganoderic acids are recognized as a special bioactive compound in *Ganoderma* which possess biological functions such as anti-cancer and anti-HIV^{2,3}. However, these two major groups of medicinal compound in *Ganoderma lucidum* are heat sensitive⁴. When exposed to heat, these compounds are easily degraded.

High quality of dried *Ganoderma lucidum* is essential either for direct consumption or further processed into pharmaceutical or health care products. Its bioactive ingredients are extracted from the fruiting body and spray dried into powder form, followed by processing the powder into capsule or incorporated into drugs and beverages. Currently *Ganoderma* processing industry applies conventional convective hot air drying (or known as oven drying) to remove water from the fruiting body before the bioactive ingredients are extracted. As hot air drying is typically operated at high temperature, which is not suitable for polysaccharides and crude ganoderic acids, it tends to cause higher loss of these active ingredients in the dehydrated *Ganoderma*. In this regard, a feasible drying method with optimum drying condition is important to retain bioactive ingredients and at the same time extending its shelf-life⁵.

The effect of heat treatment using different drying methods such as vacuum drying, hot air drying and freeze drying on the polysaccharides extracted from the fruiting body and mycelium of *Ganoderma lucidum* was determined by Lai et al. (2007). The rheological properties of polysaccharides was not affected significantly except for the extracted uronic acid, which is one of the major compound (11 -12 % dry basis) of *Ganoderma lucidum* polysaccharides⁶. The effect of drying methods on the retention of crude polysaccharides and triterpenes in concentrated *Ganoderma lucidum* extraction was reported by Cui et al., (2006). Two stage drying method using combined microwave - vacuum and conventional vacuum is found to retain most of the polysaccharides and triterpenes as compared to conventional vacuum drying. The drying time required for the two stage drying method to achieve the desired moisture content of dried product is 90% shorter than the conventional drying method whereas the retention of total water soluble polysaccharides and triterpenes is close to freeze dried product (98.96%), which is 91.53% and 89.29%, respectively⁴.

Nevertheless, the effect of other drying methods such as oven and heat pump drying on these bioactive ingredients of *Ganoderma lucidum* fruiting body has not yet been investigated.

Besides ganoderic acids and water-soluble polysaccharides, the effect of different drying methods on the biochemical quality of pretreated oyster mushrooms such as protein, carbohydrate and free amino acid has been evaluated⁷. Sun dried, fluidized bed dried and thin layer drying of mushrooms do not affect its biochemical constituents significantly but the retention of protein in the fruiting body is found to reduce significantly after pretreating with potassium metabisulphite (KMS). However, fluidized bed drying of oyster mushrooms at 50°C with 0.5% KMS is found to be superior to other drying methods in terms of higher drying rates and lower browning index with relatively high retention of biochemical constituents.

The objectives of this study are to investigate the effect of different drying methods, as well as different drying conditions of convective hot air drying, vacuum drying, heat pump drying and freeze drying on the *Ganoderma lucidum* fruiting body in terms of the retention of crude ganoderic acids and total water-soluble polysaccharides content.

MATERIAL AND METHODS

Material

Mature fruiting body of *Ganoderma lucidum* was supplied by Ganofarm Sdn. Bhd., Tanjung Sepat, Selangor Darul Ehsan, Malaysia. The production of *Ganoderma lucidum* required a minimum of 60 days cultivation in plastic bags containing wood chips and rice grains as nutrient sources for the fruiting bodies. *Ganoderma* experienced dehydration at room temperature. Therefore, the material was utilized immediately for experiment purpose, once excised from the plastic bags. The fruiting body was a kidney shaped mushroom body and was thoroughly washed to remove dirt whereas the trunk was removed from the growth medium using a vegetable slicer. A total of three fruiting bodies with average weight of 36 ± 0.1 g (Adventure OHAUS electronic balance, USA, model AR3130, range 0-310 g with accuracy of ± 0.001 g) were used for each drying experiments at different drying conditions and drying methods. The dry weight of the samples was determined using hot air convection oven at 105°C for 24 hours⁸. The average initial moisture content of the fruiting body was found to be 249.49% (dry basis).

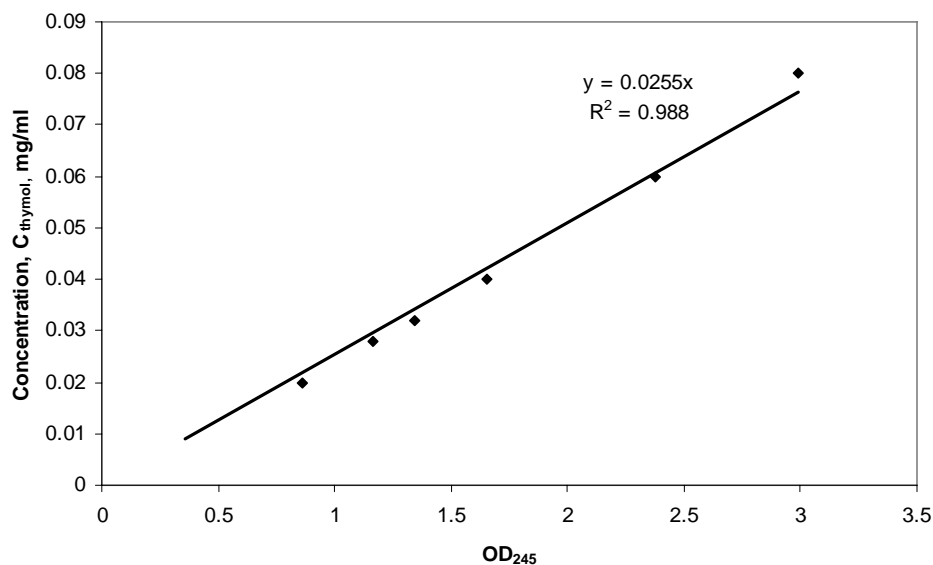


Fig. 1: Standard curve of thymol concentration (C_{thymol}) which varied linearly with optical density (OD) at 245 nm.

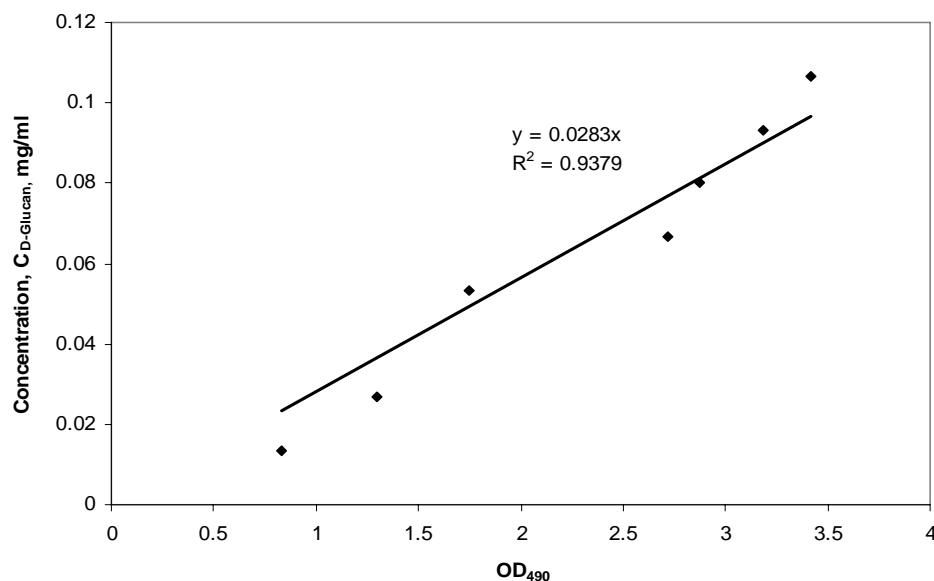


Fig. 2: Standard curve of D-Glucan concentration ($C_{\text{D-Glucan}}$) which varied linearly with optical density (OD) at 490 nm.

Drying methods

The fruiting body of *Ganoderma lucidum* was dried by different drying methods which are described below until equilibrium moisture content (EMC) was achieved.

1. Freeze drying

Freeze drying is a high cost drying method due to its intensive energy nature⁹. Hence, freeze dried products were treated as control samples. *Ganoderma* fruiting bodies were pre-frozen overnight in a freezer at -18°C before freeze dried in a laboratory freeze dryer (Alpha 1-2 LD Plus, Martin Christ, Germany) at different drying times. The primary drying temperature (T_1) was set at -40°C and

0.12 mbar (P_1) whereas the secondary drying temperature (T_2) was carried out at -50°C and 0.04 mbar (P_2). For crude ganoderic acid retention test, the total drying time for primary and secondary drying varies from 18 hours to 4 days and the highest total crude ganoderic acids was treated as control sample. The control sample for water-soluble polysaccharides test was evaluated from freeze dried *Ganoderma lucidum* at 18 hours of primary drying condition.

2. Convective hot air drying

Fresh *Ganoderma lucidum* fruiting bodies were dried in a laboratory scale hot air circulation oven (Memmert, Germany, range $20-250^{\circ}\text{C}$ with an accuracy of $\pm 0.5^{\circ}\text{C}$) at air temperatures of 50°C with and

without air circulation. Drying temperature of 50°C was selected as this drying temperature could retain most of the bioactive ingredients in *Ganoderma* fruiting body¹⁰. The air circulation was set at a velocity of 1.40 m s⁻¹. Prior to drying experiments, the oven was turned on at the selected operating condition for about 30 minutes to allow it achieved the steady state conditions. The air velocity was measured using anemometer (Hygrolog, Switzerland, with accuracy ± 0.01 m s⁻¹) while the relative humidity in the oven was measured using a humidity probe (Hygromax, Switzerland, range from 0 to 100%). The total drying time was 58 hours for oven drying without air circulation and 31 hours for drying with air circulation. The drying times were long enough to achieve EMC at different drying conditions.

3. Vacuum drying

Ganoderma fruiting bodies were dried in a laboratory scale vacuum dryer (Mettert, Germany, range 20-200°C and 10-1000 mbar) with plate temperature set at 50°C and three different pressures were set at 50 mbar, 100 mbar and 200 mbar, respectively. The total drying time to achieve EMC was ranged from 41 to 58 hours at different drying conditions.

4. Heat pump drying

Heat pump drying was conducted at mild temperature with dehumidified air (at low relative humidity) as according to the working principle of heat pump¹¹. *Ganoderma* fruiting bodies were dried in a drying chamber of a heat pump dryer. The dryer consisted of two drying chambers measuring 95 cm x 33cm x 33cm each. Drying materials were placed on a wire screen tray with size of 27 cm x 23 cm, perpendicular to the air flow. The drying chamber was operated at drying temperature of 28.4°C with 28.55% relative humidity (RH), which was measured by a humidity probe (HygroFlex, RS 232, Huntington, NY, range from 0 to 100%). Average superficial air velocity in the chamber was 4.53 m s⁻¹, which was measured using an anemometer (Rotronic, D5-U-2, Huntington, NY with accuracy ± 0.01 m s⁻¹). The sample was dried for 30 hours to its EMC.

5. Repeatability

Each of the drying experiments was replicated three times and the average results were obtained.

Determination of crude ganoderic acids

Dried *Ganoderma lucidum* fruiting bodies at EMC were ground into powder using a mechanical grinder (Retsch, SM100, Haan, Germany) which was attached with a sieve (conidur holes, 5 mm) to obtain a homogeneous powder size. The powder (17 \pm 0.1 g) was subjected to extraction using 95% (v/v) ethanol for 4 to 5 days at room temperature. The suspension which contained *Ganoderma* powder and ethanol was shook for overnight using a mechanical shaker (Model 903, PROTECH, Malaysia). After the removal of the solid powder by vacuum filtration, the supernatants were dried at 45°C under vacuum (150 mbar) in a rotary evaporator (Heidolph, 4000 series, Schwabach, Germany) until all ethanol was vaporized. The residues were then suspended in distilled water (10 ml) and later extracted with 10 ml chloroform (Fisher, Pittsburgh, PA) for 1 to 2 days. After removal of water by centrifugation (6000 rpm, 10 min.), the ganoderic acids in the chloroform extract were further extracted with 5% (v/v) NaHCO₃. HCl (2N) was added to the solution to adjust the pH of the NaHCO₃ phase to lower than 3.0. After removal of chloroform by evaporation at 45°C, crude ganoderic acids were dissolved in absolute ethanol (99.4% v/v, Fisher, Pittsburgh, PA) and the absorbance was measured at 245 nm in a spectrophotometer (Biochrom, Libra S12, UK)¹². The crude ganoderic acids content of each sample were analyzed twice for a total of three replications for each drying conditions. The average readings were obtained. Figure 1 shows the standard curve obtained from thymol (Sigma, Milwaukee, WI) and absolute ethanol as blank solution.

Determination of total water-soluble polysaccharides

Total water-soluble polysaccharides were determined based on colored reaction of polysaccharides and their derivatives with phenol and concentrated sulphuric acid. The concentration of the water-soluble polysaccharides was determined using a spectrophotometer at maximum absorption of 490 nm. Figure 2 shows the standard curve obtained from β -1,3-Glucan (Sigma, Milwaukee, WI) and distilled water as blank solution.

Dried *Ganoderma lucidum* fruiting bodies at EMC which were obtained using different drying treatments were ground into powder using a mechanical grinder (Retsch, SM 100, Haan, Germany). The powder (1 \pm 0.1 g) was subjected to hot water extraction (50 ml) at 60-65°C. The suspension that contained dried *Ganoderma* powder and water was shaken for more than 6 hours using a mechanical shaker (Model 903, PROTECH, Malaysia). After the removal of the solid powder by vacuum filtration, the supernatants were dried at 60°C under vacuum (100 mbar) in a rotary evaporator (Heidolph, 4000 series, Schwabach, Germany) until all water was vaporized. The polysaccharides were then washed with 85% ethanol which was vaporized again in the rotary evaporator at 45°C and 150 mbar. The residue was then dissolved with distilled water. The solution was then transferred to a 250 ml flask, which was then diluted to 250 ml with distilled water. A 2 ml volume of the solution was pipetted into a 10 ml centrifuge tube and 1 ml of 5% phenol was added. The mixture was shaken for 2 minutes. A 5 ml volume of concentrated sulphuric acid (98% v/v) was then added to the solution and shook for another 5 minutes. The concentration of water-soluble polysaccharides in the solution was determined quantitatively by measuring the absorbance at 490 nm using a spectrophotometer (DR 2800, Hach, USA)⁴. The water-soluble polysaccharide content of each sample was analyzed twice for a total of three replications for each drying condition. Average readings were obtained.

Statistical analysis

The results of the experiments were analyzed in triplicate by using completely randomized design. Analysis of variance was performed by SAS statistical analysis package (SAS institute Inc, SAS/STAT 9.1, 2004). Mean were compared by Tukey's Studentized Ranged (HSD) test at 95% confidence level.

RESULTS AND DISCUSSION

Effect of freeze drying time on the retention of crude ganoderic acids

Table 1 shows the retention of crude ganoderic acids content for freeze dried *Ganoderma lucidum* at primary drying temperature (T₁) of -40°C (0.12 mbar) and secondary drying (final drying) temperature (T₂) of -50°C (0.04 mbar) at different drying durations. The results show that freeze dried *Ganoderma lucidum* with 18 hours primary drying at -40°C (0.12 mbar) could retain significantly more ganoderic acids ($p < 0.05$) as compared to the longer drying period which involved secondary drying at lower freeze drying temperature and vacuum pressure. Long drying time at extremely low pressure encouraged the volatilization of ganoderic acid. This in turn caused a great loss of crude ganoderic acid content in *Ganoderma lucidum*. Drying of *Ganoderma lucidum* for 18 hours at primary drying condition without secondary drying is adequate to produce dried *Ganoderma lucidum* with high ganoderic acids retention at final moisture content (FMC) of 2.93% (d.b). This value was used as a reference when comparison of crude ganoderic acids retention in the samples processed by other drying methods was conducted.

Effect of different drying methods on the retention of crude ganoderic acids

Crude ganoderic acids retention for convective hot air drying, vacuum drying, heat pump drying and freeze drying of *Ganoderma lucidum* was determined and summarized in Table 2. It can be seen that vacuum dried *Ganoderma lucidum* could produce dried product which retained 91.38 to 96.23% of ganoderic acids content. At the same drying temperature, it was clearly shown that most of the

ganoderic acids were retained in the fruiting body which dried at high vacuum condition (50mbar), while the amount of ganoderic acids reduced accordingly with the increased of vacuum pressure from 50mbar to 200mbar. This could be due to inactivation of the ganoderic acids decomposition enzymes in high vacuum condition. According to Imshenetsky et al., (1970), enzymes exposed to high vacuum condition could reduce its activity up to 49%¹³. However, the drying time to reach equilibrium moisture content (EMC) for vacuum drying of *Ganoderma lucidum* (41 – 58 hours) was longer as compared to other methods, such as heat pump drying which only required 30 hours to reach EMC. Long drying time resulted in high operating cost. This was due to the fact that vacuum drying was

mainly stimulated by heat conduction from the heating plate alone instead of heat convection. Hence, it reduced drying rate, and prolonged the total drying time. On the other hand, convective hot air drying of *Ganoderma lucidum* without air circulation showed significant loss of ganoderic acids content ($p < 0.05$) as compared to freeze dried product. Hot air dried *Ganoderma lucidum* with air circulation at a velocity of 1.40 ms⁻¹ could retain 89.59% of ganoderic acids content whereas drying of *Ganoderma lucidum* without air circulation could only retain 71.99% of ganoderic acids. Long drying duration did not only encourage the volatilization of ganoderic acids in the sample, but also stimulate the enzyme decomposition of ganoderic acids in *Ganoderma*⁴.

Table 1: Crude ganoderic acids retention of freeze dried *Ganoderma lucidum* at different drying periods.

Drying method	T ₁ (°C) / P ₁ (mbar)	t ₁ (h)	T ₂ (°C) / P ₂ (mbar)	t ₂ (h)	OD ₂₄₅	Crude ganoderic acid content (µg / g dry wt.)	Retention (%)
Freeze drying	-40 / 0.12	48	-50 / 0.04	48	1.495 ± 0.045 ^d	5.460 ± 0.165 ^e	49.01
	-40 / 0.12	24	-50 / 0.04	24	2.238 ± 0.046 ^c	8.206 ± 0.168 ^d	73.61
	-40 / 0.12	12	-50 / 0.04	12	2.352 ± 0.055 ^b	8.649 ± 0.202 ^c	77.56
	-40 / 0.12	18	-50 / 0.04	6	2.677 ± 0.048 ^b	9.844 ± 0.176 ^c	91.74
	-40 / 0.12	18	-50 / 0.04	3	2.882 ± 0.065 ^a	10.64 ± 0.240 ^b	95.33
	-40 / 0.12	18	-50 / 0.04	0	2.992 ± 0.014 ^a	11.16 ± 0.054 ^a	100

*Mean value ± standard deviation (n = 3).

Table 2: Table shows the effect of different drying methods on the retention of crude ganoderic acids in *Ganoderma lucidum*.

Drying method	Drying condition	Total drying time (h)	EMC (% d.b.)	OD ₂₄₅	Crude ganoderic acid content (µg / g dry wt.)	Retention (%)
Convective hot air drying	T = 50°C, without air circulation	58	8.79	2.036 ± 0.137 ^d	8.028 ± 0.538 ^b	71.99
	T = 50°C, air velocity = 1.40 m s ⁻¹	31	7.33	2.572 ± 0.037 ^c	9.991 ± 0.146 ^{ab}	89.59
	T = 50°C, P = 50 mbar	41	5.07	2.821 ± 0.021 ^{ab}	10.736 ± 0.078 ^{ab}	96.23
Vacuum drying	T = 50°C, P = 100 mbar	53	7.67	2.729 ± 0.028 ^{bc}	10.433 ± 0.105 ^{ab}	93.36
	T = 50°C, P = 200 mbar	58	9.97	2.557 ± 0.090 ^c	10.185 ± 0.359 ^{ab}	91.38
Heat pump drying	T = 28.4°C, RH = 28.6%, air velocity = 4.53 m s ⁻¹	30	6.80	2.714 ± 0.002 ^{bc}	10.506 ± 0.008 ^{ab}	94.08
Freeze drying	T = -40°C, P = 0.12 mbar	42 (including freezing period)	2.93	2.992 ± 0.076 ^a	11.160 ± 0.018 ^a	100

*Mean value ± standard deviation (n = 3).

Heat pump drying of *Ganoderma lucidum* at 28.4°C with 28.6% relative humidity and air circulation with a velocity of 4.53 ms⁻¹ could retain most of the ganoderic acids content (94.08%) which was not significantly different ($p > 0.05$) from the freeze dried sample. This was because the total drying time (30 hours) was the shortest compared to the other drying methods. In addition, heat pump drying at mild temperature prevented the volatilization of ganoderic acids and inactivated the enzyme activity in terms of ganoderic acid decomposition.

Effect of different drying methods on the retention of water soluble polysaccharides

Table 3 shows the retention of total water-soluble polysaccharides of dried *Ganoderma lucidum* for different drying methods and drying conditions. There was no significant difference ($p > 0.05$) for the

retention of polysaccharides content for sample dried by convective hot air drying with and without air circulation at 50°C. This revealed that hot air drying of *Ganoderma* fruiting body with air circulation did not have significant effect on the retention of water-soluble polysaccharides during the drying process. However, the drying rate was improved and consequently reduced the total drying time. In terms of total water-soluble polysaccharides content, statistical analysis showed that vacuum dried *Ganoderma lucidum* at 50°C and 50 mbar contained significantly more water soluble polysaccharides ($p < 0.05$) as compared to other drying methods and the content was close to the water-soluble polysaccharides content of freeze dried product. This may due to the deactivation of hydrolysis enzyme at high vacuum condition. However, the retention of this bioactive ingredient decreased from 98.46% to 79.77% (Table 3) as the vacuum pressure increased from 50 mbar to 200 mbar.

Generally, the reason for the degradation of polysaccharides during drying of *Ganoderma* is due to hydrolysis, in which the polysaccharides are hydrolyzed as water is bound to the molecule¹⁴. In addition, weak acidity and hydrolysis enzymes in *Ganoderma* accelerate the hydrolysis process^{4,15} during drying of *Ganoderma*. Vacuum drying of *Ganoderma lucidum* at 50°C and 200 mbar showed significant loss of water-soluble polysaccharides ($p < 0.05$) as compared to heat pump and freeze dried products, due to high final moisture content (9.97% d.b.) and longer drying time (58 hours) which encouraged the hydrolysis process of polysaccharides. Although vacuum drying of *Ganoderma lucidum* at 50°C and 50 mbar could retain high water-soluble polysaccharides content (98.46%), the total drying time (41 hours) was longer than heat pump dried

Ganoderma lucidum. In this study, heat pump drying required only 30 hours to reach the equilibrium moisture content (EMC) with 88.52% water-soluble polysaccharides retention. Heat pump drying with dehumidified air reduced the hydrolysis degree of polysaccharides due to mild drying temperature (decelerated the activity of hydrolysis enzyme), short heat treatment time and low final moisture content (6.80% d.b) (Table 3). Since drying is an energy intensive process, heat pump drying was preferable in order to reduce the operating cost as compared to high vacuum drying process. Heat pump drying operated at mild drying temperature and low relative humidity resulted in short total drying time and relative good final product quality. In addition, heat pump drying was one of the drying methods that gave high energy efficiency¹⁶.

Table 3: Table shows the retention of water-soluble polysaccharides in *Ganoderma lucidum* dried by different drying methods.

Drying method	Drying condition	Total drying time (h)	EMC (% d.b.)	OD ₄₉₀	Water-soluble polysaccharides (mg / g dry wt.)	Retention (%)
Convective hot air drying	T = 50°C, without air circulation	58	8.79	2.781 ± 0.051 ^{cd}	0.653 ± 0.012 ^c	83.61
	50°C, air velocity = 1.40 m s ⁻¹	31	7.33	2.767 ± 0.021 ^{cd}	0.644 ± 0.005 ^c	82.46
	T = 50°C, P = 50 mbar	41	5.07	3.116 ± 0.049 ^b	0.769 ± 0.012 ^a	98.46
Vacuum drying	T = 50°C, P = 100 mbar	53	7.67	2.676 ± 0.032 ^{de}	0.660 ± 0.008 ^{bc}	84.51
	T = 50°C, P = 200 mbar	58	9.97	2.523 ± 0.039 ^e	0.623 ± 0.010 ^c	79.77
	T = 28.4°C, RH = 28.6%, air velocity = 4.53 m s ⁻¹	30	6.80	2.844 ± 0.031 ^c	0.691 ± 0.008 ^b	88.52
Heat pump drying		42				
Freeze Drying	T = -40°C, P = 0.12 mbar	(including freezing period)	2.93	3.382 ± 0.076 ^a	0.781 ± 0.018 ^a	100

*Mean value ± standard deviation (n = 3).

CONCLUSION

The effect of different drying methods on the retention of crude ganoderic acids and water-soluble polysaccharides content during drying of *Ganoderma lucidum* was investigated. It was found that freeze drying of *Ganoderma lucidum* required 24 hours pre-frozen at -18°C and 18 hours primary drying duration at -40°C (0.12 mbar) to obtain the highest retention of crude ganoderic acids at final moisture content of 2.93% (d.b). Vacuum dried *Ganoderma lucidum* could preserve most of the active ingredients but it required relatively long drying time, which is not economical feasible. Convective hot air drying of *Ganoderma lucidum* resulted in significant loss of ganoderic acids content. It was also found that air circulation during hot air drying did not show significant difference in terms of the retention of water-soluble polysaccharides content. On the other hand, heat pump drying of *Ganoderma lucidum* required the shortest total drying time compared to the drying methods mentioned above. In addition, it could retain relatively high content of crude ganoderic acids and water-soluble polysaccharides (which were close to freeze dried product) in *Ganoderma lucidum* fruiting body. Hence, heat pump drying is a potential method to preserve thermolabile pharmaceutical active ingredients of *Ganoderma lucidum* and it is economical feasible in terms of operating cost for drug manufacturing process, as compared to freeze drying and vacuum drying.

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NOMENCLATURE

EMC	Equilibrium moisture content (% d.b)
FMC	Final moisture content (% d.b)
d.b	Dry basis
OD	Optical density
RH	Relative humidity (%)
t ₁	Primary freeze drying time (hr)
t ₂	Secondary freeze drying time (hr)
T	Drying temperature (°C)
P	Drying pressure (mbar)
T ₁	Primary freeze drying temperature (°C)
T ₂	Secondary freeze drying temperature (°C)
P ₁	Primary freeze drying pressure (mbar)
P ₂	Secondary freeze drying pressure (mbar)