Agr. Nat. Resour. 57 (2023) 989–998

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Effect of drying techniques on browning index, phenolic compounds, polysaccharides, triterpenoids and antioxidant activity of tiger milk mushroom (*Lignosus rhinocerus*) sclerotium

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Article Info

Article history:
Received 13 May 2023
Revised 6 November 2023
Accepted 28 November 2023
Available online 31 December 2023

Keywords:
Antioxidant,
Drying,
Lignosus rhinocerus,
Phenolic compounds,
Triterpenoids

Abstract

Importance of the work: Drying is one method for preserving medicinal mushrooms. Many studies have found that different drying techniques affect bioactive compounds and biological activity of the product. However, the effect of drying the tiger milk mushroom (*Lignosus rhinocerus*) sclerotium has not been studied.

Objectives: To investigate drying techniques to produce a high content of bioactive compounds and levels of antioxidant activity in the tiger milk mushroom sclerotium.

Materials & Methods: The sliced sclerotium samples were dried using seven drying techniques: sun drying (SD), shade drying (SHD), freeze-drying (FD), oven drying at 40°C (OD40), oven drying at 70°C (OD70), light dry roasting (LDR), and dark dry roasting (DDR) Subsequently the dried samples were milled into individual powders. Next, the dried samples were extracted with boiling water and analyzed for browning index, total phenolic, total flavonoid, total polysaccharide content and antioxidant activity compared to the fresh sample (FSH).

Results: The DDR and OD70 techniques showed high values for the browning index and antioxidant activity (based on 2,2-diphenyl-1-picrylhydrazyl hydrate and ferric reducing antioxidant power analysis) and for the total phenolic, total flavonoid and total polysaccharide contents. In contrast, the total protein content was high in the low-temperature drying samples (FSH, SD, SHD, LDR and FD). The highest total triterpenoid content (mean ± SD; 14.52 ± 0.57 mg ursolic acid/g of dry basis) was obtained in the OD40 sample.

Main finding: DDR was a potent drying technique that enhanced the total phenolic, total flavonoid and total polysaccharide contents, as well as antioxidant activity. Thus, using the DDR technique may be the most appropriate option to preserve the quality of the sclerotium of the tiger milk mushroom for commercialization as a medicinal mushroom powder for infusion.

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https://doi.org/10.34044/j.anres.2023.57.6.09

Introduction

The tiger milk mushroom (*Lignosus rhinocerus*), a member of the Polyporaceae family, is a medicinal mushroom found in southern China, Malaysia, Thailand, the Philippines, Indonesia, Australia, New Zealand and Papua New Guinea (Lai et al., 2011). The extract of the sclerotium part of tiger milk mushroom contains various bioactive compounds with medicinal properties, such as antioxidant, antimicrobial, anticancer, anti-inflammatory, antiviral, anti-asthmatic, immunomodulatory and neuritogenic activities (Abdullah et al., 2013; Lau et al., 2015; Tan et al., 2021). With such properties, health-conscious customers are interested in consuming tiger milk mushroom fusions as a therapeutic option. Malaysians slice tiger milk mushrooms into thin sheets, boil them with additional herbs, and then drink the resulting concoction (Lee et al., 2009). Thais drink tiger milk mushroom infusion made with boiling water, similar to preparing tea or herbal tea. Nowadays, agricultural community-based companies in Thailand sell tiger milk mushrooms as a dry powder for infusion that is prepared by slicing the tiger milk mushrooms into thin strips, drying them using various techniques (such as shade-drying, sun-drying and drying in a low-temperature oven), grinding the dried product into a powder and then packing in a sealed bottle. Various techniques for drying the sliced sclerotium of tiger milk mushrooms may impact the bioactive compound contents and antioxidant activity of the mushroom extract in its infusion form. Chang et al. (2006) discovered hot air-dried tomatoes had the greatest total phenolic content. The total phenolic content and 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) inhibition activity increased in pumpkin seeds and peanut shells that had been roasted (Hassan et al., 2021; Peng et al., 2021). Pholpokakul et al. (2015) reported that drying *Spirulina platensis* in a hot-air oven increased antioxidant activity based on DPPH, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid+ and ferric reducing antioxidant power (FRAP) analyses. Piskov et al. (2020) found that microwave drying increased the ferric-reducing ability of oyster mushrooms (*Pleurotus ostreatus*). However, there has been no published report to date on the influence of drying techniques on the bioactive compound contents and antioxidant activities in the tiger milk mushroom sclerotium. Hence, the current study investigated different drying techniques to evaluate the effect of drying techniques on the bioactive compound contents and antioxidant activity of the tiger milk mushroom (*Lignosus rhinocerus* CH2) sclerotium.

Materials and Methods

Source of sclerotium

The fresh sclerotium sample of *Lignosus rhinocerus* CH2 was obtained after burying fully mycelium-colonized substrate (rubber sawdust and rice bran) for 6 mth in the soil. Once the sclerotia had been harvested, the sample was washed with tap water to remove impurities and then left to dry for 15 min at room temperature on a cheesecloth.

Preparation of sclerotium sample

Each sclerotium was sliced with a knife cutter into a square shape (height = 1.50±0.1 cm, length = 1.50±0.1 cm, thickness = 0.2 cm) and subjected to seven different drying techniques: sun drying (SD), shade drying (SHD), freeze-drying (FD), oven drying at 40°C (OD40), oven drying at 70°C (OD70), light dry roasting (LDR), and dark dry roasting (DDR), until a constant weight was obtained (Table 1). The air temperature (°C) and relative humidity (%RH) in the sun-drying and shade-drying areas were recorded using an RHT10 data logger (Extech; Nashua, NH, USA). The dried samples were milled into a fine powder and then stored in a dry place in a tightly closed container before analysis.

Moisture content determination

The moisture contents of fresh and dried sclerotium samples were determined according to method 925.10 by drying the sample at 105°C (Association of Official Analytical Chemists, 2000).

Color measurement

The color of the sclerotium samples was determined by measuring the reflectance of a 100 g of sample in terms of the L* (blackness/whiteness), a* (greenness/ redness) and b* (blueness/yellowness) values based on the CIELAB system using a ColorQuest XE colorimeter (Hunterlab; Reston, VA, USA), as described by Shan et al. (2016). Before measuring the samples, the equipment was calibrated against white and black tiles. The total color difference (ΔE) of the fresh sclerotium (L^*_0, a^*_0, b^*_0) compared to the dried sclerotium (L*, a*, b*) and the browning index (BI) were calculated using Equations 1 and 2, respectively:

$$\Delta E = \sqrt{(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2} \quad (1)$$

$$BI = \frac{[100(x - 0.31)]}{0.172}, \text{ where } x = \frac{(a^* + 1.75L^*)}{(5.645L^* + a^* - 3.012b^*)} \quad (2)$$

Extraction of sclerotium samples

Boiling water was used as a solvent to simulate the general conditions of sclerotium extraction for consumption (infusion form). Separate 0.5 g samples of fresh and ground dried sclerotia were dissolved in 20 mL of hot distilled water (100°C). The extractions were performed in an orbital shaker (150 rpm) for 15 min. The sampled sediments were removed by passing the liquid through a Whatman No.1 filter paper and then the prepared extracts were collected and stored at 4°C for further analysis.

Determination of total phenolic content

The total phenolic content (TPC) was determined according to Norra et al. (2016), with minor modifications. Briefly, 0.1 mL of the prepared sclerotium extract was mixed with 0.5 mL of Folin and Ciocalteu's phenol reagent. After 5 min, 1 mL of 7.5% (weight per volume, w/v) Na_2CO_3 solution was added to the reaction mixture and adjusted to 10 mL with distilled water. The reaction mixture was kept in the dark for 90 min and the absorbance was recorded at 765 nm using an ultraviolet-visible spectrum (UV-Vis) spectrophotometer (GENESYS 10S UV-Vis; Thermo Fisher Scientific Inc.; Madison, WI, USA). A calibration curve was created using a standard solution of gallic acid (0–100 mg/L). The results were reported as milligrams of gallic acid equivalent (GAE) per gram on a dry basis.

Determination of total flavonoid content

The total flavonoid content (TFC) of the prepared extract was determined using a colorimetric method, according to Roshanak et al. (2016). A 0.1 mL sample of the prepared sclerotium extract was mixed with 2 mL of distilled water, followed by 0.15 mL of 5% (w/v) $NaNO_2$ solution. After 6 min, 0.15 mL of 10% (w/v) $AlCl_3$ solution was added. Then 6 min later, 2 mL of 4% (w/v) NaOH was added to the mixture and the volume was adjusted to 5 mL with distilled water. The absorbance of the reaction mixture was recorded at 510 nm using the UV-Vis spectrophotometer. Catechin at different concentrations (0–100 mg/L) was used as a standard. The results were expressed as milligrams of catechin (CE) equivalent per gram on a dry basis.

2,2-Diphenyl-1-picrylhydrazyl hydrate free radical scavenging assay

The free radical scavenging ability of the prepared extract was tested based on DPPH radical scavenging assay, as described by Norra et al. (2016). Briefly, 0.1 mL of the prepared sclerotium extract was added to 2.9 mL of 0.004% (w/v) DPPH prepared in methanol. After 30 min of incubation, the absorbance at 517 nm was recorded using the UV-Vis spectrophotometer. The percentage inhibition of DPPH radical was calculated using Equation 3:

$$\% \text{ DPPH inhibition} = \frac{(A_0 - A_S)}{A_0} \times 100 \quad (3)$$

where A_S is the absorbance of the sample solution and A_0 is the absorbance of the control.

Ferric reducing antioxidant power assay

The antioxidant potential of the prepared extract was measured through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) based on ferric reducing antioxidant power (FRAP) assay, as described by Çoklar and Akbulut (2017), with some modifications. The FRAP reagent was freshly made by dissolving 300 mM acetate buffer (pH 3.6) and 10 mM 2,4,6-tripyridyl-S-triazine solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in a ratio of 10:1:1. The FRAP reagent was pre-warmed to 37°C before use in a water bath. In the reaction, 1.5 mL of FRAP reagent was mixed in 0.1 mL of the prepared sclerotium extract and incubated at 37°C for 4 min. The absorbance was recorded at 593 nm using the UV-Vis spectrophotometer. Ferrous sulfate ($FeSO_4$) in the concentration range 0–1000 µmol/L was used for the calibration curve, and FRAP values were expressed as micromoles of ferrous equivalent (µmol Fe^{2+}) per gram on a dry basis.

Determination of total triterpenoids

The total triterpenoid content was measured using a colorimetric method, according to Gupta et al. (2018). The prepared extract or ursolic acid standards (0–100 mg/L) were evaporation to dryness in a boiling water bath at 100°C. After adding 0.3 mL of 5% (w/v) vanillin/glacial acetic acid and 1 mL of perchloric acid solution into each tube, the test tubes were incubated in a water bath at 60°C for 15 min and left until they had cooled to room temperature. Then, 5 mL of glacial acetic acid was added to each tube.

The absorbance of each sample was measured at 548 nm using the UV-Vis spectrophotometer. The results were expressed as milligrams of ursolic acid equivalent per gram on a dry basis.

Determination of total protein content

The protein content was determined according to the method of Bradford (1976). A 0.1 mL sample of the prepared extract in a test tube was added with 1 mL of Bradford's reagent and mixed well. Then, the test tube was left at room temperature for 10 min, after which the absorbance of the sample was recorded at 595 nm using the UV-Vis spectrophotometer. The standard curve of bovine serum albumin (BSA) was used to calculate the protein content in each sample.

Determination of total polysaccharides

The total polysaccharide content of the prepared extract was determined using the phenol-sulfuric acid method, as described by Ma and Yu (2017) using glucose as the standard. A 1.6 mL sample of 6% (w/v) phenol was added into a test tube containing 0.1 mL of the prepared extract or glucose standard and then 7.5 mL of 97% (volume per volume) sulfuric acid was added. After 20 min, the absorbance of the sample was recorded at 490 nm using the UV-Vis spectrophotometer. Different concentrations of d-glucose (0–100 mg/L) were used as a standard. The results were expressed as milligrams of d-glucose equivalent per gram on a dry basis.

Statistical analysis

The Minitab statistical program version 19 software (Minitab Inc.; State College, PA, USA) was used for all statistical analyses of the data. Results were presented as mean ± standard deviation (SD). Differences between the samples were assessed using one-way analysis of variance in combination with Tukey's comparison test at the $p < 0.05$ significance level. Testing based on Pearson's correlation (r) was utilized to examine the correlation between the browning index, bioactive compound contents (phenolic, flavonoid, protein, polysaccharide, triterpenoid) and antioxidant activities (DPPH, FRAP). Principal component analysis was used to visualize correlations between variables.

Results and Discussion

Moisture content of dried sclerotium

The FSH sample had a moisture content of 21.93±0.60%, while the dried sclerotium samples had a moisture content range from 1.80±0.31% to 4.12±0.32%, as shown in Table 1. The FD, SD, SHD, OD40, OD70, LDR and DDR samples had moisture contents of 1.80±0.31%, 3.26±0.41%, 4.12±0.32%, 3.47±0.23%, 2.34±0.21%, 3.13±0.24% and 2.47±0.58%, respectively. The FD technique resulted in the greatest water evaporation from the sclerotium sample, while the SHD technique resulted in the lowest water evaporation. The water in the materials could have been free water or water bonded to the matrix by different forces. Free water freezes, whereas bound water does not. The freeze-drying removes water from the materials by sublimation. During the freeze-drying process, all frozen water, as well as some bound water, must be removed under a vacuum (Nowak and Jakubczyk, 2020). Therefore, the FD sample had a higher water loss than the SHD sample which was obtained by a water evaporation process via diffusion occurring on the surface and inside the material to the air. Valipour (2014) reported that the major climatic parameters affecting evapotranspiration were the %RH, temperature and wind speed. The DDR technique resulted in a greater amount of water evaporation from the SD sclerotium sample than the LDR technique. Zzaman and Yang (2014)

Table 1 Moisture content of fresh and dried *Lignosus rhinocerus* CH2 sclerotium samples using different drying techniques with details of drying process and drying time

Drying technique	Drying process	Drying time	Moisture content (%)
Fresh (FSH)	Undried fresh sample	0 min	21.93±0.60^{a}
Freeze drying (FD)	Dried in the freeze dryer at -50°C	2 d	1.80±0.31^{e}
Sun drying (SD)	Dried under direct sunlight at 37±2°C (RH 31±5.6%)	2 d	3.26±0.41^{c}
Shade drying (SHD)	Dried in the shade at 30±2°C (RH 66±2.5%)	3 d	4.12±0.32^{b}
Oven drying 40°C (OD40)	Dried in the hot air oven at 40°C	2 d	3.47±0.23^{c}
Oven drying 70°C (OD70)	Dried in hot-air oven at 70°C	1 d	2.34±0.21^{de}
Light dry roasting (LDR)	Roasted sun-dried samples at 100°C	15 min	3.13±0.24^{c}
Dark dry roasting (DDR)	Roasted sun-dried samples at 180°C	15 min	2.47±0.58^{d}

Means ± SD ($n = 3$) in a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

discovered that the roasting temperature had a significant effect on moisture loss during the roasting process. Extending the time and temperature resulted in a more rapid moisture loss. The moisture content of the OD40 sample was similar to that of the SD sample, while the moisture content of the OD70 sample was similar to that of the DDR sample. Mahayothee et al. (2020) found that increasing the drying temperature in a hot-air dryer decreased the drying time, consistent with the current results where the drying time of the sclerotium sample using the OD70 technique was shorter than for the OD40 technique. The effective dehydration process of the FD technique in the *L. rhinocerus* CH2 sclerotium sample in the current study produced similar results for *Cordyceps militaris* for which the moisture content in the FD sample (4.15±0.20%) was reported by Chimsook (2018) to be lower than in the hot-air dried sample (7.04±0.19%). In button mushrooms, Shams et al. (2022) reported that the moisture content in the FD sample (5.09%) was less than for the cabinet-dried sample (7.60%).

Color changes

The effect of the drying technique on the color of the dried sample was investigated. It was found that various drying processes resulted in a change in the sample color, as shown in Table 2. The FSH sample had the highest L* value (92.94±0.30) and the lowest BI value (10.53±0.32). The L* value decreased when the samples were dried at high temperatures, with the lowest two L* values being in the OD70 (76.87±0.23) and DDR (77.96±0.79) samples. In contrast, the values a*, b*, ΔE and BI increased with increasing drying temperature, all four parameters having their highest values in the OD70 and DDR samples. These research results were similar to those for Chaga mushroom (Lee et al., 2007) and button mushroom (Argyropoulos et al., 2008), with these two studies reporting that drying at high temperature resulted in decreased L* values and increased values for ΔE and BI, possibly as a result of browning pigment formation via the non-enzymatic Maillard reaction stimulated by heat.

Total phenolic and flavonoid contents of dried sclerotium

The TPC of the dried sclerotium samples was in the range 16.33±0.39 to 32.38±0.88 mg GAE/g dry basis (Table 3). The DDR sample had the highest TPC value of 32.38±0.88 mg GAE/g dry basis, followed by the OD70 (26.36±0.56 mg GAE/g dry basis), SD (18.82±0.79 mg GAE/g dry basis),

Table 2 Color, total color difference and browning index of *Lignosus rhinocerus* sclerotium samples prepared using different drying techniques

Drying technique	L*	a*	b*	Δ*E*	BI
Fresh (FSH)	92.94±0.30^{a}	0.03±0.02^{c}	9.59±0.31^{e}	0.00±0.00	10.53±0.32^{a}
Freeze drying (FD)	86.78±0.49^{b}	0.15±0.07^{c}	12.75±0.08^{d}	6.93±0.02^{d}	15.46±0.18^{b}
Sun drying (SD)	82.34±0.30^{c}	0.96±0.22^{b}	15.96±0.25^{bc}	12.41±0.13^{b}	21.67±0.46^{c}
Shade drying (SHD)	82.73±0.41^{c}	0.50±0.17^{bc}	15.46±0.15^{bc}	11.79±0.07^{c}	20.42±0.11^{c}
Oven drying at 40°C (OD40)	80.85±0.68^{d}	0.68±0.29^{bc}	16.37±0.76^{b}	13.88±0.25^{b}	22.48±0.06^{d}
Oven drying at 70°C (OD70)	76.87±0.23^{e}	2.21±0.53^{a}	18.35±0.46^{a}	18.44±0.32^{a}	28.50±0.44^{e}
Light dry roasting (LDR)	82.94±0.45^{c}	0.92±0.26^{b}	14.77±0.19^{c}	11.30±0.14^{c}	19.76±0.27^{c}
Dark dry roasting (DDR)	77.96±0.79^{e}	2.08±0.26^{a}	17.79±0.93^{a}	17.10±0.21^{a}	25.75±0.13^{e}

L^*_0, a^*_0 and b^*_0 color values of fresh sclerotium used for determining total color difference (ΔE) were 92.94 ± 0.30, 0.03 ± 0.02 and 9.59 ± 0.31, respectively.

Means ± SD (n = 3) within each row superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 3 Total phenolic content, total flavonoid content, DPPH inhibition and total triterpenoid content of *Lignosus rhinocerus* sclerotium extract prepared using different drying techniques

Drying technique	Total phenolic content (mg GAE/g dry basis)	Total flavonoid content (mg CE/g dry basis)	DPPH inhibition (%)	FRAP value (μM Fe(II)/g dry basis)
Fresh (FSH)	16.33±0.39^{e}	4.29±0.49^{e}	35.11±2.82^{b}	15.55±0.78^{d}
Freeze drying (FD)	18.57±0.90^{cd}	5.72±0.13^{de}	39.88±2.34^{b}	14.92±0.25^{d}
Sun drying (SD)	18.82±0.79^{c}	17.14±0.77^{a}	40.63±3.21^{b}	17.61±0.29^{c}
Shade drying (SHD)	17.57±0.25^{cde}	7.15±0.24^{cd}	38.94±2.07^{b}	15.09±0.31^{d}
Oven drying at 40°C (OD40)	16.57±0.69^{de}	7.34±0.17^{cd}	37.53±1.41^{b}	14.53±0.18^{d}
Oven drying at 70°C (OD70)	26.36±0.56^{b}	8.57±0.42^{c}	71.06±2.37^{a}	23.29±0.23^{b}
Light dry roasting (LDR)	17.57±0.97^{cde}	7.13±0.28^{cd}	37.76±2.19^{b}	14.36±0.21^{d}
Dark dry roasting (DDR)	32.38±0.88^{a}	14.29±0.89^{b}	70.79±2.62^{a}	31.24±0.27^{a}

GAE = Gallic acid equivalent; CE = Catechin equivalent

Means ± SD (n = 3) within each column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

FD (18.57±0.90 mg GAE/g dry basis), SHD (17.57±0.25 mg GAE/g dry basis), LDR (17.57±0.97 mg GAE/g dry basis), OD40 (16.57±0.69 mg GAE/g dry basis) and FSH (16.33±0.39 mg GAE/g dry basis) samples, respectively. The TFC of the dried sclerotium samples was in the range 4.29±0.49 to 17.14±0.77 mg CE/g dry basis (Table 3). The highest TFC was in the SD sample (17.14±0.77 mg CE/g dry basis) followed by the DDR (14.29±0.89 mg CE/g dry basis) and OD70 (8.57±0.42 mg CE/g of dry basis) samples. The FSH sample had the lowest TFC of 4.29±0.49 mg CE/g dry basis. Similar to this study, Roshanak et al. (2016) reported that the oven-dried green tea sample at 60°C had the highest TPC (209.17 mg GAE/g dry basis), while the fresh green tea sample had the lowest TPC (50.79 mg GAE/g dry basis). The FD, SD and SHD green tea samples had increased TPC values compared to the fresh sample. In tomatoes, the highest TPC was in the hot-air-dried sample, followed by the freeze-dried and fresh samples (Chang et al., 2006). Hassan et al. (2021) reported that the TPC and TFC values for boiled and roasted peanut shells of the Sodari and Ghabiash cultivars were higher than in the raw samples. Increases in the phenolic and flavonoid contents upon drying at high temperatures was probably due to the breakdown of the phenolic and flavonoid compounds in the matrix during the drying process due to the destruction of cell structure. In addition to the release of bound phenolic compounds, the TPC increased in roasted pumpkin seeds because the compounds derived from the Maillard reaction reacted with Folin-Ciocalteu reagent (Peng et al., 2021). In addition, enzyme denaturation at high drying temperatures resulted in high concentrations of phenolic and flavonoid compounds in cells, whereas the fresh samples contained active enzymes, so any biochemical reactions produced low phenolic and flavonoid compounds in the cells (Hassan et al., 2021; Roshanak et al., 2016). The increase in the flavonoid content in the SD sample could have been due to UV stimulation from sunlight. Vidović et al. (2015) reported UV-B-induced flavonoids in *Plectranthus coleoides* leaf tissue. Caldwell (1971) found that alpine plants at high altitudes and tropical plants from UV-exposed regions had higher flavonoid contents than plants from other regions. In addition, other factors, such as the thermal processing technique, heating temperature, heating time and food matrix composition, impacted the thermal stability of various flavonoids (Gao et al., 2022). According to Chaaban et al. (2017), differences in the flavonoid structure (degree and position of hydroxylation, presence of a substituent) could alter their heat processing stability. They reported that when heated to 100°C, 110°C, 120°C and 130°C, naringin had greater stability than rutin, with no degradation of naringin below 100°C and less than 2% at 100°C. Only 20% of the naringin content was reduced at 130°C. In addition, the newly formed degradation products would differ based on the type of degradation (temperature, oxygen, light) and the structure of the native flavonoid.

Antioxidant activity of dried sclerotium

The percentage inhibition in the DPPH and FRAP values represent antioxidant activities. It was found that the antioxidant activities increased with increasing temperature. The DDR and OD70 samples had the highest DPPH inhibition values of 70.79±2.62% and 71.06±2.37%, respectively, (Table 3). The FRAP values showed a similar trend to DPPH inhibition. The DDR and OD70 samples had FRAP values of 31.24±0.27 µM Fe(II)/g dry basis and 23.29±0.23 µM Fe(II)/g dry basis, respectively, (Table 3). At the same time, the FSH sample had DPPH inhibition of 35.11 ± 2.82% and a FRAP value of 15.55 ± 0.78 µM Fe(II)/g dry basis. The results from the current experiment were similar to the research results using roasted peanut kernel flour and roasted pumpkin seeds, indicating that increases in the TPC, TFC and Maillard reaction products caused an increase in DPPH inhibition at a high roasting temperature (Win et al., 2011; Peng et al., 2021). The SD sample had a high flavonoid content and moderate antioxidant activity, perhaps because the new products formed as a result of flavonoid degradation due to UV radiation from sunlight had lower antioxidant activity. Chaaban et al. (2017) discovered that the degradation products formed during flavonoid degradation influenced antioxidant activity in three ways: 1) antioxidant activity could decline because the degradation products had less antioxidant activity; 2) antioxidant activity could be maintained at a consistent level because the degradation products had the same antioxidant activity as native flavonoids; and 3) antioxidant activity could increase because the degradation products had higher antioxidant activity. Although flavonoid structural changes affect antioxidant activity, antioxidant activity is unrelated to flavonoid content; for example, the antioxidant activity of a quercetin solution remains constant after a 4 h heat treatment at 100°C, despite the quercetin being completely degraded (Ioannou et al., 2020).

Total protein, polysaccharide and triterpenoid contents of dried sclerotium

The highest protein content was obtained in the FSH sample (2.33±0.05 mg BSA/g dry basis), with the lowest for the DDR sample (1.51±0.02 mg BSA/g dry basis), as shown in Table 4. These results indicated that increasing the drying temperature in the OD70 and DDR techniques resulted in a decrease in the protein content of the sample, which was consistent with the report on button mushroom findings where high-temperature drying caused degradation and denaturation of protein (Shams et al., 2022). On the other hand, the decreases in the protein contents in the OD40, SD and SHD samples might have been due to protein oxidative degradation that was either directly or indirectly related to environmental conditions, such as UV radiation, the temperature range of 30–37°C, humidity in the sample, oxygen in the air and microbial activity during the long drying times (Davies, 2016; Chen et al., 2022). Regarding the FD technique, Roy and Gupta (2004) reported that small ice crystals and a relatively large surface area of an ice-liquid interface were formed during fast freezing, increasing the exposure of protein molecules to the ice-liquid interface and thus increasing protein damage.

The total polysaccharide content ranged from 670.79±5.76 mg glucose/g dry basis to 1,508.23±11.59 mg glucose/g dry basis (Table 4). The DDR sample had the highest total polysaccharide content of 1,508.23±11.59 mg glucose/g dry basis, followed by the OD70 sample (900.46±8.90 mg glucose/g dry basis) compared to the other drying techniques. The FSH and OD40 samples had the lowest polysaccharide contents of 674.53±8.45 mg glucose/g dry basis and 670.79±5.76 mg glucose/g dry basis, respectively. These results could imply that the high temperature affected the cell structure, causing the release of polysaccharides from the matrix. Dong et al. (2019) reported a similar result where their high-temperature microwave-dried sample at 700 W had a higher polysaccharide content than their other samples being microwave-dried at 420 W, hot-air-dried at 50°C, freeze-dried and hot air-dried at 70°C, respectively. Furthermore, the current results agreed with the report of Zheng et al. (2020), who found that the modification of the cell wall polysaccharide structure using ultrasonic-assisted extraction led to an increase in the polysaccharide yield of *Ganoderma lucidum*. However, the yield of polysaccharides was directly proportional to the increase in temperature.

The total triterpenoid content ranged from 7.25±0.44 mg ursolic acid/g dry basis to 14.52±0.57 mg ursolic acid/g dry basis (Table 4). The triterpenoid content increased with the drying temperature, with the highest amount reported in the OD40 sample (14.52±0.57 mg ursolic acid/g dry basis). Ramos et al. (2003) found that water loss and component segregation during drying reduced cell wall rigidity because the cell walls were potentially damaged or disrupted. For the reasons mentioned above, damaged or disrupted cells could affect the release of triterpenoid compounds. The various drying techniques had different effects on alterations in the cell wall structure. An increase in temperature after reaching the optimum would result in a reduction in the triterpenoid content because the higher temperature promoted the breakdown of triterpenoid compounds. Therefore, the amounts of triterpenoid content in the OD70, LDR and DDR samples were 9.11±0.72 mg ursolic acid/g dry basis, 9.53±0.31 mg ursolic acid/g dry basis and 7.67±0.43 mg ursolic acid/g dry basis, respectively.

Table 4 Total protein content, total polysaccharide and total triterpenoid content of *Lignosus rhinocerus* sclerotium extract prepared by different drying techniques

Drying technique	Total protein content (mg BSA/g dry basis)	Total polysaccharide content (mg glucose/g dry basis)	Total triterpenoid content (mg ursolic acid/g dry basis)
Fresh (FSH)	2.33±0.05^{a}	674.53±8.45^{e}	9.53±0.54^{b}
Freeze drying (FD)	1.70±0.02^{c}	724.71±6.78^{d}	8.75±0.62^{bcd}
Sun drying (SD)	1.81±0.04^{b}	780.02±4.25^{c}	7.25±0.44^{d}
Shade drying (SHD)	1.77±0.04^{b}	740.11±6.02^{d}	8.49±0.73^{bcd}
Oven drying at 40°C (OD40)	1.71±0.03^{c}	670.79±5.76^{e}	14.52±0.57^{a}
Oven drying at 70°C (OD70)	1.67±0.05^{c}	900.46±8.90^{b}	9.11±0.72^{bc}
Light dry roasting (LDR)	1.83±0.02^{b}	722.61±6.32^{d}	9.53±0.31^{b}
Dark dry roasting (DDR)	1.51±0.02^{d}	1508.23±11.59^{a}	7.67±0.43^{cd}

BSA = Bovine serum albumin

Means ± SD (n = 3) within each column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Pearson's correlation coefficients of biologically active compounds and antioxidant activity of fresh and dried sclerotium dried using various techniques

The statistical analysis in Table 5 indicates a high positive correlation between browning index and DPPH activity (r = 0.760, $p < 0.05$) and moderate positive correlations between browning index and total phenolic content, total flavonoid content, FRAP activity and total polysaccharide content (r = 0.536–0.675). These results suggested that drying a sclerotium sample at a high temperature resulted in the formation of the brown pigment produced by the Maillard reaction. Nooshkam et al. (2019) revealed that melanoidin and amadori products produced by the Maillard reaction affected antioxidant activities. A very high significant correlation was noted between the total phenolic content and DPPH (r = 0.950, $p < 0.001$) and FRAP activity (r = 0.984, $p < 0.001$). The total flavonoid content showed a low correlation with DPPH (r = 0.399), but a moderate correlation with FRAP activity (r = 0.561). According to these findings, phenolics were the predominant constituents, whereas flavonoids were minor constituents that contributed to antioxidant activity, similar to the research results reported by Azieana et al. (2017). The total protein and triterpenoid contents were negatively correlated with browning index, total phenolic content, total flavonoid content, total polysaccharide content, DPPH and FRAP activity. A high correlation between total polysaccharide content and total phenolic content was observed (r = 0.942, $p < 0.001$). The results supported the findings that polyphenols had a high correlation with the polysaccharide content in the mushroom extract (r = 0.82) and the relationship appeared linear, as was also reported by Song and Griensven (2008). Polysaccharide-polyphenol conjugates formed by H-bonding or hydrophobic interactions could be one of the factors influencing polysaccharide antioxidant activity (Li et al., 2007; Wang et al., 2016). The total polysaccharide content had a high positive correlation with DPPH (r = 0.795) and FRAP (r = 0.959, $p < 0.001$). Higher levels of polysaccharides increased ferric reduction ability but not DPPH radical scavenging activity (Table 3). These findings suggested that polysaccharides play an important role in reducing capacity rather than in free radical scavenging activity. In addition, many published research results have commented that polysaccharides contained in mushrooms and plants have the potential to be used as natural antioxidants. (Xiao et al., 2012; Liu et al., 2014; Zou et al., 2015; Mu et al., 2020).

Principal component analysis

The principal component (PC) analysis results are presented in Fig. 1. PC1 and PC2 at 66.10% and 15.20%, respectively,

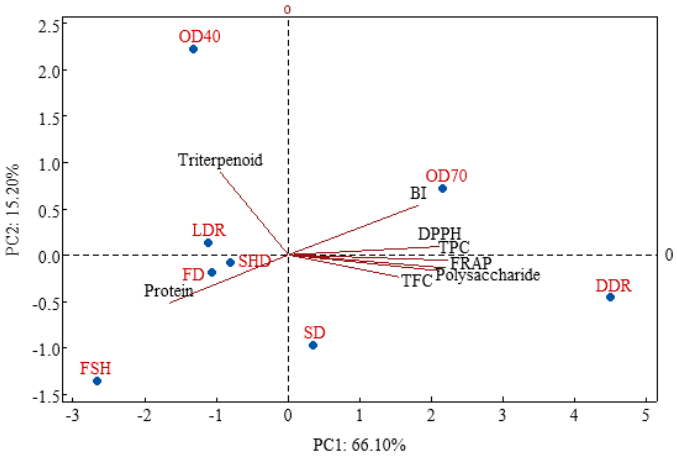

Fig. 1 Principal component (PC) analysis biplot of physio-biochemical properties of fresh and dried samples dried using various techniques, where red text indicates various drying techniques (see Table 1) and BI = browning index, DPPH = 2,2-diphenyl-1-picrylhydrazyl hydrate free radical scavenging assay, TPC = total phenolic content, FRAP = ferric reducing antioxidant power assay, and TFC = total flavonoid content

Table 5 Correlation between phenolic contents, antioxidant activity, anti-inflammatory activity and anticancer activity of fresh and dried *Lignosus rhinocerus* sclerotium samples dried using various drying techniques

	BI	TPC	TFC	DPPH	FRAP	Protein	TPS	TP
BI								
TPC	0.675							
TFC	0.548	0.505						
DPPH	0.760*	0.950***	0.399					
FRAP	0.622	0.984***	0.561	0.912**				
Protein	-0.797*	-0.610	-0.461	-0.582	-0.518			
TPS	0.536	0.942***	0.562	0.795	0.959***	-0.563		
TP	-0.020	-0.396	-0.462	-0.307	-0.397	0.058	-0.406	

BI = browning index; TPC = total phenolic content; TFC = total flavonoid content; DPPH = 2,2'-diphenyl-1-picrylhydrazyl activity; FRAP = ferric ion reducing antioxidant power; TPS = polysaccharide content; TP = triterpenoid content.
* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$)

explained 81.30% of the total variance. The DDR and OD70 samples correlated with the browning index (BI), total phenolic content (TPC), total flavonoid content (TFC), total polysaccharide content and antioxidant activity (DPPH and FRAP). The OD40 sample correlated with the total triterpenoid content. The protein content was also related to the FSH, SD, SHD, LDR and FD samples.

In conclusion, the current work focused on evaluating the impact of different drying techniques on the bioactive compound contents and antioxidant activity of tiger milk mushroom sclerotium samples. The study showed that that the different drying techniques resulted in varying the bioactive compound contents and antioxidant activity. Among the drying techniques investigated, the DDR technique resulted in higher levels of phenolic content, flavonoid content, polysaccharide content and antioxidant activity; however, the protein content was low. Thus, using the DDR technique may be the most appropriate option to preserve the quality of the sclerotium for commercialization as a medicinal mushroom powder for infusion.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was supported by the National Research Council of Thailand (NRCT, Project number 122705).

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