A comparative study of conventional and supercritical carbon dioxide extraction methods for the recovery of bioactive compound from Lion's Mane mushroom (*Hericium erinaceus*)

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Abstract. Lion's Mane mushroom (*Hericium erinaceus*), LM, is a medicinal mushroom which has various bioactive compounds within its fruiting bodies. However, during the cultivation, a large amount of the irregular-shape LM (Ir-LM) was generated. This mushroom type was considered a mushroom by-product. In this study, conventional solvent extraction (Maceration) and supercritical fluid extraction using carbon dioxide (SCFE-CO_2) were performed to compare the recovery of ergosterol, hericenone C, and hericene A content from Reg-LM and Ir-LM fruiting bodies. Furthermore, two extraction conditions (40°C at 200 bar and 70°C at 350 bar) were conducted for the SCFE-CO_2 technique. The results showed that SCFE-CO_2 methods produced a higher recovery of ergosterol and hericenone C as compared to the Maceration techniques. SCFE-CO_2 extracts were determined for their antioxidant activities. The DPPH radical scavenging activity of the extract from 70°C at 350 bar was significantly higher ($p < 0.05$) than the extract obtained from 40°C at 200 bar. The results revealed the use of green technology supercritical fluid extraction using carbon dioxide to recover bioactive compounds from mushroom by-products and apply for high-value added products.

Keyword. Lion's Mane mushroom (*Hericium erinaceus*), Ergosterol, Conventional solvent extractions, Supercritical fluid extraction using carbon dioxide, Antioxidant activity

1 Introduction

Lion's Mane mushroom (*Hericium erinaceus*), LM, is an important medicinal mushroom containing many bioactive compounds which have been used for medicines and food supplements [1]. Several bioactive compounds belonging to the genus *Hericium* have been isolated from LM mushroom [2]. The compounds indicate responses on antitumor, antibacterial, hypoglycemic and neuroprotective [3]. Gasecka *et al.* 2020 [4] reported that more than 4.5 mg/g dry weight of ergosterol was found in the fruiting bodies of fresh LM mushroom. The anti-inflammatory activity of ergosterol is correlated to the suppression of the proinflammatory cytokines including TNF-α, IL-6 and IL-1β [5]. Hericenone L isolated LM mushroom fruiting bodies show cytotoxic activity against the EC109 cell line by the MTT assay [6]. Kawagishia *et al.* 1991 [7] reported that hericenones C, D and E can stimulate the synthesis of nerve growth factor (NGF). Hericene A, one of the bioactive compounds in LM mushroom, strongly inhibited α-glucosidase activity with an IC_{50} of 6.7 μM [8].

Nowadays, mushrooms are cultivated for sustainable production and consumption, by sometimes using agricultutal waste as substrate, which fit to the concept of circular economy [9]. However, irregular-shape LM (Ir-LM), considered a by-product, was found during mushroom cultivation. These by-products are sold at low prices in the market. Aguayo *et al.* 2017 [10] reported that high amounts of button mushroom (*Agaricus Bisporus*) fruiting bodies by-product were generated during mushroom production (representing around 20% of total production). These button mushroom by-products were discarded due to their misshapen caps or stalks that do not meet the specifications set by the retailers. However, amounts of β-glucan 1.01 g/100 g dry mass can be obtained from these button mushroom by-products. β-glucan is a soluble dietary fiber that can enhance immune function and lower blood cholesterol [10]. Therefore, an investigation for alternative and profitable solutions is required. Mushroom by-products could be used for the preparation of extracts enriched in bioactive compounds that could be applied by the pharmaceutical and food industry.

Several extraction methods have been developed with the goal of producing extracts with higher yields and reduced costs. Organic solvents including methanol, ethanol, and acetone are used in extraction. The usage of

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such solvents, on the other hand, is hazardous to human health as well as the environment [11]. Supercritical fluid extraction using carbon dioxide (SCFE-CO_2) is a promising method for extracting natural oils and oleoresins. SCFE-CO_2 uses carbon dioxide as the extraction solvent under supercritical conditions. The critical temperature and pressure for carbon dioxide are 31.1 °C and 72.8 bar, which enables it to preserve the sensitive heat compounds and also their quality. Carbon dioxide is inert, non-toxic, non-corrosive, non-flammable and recyclable [12].

The optimal condition for the extraction of ergosterol from *Lentinula edodes* by SCFE-CO_2 was reported as 350 bar and 70 °C, providing the highest ergosterol at 18% (w/w) or 180 mg/g dw [13]. Prasad *et al.* (2017) [11] investigated the effect of SCFE-CO_2 on *L. edodes* extract production and antioxidant activity. They found supercritical extracts gave low yields (0.56 % when pure CO_2 was used as the solvent and 1.02 % when 5% methanol was added as a co-solvent) but produced high quality extracts [strong antioxidant activity, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, polyphenol content, and ferric reducing antioxidant power (FRAP)] with a greater number of organic compounds than extracts obtained by the solvent extraction. However, there are very few studies on the extraction of bioactive compounds from LM mushroom by SCFE-CO_2. Parada et al. 2015 [14] revealed that the temperature and pressure at 40°C, 200 bar could provide the LM extract with high antioxidant properties, while the use of 70°C, 350 bar by Morales *et al.* 2017 [13] exhibited the highest ergosterol content from shiitake mushrooms. The recovery of extracts enriched in bioactive compounds from the mushroom by-product and used as additional ingredients in foods or beverages would help value-adding the produces, reduce production and waste management costs and also environmental impact.

In this study, conventional solvent extraction (Maceration) and supercritical fluid extraction using carbon dioxide (SCFE-CO_2) were performed to compare the recovery of ergosterol content and other major compounds from Ir-LM fruiting bodies. Furthermore, two extraction conditions (40°C at 200 bar and 70°C at 350 bar) were conducted for the SCFE-CO_2 technique by varying pressure and temperature. The antioxidant capacities of the extracts from each SCFE-CO_2 condition were determined.

2 Materials and methods

2.1 Materials

Regular-shape (Reg-LM) and irregular-shape (Ir-LM) fruiting bodies of LM were purchased from Fresh and Friendly Farm Co., Ltd. at Thanyaburi district in Pathum Thani province, Thailand, and stored at -20°C in dark condition until further use. Ergosterol (95%) was obtained from Sigma-Aldrich Química (Madrid, Spain). Gallic acid (98%) was procured from Sigma-Aldrich, USA. Folin-Ciocalteu reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sisco Research Laboratories Pvt. Ltd., India. Sodium carbonate (99.5% pure) was purchased from Merck, India. Trolox reagent was obtained from M Tedia, USA. Solvents such as water (HPLC grade) and methanol (HPLC grade) were obtained from LAB-SCAN (Gliwice, Polland) and absolute ethanol was from PanReac (Barcelona, Spain). Carbon dioxide gas was supplied by the Bombay Carbon Dioxide Gas Company, Mumbai, India. The reagents and solvents were of analytical quality.

2.2 Mushroom preparation

Mushroom fruiting bodies were lyophilized by a vacuum freeze drier (GFD30S, Grisrianthong, Thailand). The freeze-dried samples were milled and sieved to the particle size of around 75-150 µm and stored in a container at -20 °C until analysis. Overview of the experiments and analyses carried out in this study for the conventional method (Maceration) and supercritical fluid extraction using carbon dioxide (SCFE-CO_2) of LM mushroom is shown in Figure 1.

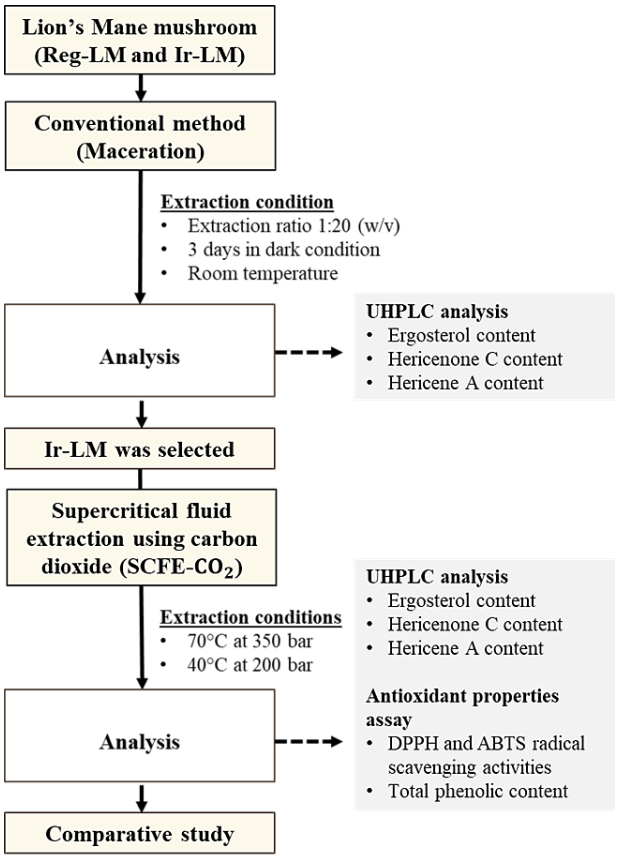

Figure 1. Overview of the experiments and analyses carried out in this study for the conventional method (Maceration) and supercritical fluid extraction using carbon dioxide (SCFE-CO_2) of Lion's Mane mushroom.

2.3 Extraction of bioactive compounds by conventional method (Maceration)

Dried powder of Reg-LM and Ir-LM, 10 g, were extracted using 200 mL absolute ethanol at room temperature (25 ±

3 °C) for 3 days in a dark condition. The sample was stored in the dark to prevent the transformation of ergosterol into vitamin D_2 or a variety of photoirradiation products such as previtamin D_2, tachysterol, and lumisterol [15]. The crude extracts were then filtered and concentrated to dryness using a rotary evaporator (R114, Buchi, Switzerland) at a controlled temperature (50 ± 1 °C) and pressure (150 bar). Ergosterol, hericenone C, and hericene A in the extract were determined and calculated for their content in the mushroom samples.

2.4 Extraction of bioactive compounds by supercritical fluid extraction using carbon dioxide (SCFE-CO_2)

Supercritical fluid extraction using carbon dioxide was used to extract the mushroom (Ir-LM). Carbon dioxide with a purity of > 99.9% was used. The extraction was carried out on Applied Separations, laboratory-scale supercritical equipment, (Allentown, PA, USA). The extractions were performed at two conditions: 40°C and 200 bar, and 70°C and 350 bar. The conditions for the extraction were from Parada *et al.* 2015 [14] and Morales *et al.* 2017 [13]. The extraction time was 40 min with a flow rate of CO_2 at 4 mL/min. Polypropylene wool and the sample were put in the vessel before being placed in the oven. The extracts were collected and stored at -20°C until further analysis. Storage at -20°C appeared to be appropriate for ergosterol extracts from mushrooms because it could reduce ergosterol oxidation [16]. The SCFE-CO_2 extracts and mushroom powder before and after the extraction were shown in Figure 2.

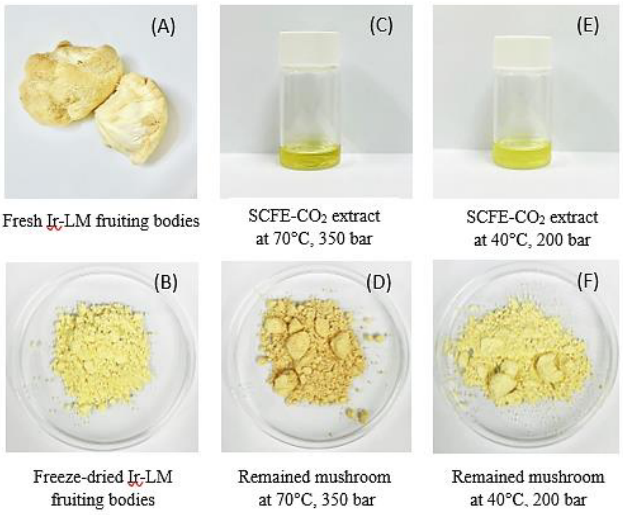

Figure 2. Fresh (A) and freeze-dried Ir-LM fruiting bodies (B); their extracts by SCFE-CO2 at 70°C, 350 bar (C), 40°C, 200 bar (E); and mushroom remained after extraction (D, F).

2.5 Analysis

2.5.1 UHPLC analysis

Determination of ergosterol, hericenone C and hericene A in the extract were performed using ultra high-performance liquid chromatography, UHPLC (Acquity Arc HPLC, waters, UK). The UV-VIS LC detector was connected to the UHPLC system, which was equipped with an Aquity-C18 (100 x 3 mm) analytical column. The mobile phase consisted of a mixture of methanol and water at a ratio of 98:2. The chromatograms were detected at 282 nm. The biologically active compounds content in the LMs as mg/g extract and mg/g dried LM calculated as mg of each bioactive compound (calculated from the calibration curve of standard ergosterol) in 1 g of the extract or 1 g of dried LM.

2.5.2 DPPH scavenging activity

To determine the antioxidant capacity of SCFE-CO_2 extracts, DPPH radical scavenging assay was adopted from a previous study with minor modification [17]. Briefly, DPPH radical solution was prepared by dissolving DPPH in absolute methanol at a concentration of 0.5 mM. The experiments were conducted in a 96-well plate. In each well, an aliquot (150 µL) of the DPPH solution was added to 50 µL of each extract. The plate was vigorously shaken and incubated in the dark for 30 min at room temperature. Trolox was used to constructing a calibration curve. The absorbance of the solution was measured on a microplate reader at 540 nm. All the experiments were performed in triplicates and calculated as mg TE /g dried LM: The scavenging capacity was calculated using Eq. 1.

$$\%\ Inhibition = [(A_{\text{control}} - A_{\text{sample}}) / A_{control}] \times 100 \quad (1)$$

Where $A_{control}$ is the absorbance of the control that contained all reagents except the samples. A_{sample} is the absorbance of the mushroom extracts with reagents added.

2.5.3 ABTS scavenging activity

The ABTS radical scavenging assay was modified slightly from Ahmed et al. 2012 [18]. ABTS was prepared by mixing ABTS with $K_2S_2O_8$ solution and allowed the mixture to react at room temperature for 16 h. The ABTS solution was diluted with methanol to obtain the absorbance of 1.1-1.2 at 750 nm. One hundred microliters of ABTS solution were mixed with 50 µL of the extract in 96-well microtiter plates. The absorbance was monitored for 30 min at room temperature. Methanol was used as a blank. All measurements were performed in triplicate. The antioxidant activity was determined from the calibration curve of Trolox and calculated as mg TE/g dried LM followed Eq. 1.

2.5.4 Total phenolic content (TPC)

Total phenolic content (TPC) of SCFE-CO_2 extracts (40°C, 200 bar and 70°C, 350 bar) was evaluated using the Folin-Ciocalteu reagent. A 96-well microplate reader (Bio-Rad, iMark, USA) was used to determine the TPC value by measuring the absorbance at 750 nm. Gallic acid was used as a standard, and the results were calculated as gallic acid equivalents (GAE)/g of dry weight sample [19]. All experiments were performed in triplicates.

2.6 Statistical analysis

Data were analysed using the SPSS 11.6 for Windows (SPSS Inc.) by one-way analysis of variance (ANOVA). An independent t-test was used to compare the means of each treatment and the Scheffe test at $p < 0.05$ significance level was applied to compare the differences between each sample.

3 Results and discussion

3.1. Extraction of bioactive compounds by a conventional method

The content of ergosterol, hericenone C, and hericene A content in the extract from LM mushrooms are presented in Table 1. The Maceration of the LM mushroom revealed that Ir-LM contained significantly higher ($p < 0.05$) ergosterol content (2.09 ± 0.03 mg/g dried LM) than that of Reg-LM (1.74 ± 0.02 mg/g dried LM). Therefore, Ir-LM was selected for further study using SCFE-CO_2 at varying extraction times and temperatures. Ir-LM is considered as by-product because their inferior morphology (including misshapen caps) does not meet the specifications set by retailers, and is sold at low prices in the mushroom market. Therefore, alternative solutions like the SCFE-CO_2 methodology are required to increase the value of these mushroom by-products from the mushroom farm. However, the Reg-LM was not selected for SCFE-CO_2 in this research because the regular-shape LM can be sold fresh at a high price.

3.2 Extraction of bioactive compounds by supercritical fluid extraction using carbon dioxide

The extraction of bioactive compounds in Ir-LM obtained from Maceration (Table 1) and SCFE-CO_2 extraction (Table 2) methods were compared. The comparison study was performed based on the content of each specific bioactive compound including ergosterol, hericenone C, and hericene A which appeared in the extracts from both maceration and SCFE-CO_2 methods. The information is presented as mg/g of dried LM and mg/g of extract.

When expressing the results in mg/g dried LM, the extract from Maceration presented higher ergosterol content (2.09 ± 0.03 mg/g dried LM) than those of SCFE-CO_2 extracts, 1.11 ± 0.01 mg/g dried LM and 1.10 ± 0.01 mg/g dried LM, for 70°C at 350 bar and 40°C at 200 bar, respectively. This result might be caused by the extraction time of SCFE-CO_2 used in this study (40 min). However, further investigation on the effects of extraction time is required. Parada *et al.* 2015 [14] revealed that the extraction yield of Ir-LM extracted by SCFE-CO_2 (20 MPa, 40 °C) with pure CO_2 was increased with an increase in the extraction time (during 2 h). Longer extraction times allow supercritical fluid carbon dioxide (SCF-CO_2) to penetrate deeper into the sample matrix, resulting in greater solute dissolution and interaction with SCF-CO_2 over time [20].

Interestingly, ergosterol content in the Ir-LM extract obtained from SCFE-CO_2 (Table 2) at 70°C, 350 bar was the highest (61.15 ± 0.11 mg/g extract) when expressing the results as mg/g extract, followed by SCFE-CO_2 at 40°C, 200 bar (60.19 ± 0.31 mg/g extract) and Maceration (22.17 ± 0.31 mg/g extract). The contents of hericenone C and hericene A in the extracts obtained from both Maceration and SCFE-CO_2 also showed the same trend with the ergosterol content. Besides, Ir-LM extract obtained from SCFE-CO_2 for 70°C at 350 bar exhibited the highest hericenone C (43.35 ± 0.06 mg/g extract) and hericene A (6.53 ± 0.04 mg/g extract) content, followed by SCFE-CO_2 for 40°C at 200 bar (Table 2). The results represented that SCFE-CO_2 yielded a higher purity of ergosterol, hericenone C, and hericene A than the use of Maceration. The possible reason for the lower purity of bioactive compounds in the Ir-LM extract obtained by Maceration might be because the polar solvent (ethanol) used for the extraction can also be polyphenols or other compounds [21]. HPLC chromatograms of ergosterol standard and the extracts of Ir-LM by both extraction techniques are shown in Figure 3.

Table 1. Ergosterol, hericenone C, and hericene A concentrations in regular-shape (Reg-LM) and irregular-shape (Ir-LM) Lion's Mane mushroom extracted by conventional method (Mar).

Dried sample	Extract (g)	Extraction yield (%)	Bioactive compound	mg/g dried LM	mg/g extract
Reg-LM-Mar 10 g	1.04	10.40	Ergosterol	1.74 ± 0.02^{b}	15.72 ± 0.17^{b}
			Hericenone C	0.44 ± 0.01^{c}	3.99 ± 0.06^{c}
			Hericene A	0.03 ± 0.01^{f}	0.26 ± 0.01^{e}
Ir-LM-Mar 10 g	0.90	9.41	Ergosterol	2.09 ± 0.03^{a}	22.17 ± 0.31^{a}
			Hericenone C	0.35 ± 0.01^{d}	3.74 ± 0.14^{c}
			Hericene A	0.07 ± 0.01^{e}	0.74 ± 0.01^{d}

Different superscripts in the same column of each unit mean a significant difference at $p < 0.05$

Table 2. Ergosterol, hericenone C, and hericene A concentrations in irregular-shape Lion's Mane (Ir-LM) mushroom extracted by supercritical fluid extraction using carbon dioxide (SCFE-CO_2).

Dried sample	Extract (mg)	Extraction yield (%)	Bioactive compound	mg/g dried LM	mg/g extract
Ir-LM-SCFE-CO_2 (70°C, 350 bar) 3 g	52.20	1.74	Ergosterol	1.11 ± 0.01^{a}	61.15 ± 0.11^{a}
			Hericenone C	0.79 ± 0.01^{b}	43.35 ± 0.06^{b}
			Hericene A	0.12 ± 0.01^{d}	6.53 ± 0.04^{d}
Ir-LM-SCFE-CO_2 (40°C, 200 bar) 3 g	52.46	1.75	Ergosterol	1.10 ± 0.01^{a}	60.19 ± 0.31^{a}
			Hericenone C	0.32 ± 0.01^{c}	25.81 ± 0.08^{c}
			Hericene A	0.09 ± 0.01^{e}	4.75 ± 0.01^{e}

Different superscripts in the same column of each unit mean a significant difference at $p < 0.05$

The extraction of non-polar molecules is ensured by SCFE-CO_2 because supercritical fluid carbon dioxide (SCF-CO_2) is non-polar [12]. However, the CO_2 molecule

at the critical state exhibits low polarity due to the presence of a quadrupole moment. Thus, it can dissolve some polar compounds at strong extraction conditions [22]. Ergosterol has four ring structure with a hydrophilic hydroxyl, and an acyl side chain [23]. Song et al. 2009 [18] reported that SCFE-CO_2 exhibits dominance in adjustable polarity, and has the ability to extract ergosterol from *Ganoderma lucidum*.

There are very few reports on the extraction of polar compounds using carbon dioxide in a supercritical state as in SCFE-CO_2, due to the fact that SCF-CO_2 is a non-polar solvent. However, SCF-CO_2 might dissolve the slight polar compound like ergosterol under specific conditions of temperature and pressure. These are two possible reasons to support the result, firstly, the presence of a quadrupole moment in the CO_2 molecule at the supercritical state. Secondly, under appropriate conditions, the high vapor pressure of polar solute may significantly affect the SCFE-CO_2 and increase the extractive efficiency and the extract properties [18,22-24]. In addition, Wrona et al. 2019 [25] postulated that the extraction of polar compounds by non-polar solvent may be induced by the diffusion mechanism of bioactive chemicals from the internal sections of the plant matrix due to the high pressure and temperature used.

Another possible reason for higher bioactive compound contents in SCFE-CO_2 from 70°C at 350 bar is an increase in pressure increases the density of SCF-CO_2 which in turn increase the solvation capacity of SCF-CO_2 and leads to solubilization of more solute. Higher pressure extraction reduces the intermolecular distances between the solute and SCF-CO_2, allowing a higher mass transfer between them [26].

The effect of temperature and pressure on the color of SCFE-CO_2 extracts and remaining mushrooms after extraction were obvious as presented in Figure 2. Lower temperature and pressure (40°C, 200 bar) gave a lighter yellow-color extract (Figure 2E) and remaining mushroom (Figure 2F), while higher temperature and pressures (70°C, 350 bar) gave deep yellow-color extract (Figure 2C) and remaining mushroom (Figure 2D). Chang *et al.* 2021 [27] reported that stir-fried LM mushroom had significantly higher ($P < 0.05$) L* and +a* values than microwaved, steamed, and boiled cooking techniques. This could be due to the formation of Maillard reaction products. Besides, heat treatment might also induce non-enzymatic oxidative changes in polyphenol, resulting in the development of darker compounds [28]. This might be the possible reason for the darker color shown in the mushroom cake extracted by SCFE-CO_2 at 70°C, 350 bar.

Nevertheless, one of the major chromatogram peaks (Rt = 5.368) observed in Ir-LM extract from Maceration did not appear in both SCFE-CO_2 chromatograms as presented in Figure 3. This peak appeared at a shorter retention time (Rt) than hericenone C, ergosterol, and hericene A peak that represented a high polar compound. Reversed phase-ultra-high-performance liquid chromatography exhibits a strong attraction between the polar solvent and polar molecule in the mixture being passed through the column. Therefore, the polar molecules move through the column more quickly than non-polar compounds [29]. To extract this compound (Rt = 5.368) by SCFE-CO_2, the extraction temperature and pressure should be optimized or the modifiers such as ethanol could be used to increase the polarity of the solvent mixture to extract higher polar compounds including ergosterol.

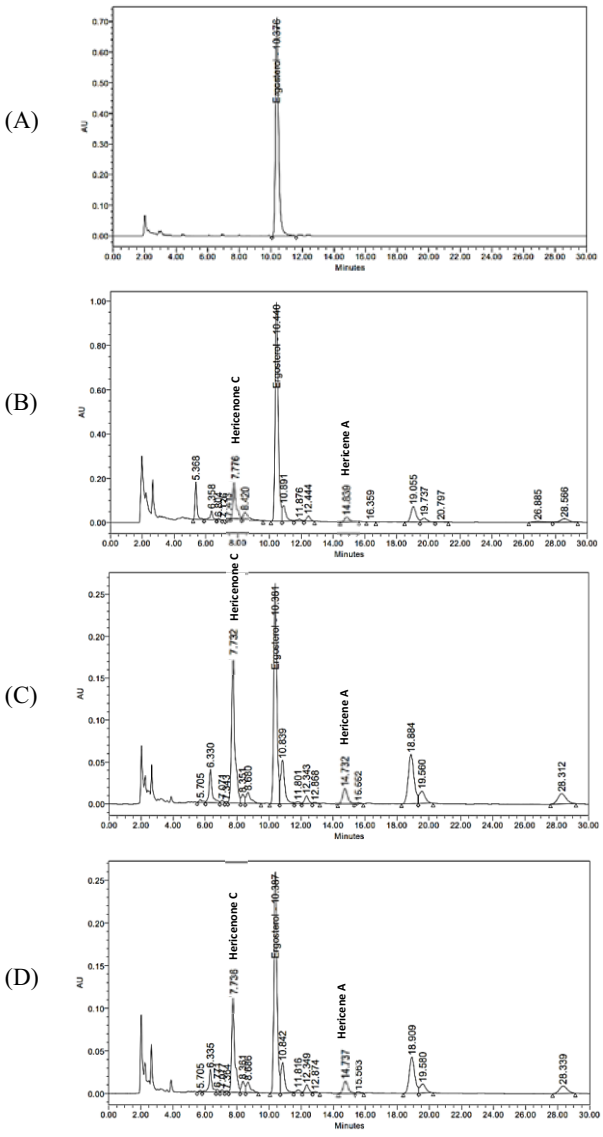

Figure 3. HPLC chromatogram of ergosterol standard (A), bioactive compounds extracted from Ir-LM by Maceration (B), SCFE-CO_2 at 70°C, 350 bar (C), and SCFE-CO_2 at 40°C, 200 bar (D).

Modifiers cause the sample matrix to swell, resulting in the increased mass transfer rate between the solute and solvent. Modifiers can also enhance the extract flow better by lowering its viscosity. All these factors work together to increase yield [30]. Selectivity of the supercritical extraction fluid is the main factor in determining the functional qualities of the final extract. Changing the extraction conditions, such as temperature, pressure, and type and concentration of co-solvent could allow a selective extraction of the desired compounds from biomass [31].

Mazzutti *et al.* 2012 [32] used supercritical fluid extraction with pure CO_2 and CO_2 plus 2.5 %, 5.0 %, and 10.0 % (w/w) ethanol as a co-solvent to extract *Agaricus brasiliensis* mushrooms. They discovered that the optimal conditions for obtaining high yields (1.19 %) using pure CO_2 were 30 MPa and 323.15 K. The extraction yields were much higher, reaching 4.2 % when 10% ethanol was used as a co-solvent to recover polar molecules. Ergosterol extraction from *Pleurotus pulmonarius* mushrooms was investigated using the SCFE-CO_2 [33]. Compared to ultrasound-assisted extraction with water as solvent (UAEW) (0.9 mg/g) and SCFE-CO_2 without co-solvent, the results showed that SCFE-CO_2 with co-solvent (5.0 % EtOH) led to the best acceptable approach to generate the extracts rich in ergosterol content, reaching a value of 40.1 mg/g.

3.3 Antioxidant activity and TPC of Ir-LM extract obtained from SCFE-CO_2

DPPH, ABTS scavenging ability, and total phenolic contents of SCFE-CO_2 extracts are revealed in Table 3. The mushroom extract by SCFE-CO_2 was diluted with absolute methanol to determine their antioxidant properties. The Ir-LM extract obtained from SCFE-CO_2 at 70°C at 350 bar (0.09 ± 0.01 mg TE/g dried LM) was found significantly higher in DPPH radical scavenging activity than the extract obtained at 40°C, 200 bar (0.07 ± 0.01 mg TE/g dried LM). The IC_{50} by DPPH of both SCFE-CO_2 extracts were 10.26 mg/ml and 12.99 mg/ml of SCFE-CO_2 with 70°C at 350 bar and 40°C at 200 bar, respectively. However, no significant difference in ABTS scavenging ability and total phenolic contents between both SCFE-CO_2 extracts was observed (Table 3).

Table 3. The antioxidant activities and total phenolic content of Ir-LM were extracted by supercritical fluid extraction using carbon dioxide (SCFE-CO_2).

Extract	DPPH (mg TE/g dried LM)	ABTS (mg TE/g dried LM)	Total phenolic content (mg GAE/g dried LM)
Ir-LM-SCFE-CO_2 (70°C, 350 bar)	0.09 ± 0.01^{a}	0.11 ± 0.01^{a}	0.20 ± 0.01^{a}
Ir-LM-SCFE-CO_2 (40°C, 250 bar)	0.07 ± 0.01^{b}	0.11 ± 0.01^{a}	0.19 ± 0.01^{a}

Different superscripts in the same column mean significant difference at $p < 0.05$
DPPH: 2,2-diphenyl-1-picrylhydrazyl
ABTS: 3-ethylbenzthiazoline-6-sulphonic acid

Dupont *et al.* 2021 [34] described that the presence of two double bonds in the B-ring of ergosterol could confer antioxidant properties. The antioxidant activity of the lipophilic fraction of button mushrooms was shown to be dominated by ergosterol [35]. Recently, there is an increasing interest in bioactive compounds from natural sources including antioxidants from Sacha Inchi oil [36-37], bamboo mushroom [38] and LM obtained from maceration [39-40]. The antioxidant activities of both SCFE-CO_2 extracts might depend on the ability of other compounds in the crude extracts including phenolic compounds. Total phenolic contents were found in both Ir-LM extract obtained from SCFE-CO_2 with 70°C, 350 bar (0.20 ± 0.01 mg GAE/g dried LM) and the extract obtained from SCFE-CO_2 at 40°C, 200 bar (0.19 ± 0.01 mg GAE/g dried LM).

4 Conclusion

Irregular-shape Lion's Mane mushroom extracts obtained by supercritical extractions from 70°C at 350 bar presented the highest purity of the ergosterol, hericenone C, and hericene A contents when compared to the extract obtained by SCFE-CO_2 from 40°C at 200 bar and the conventional solvent extraction or Maceration technique. The extracts obtained by SCFE-CO_2 from 70°C at 350 bar also showed a higher antioxidant activity determined by the DPPH method than the extract obtained from 40 °C at 200 bar. However, the SCFE-CO_2 method with two extraction conditions used for the extraction of bioactive compounds in this research proved to be more promising than conventional methods used in the purification of interesting compounds in Ir-LM. This research enables the extraction of bioactive compounds from mushroom by-products by SCFE-CO_2, a green extraction technology, for industrial scale applications.

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