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# Superior component compositions and antioxidant activity of Volvariella volvacea oil compared to those of Agrocybe cylindracea and two Lentinula edodes oils

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# Abstract

The biological activity of an oil not only depends on its fatty acid composition but also the lipid composition and trace components. In this paper, to select the optimal mushroom oil, the component compositions (fatty acids, lipids, polyphenols, flavones, tocopherols, and unsaponifiable matters) and antioxidant activities in vitro of four mushroom oils (*Agrocybe cylindracea*, two *Lentinula edodes*, and *Volvariella volvacea*) were investigated and compared. The results showed that the four tested oils had the same fatty acid composition in different amounts, but the lipid component, minor components, and free radical scavenging activity in the tested oils varied widely depending on the type of mushroom. Overall, *Volvariella volvacea* oil was considered superior to the other three tested oils, as it had the largest contents of polar lipids, diglycerides, polyunsaturated fatty acids (74.38%), unsaponifiable matter (319.09 mg/kg), total phenols (124.08 mg/100g), tocopherols (139.86 mg/100g), as well as the highest ABTS and FRAP values (349.45 and 3801.70  $\mu$ mol Trolox/100g). This finding suggests that *Volvariella volvacea* oil is a promising resource that should be further researched.

#### KEYWORDS

antioxidant capacity in vitro, lipid composition, minor elements, mushroom oil

# 1 | INTRODUCTION

With the increasing desire for greater sustainability and food security, more and more new resources have been developed to enrich food diversity (Rosmiza et al., 2016); thus, mushrooms have gained attention because of their high nutritional value (rich in protein, carbohydrates, edible fiber, vitamins, and phytosterols), medicinal activity (antitumor, hipocolesterolemic, antioxidant, antifungal, and antimicrobial), and industrial application (Kitzberger et al., 2007, 2009; Sugui et al., 2003). Apart from their appearance as food ingredients at dinner tables, mushrooms are also applied in cosmetics (Wu et al., 2016), health care (Lu et al., 2020), and pharmaceutical fields (Valverde et al., 2015). At present, the mushroom industry is regarded as important for rural areas to prosper because it benefits agriculture and increases farmers' income in many countries. Therefore, it is necessary to discuss any approach to enhance the added value of edible mushrooms.

Given the early studies on their chemical composition and nutritional qualities, mushrooms are labeled "high in proteins, vitamins, fibre and minerals" but "low in calories, fats, and essential fatty acids" (Agrahar-Murugkar & Subbulakshmi, 2005; Sanmee et al., 2003). Compared with macronutrients such as polysaccharides, polypeptides, and dietary fiber, oil (or lipid), accounting for 1.75–15.5% of the dry weight of mushrooms (Hong et al., 1988), is often ignored by researchers, resulting in insufficient studies. However, oil (or lipid) is an important carrier of fat-soluble active constituents (i.e.,

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tocopherols, ergosterols, terpenoids, and so on), which play an important role in the function of mushrooms, as the fat-soluble components of mushrooms have been reported to present biological activities, such as antioxidant, antitumor, and immunomodulatory activities. Therefore, to evaluate the medicinal value of mushrooms comprehensively, it is essential to study mushroom oil.

Regarding the existence of fat-soluble active constituents, the biological activity of mushroom oil is also separately affected by these trace concomitant contents, in addition to being affected by fatty acid and lipid composition (Zheng et al., 2017). Hence, when we need to explore the potential application value of mushroom oil, it is crucial to investigate the extract composition and content of the lipids and microcomponents in mushroom oil.

Early in 2013, L.edodes constituted 22% of the world's total output and was affirmed as the most widely cultivated mushroom (Royse et al., 2017). However, during the initial cultivation period, L.edodes was only cultivated in certain regions with suitable climate, its limited production could not meet the increasing demand of people, and it was the shortage of L.edodes that intensified the expansion of cultivation areas. Flower mushrooms are the product of abnormal growth of L. edodes, and unsuitable planting environmental conditions (i.e., low temperature and humidity) could promote the transformation of L. edodes to flower mushrooms. In contrast to conventional L.edodes, which have smooth pileipellis, flower mushrooms are produced only when the fleshy cells of *L.edodes* rupture to form chrysanthemum-like pilei (Cao, 2000). Although flower mushrooms are 5-8 times more expensive than conventional L.edodes, they are still preferred by global consumers owing to the higher nutritional value and superior characteristics (thick pilei, tender meat, and beautiful appearance) (Liu et al., 2022). With an output accounting for 5% of the world's total production, Volvariella volvacea is deemed the second most widely cultivated mushroom. Similar to L.edodes, Volvariella volvacea and Agrocybe cylindracea have also recently been accepted by more and more consumers because of their delicious taste and high nutritive value (Jia et al., 2020). Therefore, based on their enormous output and high popularity, these four mushrooms (Agrocybe cylindracea, L. edodes, flower mushroom, and Volvariella volvacea) occupy a pivotal position in the mushroom industry.

In this study, oils of Agrocybe cylindracea, conventional L. edodes, flower mushroom, and Volvariella volvacea were extracted to investigate their exact nutritional composition and free radical scavenging ability. By comparing all tested indicators, the oil with the highest development value was selected, providing theoretical data for the future application of mushroom oil.

# 2 | MATERIALS AND METHODS

#### 2.1 | Materials

Dried mushrooms (Agrocybe cylindracea, conventional L. edodes, flower mushroom, and Volvariella volvacea) were provided by Xi'an ZHONGKEDA Biotechnology Co., Ltd (a company selling plant extracts and animal extract production specifically). Standards of α-, γ-, and δ-tocopherols, Trolox, and cholestan-3-ol (purity >98%) were purchased from Toronto Research Chemicals Inc (Shanghai, China). The antioxidant reagents 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), and tripyridyltriazine (TPTZ) were provided by Sigma-Aldrich Chemical Co., Ltd. (Shanghai, China), while the other reagents and solvents were offered by Shanghai Maclin Biochemical Technology Co., Ltd.

# 2.2 | Mushroom oil preparation

The solvent extraction method was used to prepare the mushroom oil. Specifically, the mushroom sample was washed, dried, and smashed through an 80-mesh sieve to extract oil using petroleum ether (boiling range,  $60-90^{\circ}$ C) at a ratio of 4:1 (V/W), after 4h of oil extraction at room temperature, and the mushroom oil was mainly distributed into the supernatant, which needed to be isolated from the mushroom sediment by centrifugation. After removing the solvent from the supernatant, mushroom oil was obtained and stored at -20°C for analysis.

### 2.3 | Oil Characteristics

# 2.3.1 | Oil yield

Oil yield (%) was calculated by the mass ratio of oil to the mushroom sample after oil extraction.

# 2.3.2 | Lipid separation

According to Heinzelmann et al. (2014), a silica gel column was applied to separate the polar lipids (phospholipids and glycolipids) from the neutral lipids (glycerides) of mushroom oil, namely the polar lipids and neutral lipids of mushroom oil were separated by a silica gel column ( $30 \times 400$  mm) containing approximately 35 g active silica gel. After adding 25 mg/mL oil chloroform solution into the column, chloroform, acetone, and methanol were used to wash the column in turn to obtain fractions of glycerides, glycolipids, and phospholipids, respectively.

# 2.3.3 | Fatty acid composition

GC-MS was applied to determine the fatty acid composition of the mushroom oil and the procedures were performed according to Kang et al. (2019) with some modifications. In brief, a 20mg oil sample and 3mL sodium methoxide solution (0.5 M) were mixed together to enable saponification for 30min at 70°C, and then, 1mL boron trifluoride diethyl ether solution was added into the mixture for water bath heating at 80°C for 30min, which enhanced the production of the fatty acid methyl ester. After the alkaline reaction liquid cooled

to room temperature, 3 mL of heptane was added to extract the fatty acid methyl ester from mixture. After the evaporation of solvent, the dried oil extract was re-dissolved, filtered (13 mm, 0.22  $\mu$ m), and injected into the GC–MS for fatty acid analysis.

GC-MS for determining the fatty acid composition was equipped with a DB-5 column  $(30 \text{ m} \times 0.25 \text{ µm})$ , and the temperatures of the injector and detector were both set at 290°C. At a sample loading of 1.0 µL, the sample was analyzed at a split ratio of 100/1 and an oven temperature program containing  $180^{\circ}$ C (2 min),  $10^{\circ}$ C/min to 230°C (1 min), 3°C/min to 236°C (1 min), 6°C/min to 260°C (2 min), and 5°C/min to 270°C (2 min). Temperatures of ion source and transmission line were 250°C and 280°C, respectively, for El lonization mode at a scan range of 100-350 amu.

# 2.3.4 | Lipid composition

A Thermo Scientific UltiMate 3000 HPLC equipped with an ACQUITY UPLC BEH C18 column ( $100 \text{ mm} \times 2.1 \text{ mm}$ ,  $1.8 \mu \text{m}$ , Waters, UK) and a high-resolution tandem mass spectrometer Q Exactive (Thermo Scientific) was used to analyze triacylglycerol composition of the lipid composition of the mushroom oils. After setting the injection volume, column oven temperature, and flow rate at  $4 \mu L$ ,  $35^{\circ}$ C,

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and 0.4 mL/min, respectively, the oil sample was tested by a gradient elution program containing mobile phase A (water, 0.1% formic acid) and mobile phase B (acetonitrile, 0.1% formic acid). The exact elution conditions were as follows: 0–0.5 min, 5% B; 0.5–7 min, 5% to 100% B; 7–8 min, 100% B; 8–8.1 min, 100% to 5% B; and 8.1–10 min, 5% B.

The mass spectrometer was operated in both positive and negative ion modes. At maximum injection times of 100 ms and 80 ms, precursor spectra (70–1050 m/z) and fragment spectra were collected at resolutions of 70,000 and 17,500, respectively, to hit AGC targets of  $3e^6$  (precursor) and  $1e^5$  (fragment). DDA mode was brought to acquire data in the top three configurations, and the stability of the UPLC-MS was evaluated by a quality control sample after every 10 tested samples.

# 2.3.5 | Total phenol, flavone, and tocopherol content

0.5 mL of 90% aqueous methanol was used to extract total phenol and flavone from 50 mg of mushroom oil successively three times, and the methanol supernatant was collected to evaporate to dryness. After redissolving the dryness into 0.5 mL of methanol, the mixed methanol solution (i.e., the oil methanol extract) was filtered

**TABLE 1** Oil yield and lipid category distribution of four test mushroom oils.

	Mean $\pm$ SD ( $n = 3$	3)		
%	Agrocybe cylindracea	Conventional Lentinula edodes	Flower mushroom	Volvariella volvacea
Oil yield	$2.98 \pm 0.24^{a}$	$2.17\pm0.11^{\text{b}}$	$1.84 \pm 0.07^{\circ}$	$1.31\pm0.02^d$
Glycerides	$81.63 \pm 3.50^{a}$	$85.04 \pm 3.79^{a}$	$84.53 \pm 0.96^{a}$	$67.44 \pm 5.96^{b}$
Glycolipids	$15.84 \pm 1.30^{b}$	$12.35 \pm 2.74^{\circ}$	$12.92 \pm 2.66^{c}$	$29.53 \pm 0.18^{a}$
Phospholipids	$2.53\pm0.06^{\rm b}$	$2.61 \pm 1.04^{b}$	$2.56 \pm 1.01^{\text{b}}$	$3.02 \pm 0.67^{a}$

*Note*: Datas with different superscript letter mean a significant difference (p < .05) betwwen two samples, while that with same letter signifys no significant difference.

### TABLE 2 Fatty acid compositions of four test mushroom oils.

	Content (%)/m	nean $\pm$ SD ( $n = 3$ )						
	Agrocybe cyli	ndracea			Conventional	Lentinula edode	S	
Fat acid	Glycerides	Glycolipids	Phospholipids	Oil	Glycerides	Glycolipids	Phospholipids	Oil
C <sub>14:0 (Myristic)</sub>	$0.83 \pm 0.22$	nd	nd	$0.42 \pm 0.16$	$0.81 \pm 0.03$	$2.70 \pm 0.02$	nd	$0.71 \pm 0.27$
C <sub>16:0 (Palmitic)</sub>	$13.36\pm0.05$	$17.44 \pm 0.05$	$24.67 \pm 0.66$	$15.13 \pm 0.03$	$12.76 \pm 0.23$	$27.19\pm0.17$	$17.38 \pm 0.17$	$16.01 \pm 0.02$
C <sub>16:1 (Palmitoleic)</sub>	nd	nd	nd	nd	$0.74 \pm 0.15$	nd	nd	nd
C <sub>18:0 (Stearic)</sub>	$1.27 \pm 0.32$	$2.42 \pm 0.09$	$10.03 \pm 2.07$	$1.55\pm0.36$	$1.35\pm0.20$	$2.22 \pm 0.59$	$3.21 \pm 0.63$	$1.54 \pm 0.03$
C <sub>18:1 (Oleic)</sub>	$17.90\pm0.11$	$20.51\pm0.02$	$24.95 \pm 0.60$	$19.51\pm0.08$	$9.16 \pm 0.26$	$9.20 \pm 0.07$	$9.12 \pm 0.07$	$10.18\pm0.06$
C <sub>18:2 (C9, t11)</sub>	$34.02 \pm 0.19$	$30.68 \pm 0.02$	$17.07 \pm 0.28$	$32.38 \pm 0.13$	$34.72 \pm 0.30$	$23.12 \pm 0.13$	$29.57 \pm 0.13$	$32.46 \pm 0.08$
C <sub>18:2 (C9, 12)</sub>	$32.62 \pm 0.18$	$28.96 \pm 0.04$	$23.28 \pm 0.53$	$31.00\pm0.07$	$40.46 \pm 0.33$	$35.57 \pm 0.20$	$40.72\pm0.26$	$39.10 \pm 0.13$
SFAs	$15.47 \pm 0.47$	$19.86 \pm 0.04$	$34.70 \pm 1.41$	$17.11 \pm 0.27$	$14.92 \pm 0.32$	$32.11 \pm 0.40$	$20.59 \pm 0.46$	$18.26 \pm 0.26$
MUFAs	$17.90 \pm 0.11$	$20.51\pm0.02$	$24.95 \pm 0.60$	$19.51 \pm 0.08$	$9.89 \pm 0.19$	$9.20 \pm 0.07$	$9.12 \pm 0.07$	$10.18 \pm 0.06$
PUFAs	$66.64 \pm 0.36$	$59.63 \pm 0.05$	$40.35 \pm 0.81$	$63.38 \pm 0.19$	$75.18 \pm 0.36$	$58.69 \pm 0.33$	$70.29 \pm 0.39$	$71.56 \pm 0.21$

Abbreviations: Glycer: Glycerides; Glyco: Glycolipids; Phospho: Phospholipids.

2.3.6

through a hydrophilic syringe membrane filter (0.45 µm) for analysis (Grajzer et al., 2020). The spectrophotometric determination of total phenol and total flavones was carried out according to Choo et al. (2007) and Siger et al. (2008), respectively. A 0.1g/mL oil n-hexane solution was filtered for tocopherol analysis, which requires an HPLC device equipped with a silica gel column ZORBAX RX-SIL (4.6 mm×250 mm, 5 µm) and ultraviolet detector (Cunha et al., 2006). At a flow rate of 1mL/min, the temperature of the column was set at 35°C, with n-hexane/isopropanol/ glacial acetic acid at 99/0.2/0.8 (V/V/V) as the only mobile phase, and 10 µL of sample solution was analyzed by a 20 min isocratic elution procedure. The tocopherol content in mushroom oil was deter-After saponifying the mushroom oil by alkaline hydrolysis, the mixture containing alcoholic potassium hydroxide was extracted by nhexane to obtain unsaponifiable matter. To ensure full saponification was detected at 734 nm. and precise quantification, the oil sample (0.2g) was mixed with 3 mL of potassium hydroxide solution (2 M) and 0.5 mL of internal standard ( $5\alpha$ -cholestane, 0.1 mg/mL) and heated for 30 min at 80°C. The hexane solvent was evaporated to dryness and 200µL BSFA-TMCS was added for silanization. Based on the internal standard method of quantitative analysis, GC-MS was applied to analyze the exact

#### 2.3.7 | Antiradical scavenging activity

composition of the unsaponifiable matter.

mined by the external standard method.

Unsaponifiable matter

Spectrophotometry was used to detect the antiradical scavenging activity of mushroom oil, which was evaluated comprehensively by three indicators: DPPH radical scavenging activity, ABTS radical cation decolorization assay, and the reducing ability of ferric ions in this research. With Trolox methanol solutions at different concentrations as antioxidant standards, all antioxidant indicators of mushroom oil in vitro were quantified by Trolox.

Measurement of DPPH radical scavenging activity was operated according to Nafis et al. (2019) with slight modification. Equal volumes of oil methanol extract (0.2 g/mL) and DPPH-methanol solution (0.4 mmol/L) were mixed in the dark for 30 min. With Trolox methanol solutions (10-180µmol/L) as standards, the DPPH radical scavenging activity of the oil sample was measured by the absorbance at 517 nm.

The ABTS radical cation decolorization assay of mushroom oil was performed based on a study by Huang et al. (2021) with a slight change. A working solution containing ABTS (3.5 mmol/L) and potassium persulfate (1.3 mmol/L) was stored at room temperature for 12-16h before use. After adjusting the absorbance at 734nm to 0.70, the working solution (200µL) was mixed with oil methanol extract (0.2g/mL,  $10\mu L$ ) and reacted for 20min at 30°C, and Trolox methanol solution (200-800 µmol/L) was also used to quantify the ABTS radical cation decolorization assay of the oil sample, which

The reducing ability of ferric ions in mushroom oil was measured according to the modified method of Zouirech et al. (2022). Briefly, a working solution was prepared by mixing sodium acetate trihydrate acetic acid solution (0.3 mol/L), TPTZ hydrochloric acid (10 mmol/L), and FeCl<sub>2</sub> solution (20mmol/L) at ratio of 10:1:1. Similar to the operation of ABTS, the reducing ability of ferric ions in mushroom oil was calculated by the absorbance at 593 nm, after mixing 200 µL of working solution and 10µL of oil methanol extract at 37°C for a 10min reaction.

#### 2.3.8 Statistical analysis

All experiments were performed in triplicate, and the results were calculated as the means and standard deviations. SPSS Statistics

Flower mushro	om			Volvariella Volv	/acea		
Glycerides	Glycolipids	Phospholipids	Oil	Glycerides	Glycolipids	Phospholipids	Oil
$0.73 \pm 0.19$	$1.15 \pm 0.33$	nd	$0.72 \pm 0.18$	$1.91 \pm 0.01$	$2.51 \pm 0.02$	$3.52 \pm 0.28$	$2.34 \pm 0.01$
$15.82 \pm 0.20$	$17.79 \pm 0.07$	$19.27 \pm 0.03$	$13.68 \pm 1.22$	$14.43 \pm 0.07$	$16.54 \pm 0.02$	$34.17 \pm 0.22$	$17.61 \pm 0.04$
nd							
$1.51 \pm 0.10$	$1.84 \pm 0.13$	$6.01 \pm 0.12$	$1.18 \pm 0.54$	$2.01 \pm 0.01$	$1.76\pm0.10$	$6.24 \pm 0.60$	$1.82 \pm 0.44$
$14.09\pm0.05$	$21.68\pm0.08$	$15.74\pm0.04$	$18.08 \pm 1.11$	$3.43 \pm 0.03$	$3.64 \pm 0.03$	$10.99 \pm 0.60$	$3.86 \pm 0.02$
$31.52 \pm 0.03$	$31.17\pm0.11$	$29.08 \pm 0.05$	$30.17 \pm 1.71$	$8.53 \pm 0.04$	$8.75 \pm 0.05$	nd	$8.38 \pm 0.03$
$36.34 \pm 0.06$	$26.37 \pm 0.09$	$29.90 \pm 0.04$	$36.14 \pm 4.60$	$69.70 \pm 0.13$	$66.80 \pm 0.04$	$66.80 \pm 0.04$	$66.00 \pm 0.37$
$18.06 \pm 0.05$	$20.78 \pm 0.28$	$25.28 \pm 0.11$	$15.48 \pm 0.44$	$18.35 \pm 0.08$	$20.81 \pm 0.11$	$43.94 \pm 0.36$	$21.76 \pm 0.41$
$14.09\pm0.05$	$21.68\pm0.08$	$15.74 \pm 0.04$	$18.08 \pm 1.11$	$3.43 \pm 0.03$	$3.64 \pm 0.03$	$10.99 \pm 0.06$	$3.86 \pm 0.02$
$67.85 \pm 0.09$	$57.54 \pm 0.20$	$58.98 \pm 0.08$	$66.47 \pm 0.19$	$75.22\pm0.11$	$75.55 \pm 0.08$	$45.08 \pm 0.30$	$74.38 \pm 0.40$

software was applied to find differences in data between groups, and *p*-values <.05 were regarded as statistically significant.

# 3 | RESULTS AND DISCUSSIONS

### 3.1 | Oil yield and distribution of lipid category

As shown in Table 1, the oil yield of the four mushrooms ranged from 1.31 to 2.98%. Similar to glycerides, polar lipids containing glycolipids and phospholipids were also regarded as the main lipid composition of mushroom oil because of the 14.96-32.55% percentage, conforming to the low lipid content (Vidović et al., 2011) and high polar lipid proportion (Hanuš et al., 2008) in most mushrooms. *Agrocybe cylindracea* had the highest oil yield (2.98%), while *Volvariella volvacea* had the lowest oil content (1.31%). Even so, *Volvariella volvacea* oil containing 29.53% glycolipids and 3.02% phospholipids was still believed to have a better emulsifying property, which was reported to be positively correlated with the percentage of polar lipids in the oil.

# 3.2 | Fatty acid composition

Although the oil yield of mushrooms was low, the mushroom oils were still abundant in unsaturated fatty acids (Yilmaz et al., 2006). According to Table 2, four tested mushroom oils were identified to possess seven fatty acids, where three fatty acids containing C16:0 (12.76-34.17%), C18:1 (3.43-24.95%), and C18:2 (40.35-75.55%) occupied the dominant position. In spite of the same molecular composition, the content of fatty acids varied from different lipid fraction as well as the mushroom species. For example, glycerides are apt to possess more polyunsaturated fatty acids (PUFAs) than polar lipids, and saturated fatty acids are more easily enriched in phospholipids sections apart from flower mushroom oil. More specifically, Agrocybe cylindracea contained 66.64%, 59.63%, 40.35%, and 63.38% PUFAs in glyceride, glycolipid, phospholipid, and whole oil, respectively. Compared to the 63.38-71.56% PUFA content in most tested mushroom oils, the 74.38% PUFA content endowed Volvariella volvacea oil with a special activity benefit to people who have hypertension, coronary heart diseases, ulcerative colitis, rheumatoid arthritis, chronic obstructive pulmonary diseases, and Crohn's disease (Fan & Chapkin, 1998; Kavishree et al., 2008).

# 3.3 | Composition of glycerides, glycolipids, and phospholipids

The exact lipid molecular compositions of the four mushroom oils were detected by UPLC/MS, 62 glycerides, 7 glycolipids, 7 phospholipids, and 3 sterols were identified, as shown in Table 3. The area normalization method was applied to quantify the different lipid components, and glycerides with more than 95% content, were thought to be the majority of four mushroom oils, which were found to represent a larger percentage of phospholipids than glycolipids.

### 3.3.1 | Analysis of glyceride composition

A total of 21 triglycerides (TAGs), 9 diglycerides (DAGs), 8 monoglycerides (MAGs), 10 free fatty acids (FFAs), and 14 ether-glycerides were detected in the tested mushroom oils. But the shared lipid components were made up of 7 FFAs, 5 TAGs, 5 DAGs, 3 MAGs, and 3 ether-DAGs. More FFAs (63.73-70.26%) were found than TAGs (13.22-18.44%), and linoleic acid (C18:2) at 33.35%-54.41% content was taken as the most important FFA of the four mushroom oils, conforming to the results above in Section 3.2 and a previous report (Gunc Ergonul et al., 2013; Kavishree et al., 2008). Similar to the FFAs distribution, the four mushroom oils also had the same dominant DAGs (i.e., C15:1/18:2) at 30.08-46.55%. Apart from FFA and DAG, the main ingredient of other glycerides section varied by mushroom species. For example, the TAG and ether-DAG with the largest contents in Volvariella volvacea oil were C16:1/18:2/20:3 (34.58%) and C o-19:2/18:3 (27.51%), respectively, while those in conventional L. edodes oil were C15:0/18:2/18:3 (23.00%) and C o-19:2/18:2 (33.92%).

Overall, DAG content and ether-glyceride (at 7.64–17.59% and 2.07–13.76% content) were found to be the most variable components of glyceride among the four oils. For example, the largest content of DAG (17.59%) but the lowest ether-glyceride (2.07%) appeared in *Volvariella volvacea* glyceride, but the opposite situation was only shown in conventional *L. edodes* glyceride. As ether-glyceride and DAG were mainly applied as cellular mediators (Snyder et al., 2002) and antiobesity substances (Lee et al., 2020), respectively, it was natural to assume that conventional *L. edodes* oil and *Volvariella volvacea* oil performed different functionalities, that is, cell signaling regulation and weight loss. Mushroom glycerides were first reported to contain ether-glyceride at a content of 2.07–13.76% and conventional *L. edodes* oil was proved to be an excellent source of ether-glyceride.

# 3.3.2 | Analysis of glycolipid, phospholipid, and sterol composition

Three minor lipids (≤2.10%) containing glycolipids, phospholipids, and sterols of the tested mushroom oils were analyzed in Table 3b, and the exact molecular compositions and contents were detected in different species. However, in any case, glyceryl phosphatide, cerebroside (Cer), and phytosterol esterified with linoleic acid (C18:2) were the main polar lipids of mushroom oils. Compared to other mushroom oils, *Volvariella volvacea* oil was discovered to have more diverse polar lipid molecules, that is, 6 glycolipids, 5 phospholipids, and 3 sterol esters. More phospholipids than glycolipids and

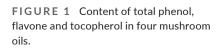
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Con	Content (%)/mean $\pm$ SD ( $n=3$ )	i±SD (n=3)														G et <i>i</i>
Ŷ	RT	MS2Metabolite	m/z	AC	LE	FM	^>	٩	RT	<b>MS2Metabolite</b>	m/z	AC	LE	FM	^^	۸L.
(a) G	slycerides com	(a) Glycerides compositions of four test mushroom oils $(\%)$	mushroom	oils (%)												
	Glycerides			$96.00\pm4.07^a$	$97.26 \pm 4.86^{a}$	$96.15 \pm 2.83^{a}$	$94.61\pm2.07^a$									
	TAG			$18.44 \pm 1.03^{a}$	$13.22 \pm 0.53^{\circ}$	$17.39\pm1.81^{\rm ab}$	$16.52\pm1.82^{\rm b}$									
1	7.09	15:0_18:2_18:3	856.74	9.03±0.26 <sup>bc</sup>	$23.00 \pm 2.55^{a}$	$10.44 \pm 0.76^{b}$	$8.76 \pm 1.28^{\circ}$	12	7.99	$18:1\_18:1\_19:2$	914.81	pu	$3.63 \pm 0.67$	pu	pu	
2	7.32	15:1_16:0_18:2	832.73	$1.94\pm0.3^{d}$	$5.60 \pm 1.47^{\rm a}$	$2.41\pm0.10^{\circ}$	$3.71 \pm 0.64^{b}$	13	8.01	16:0_18:2_18:2	872.77	$4.61 \pm 0.33^{b}$	$2.12\pm0.11^{\circ}$	pu	$12.25 \pm 0.58^{a}$	
ო	7.48	15:0_18:1_18:2	860.77	$7.83 \pm 0.49^{b}$	$21.51 \pm 1.26^{a}$	8.40±0.65 <sup>b</sup>	pu	14	8.07	18:1_18:2_21:2	940.83	$6.48 \pm 0.13^{a}$	pu	$3.51 \pm 0.19^{\rm b}$	nd	
4	7.51	15:1_16:0_18:1	834.75	$2.07\pm0.17^{c}$	$6.79 \pm 0.41^{a}$	$2.51 \pm 0.20^{b}$	pu	15	8.17	$18:1\_18:1\_18:2$	900.79	$10.78 \pm 0.94^{a}$	$4.51\pm0.07^{\circ}$	$9.34 \pm 0.34^{a}$	$5.06 \pm 0.38^{\rm b}$	
2	7.58	$18:2_18:3_19:1$	910.78	nd	$2.64 \pm 0.16$	pu	pu	16	8.19	16:0_18:1_18:2	874.78	$4.90 \pm 0.30^{a}$	$2.1\pm0.37^{c}$	pu	$3.18 \pm 0.21^{\rm b}$	
9	7.63	18:2_18:2_18:3	894.75	nd	pu	pu	$14.89 \pm 0.76$	17	8.25	18:1_18:2_21:1	942.84	$5.96 \pm 0.16$	nd	pu	pu	
~	7.65	16:0_17:1_18:1	862.78	$3.65 \pm 0.78^{\rm b}$	$7.27 \pm 1.43^{a}$	pu	pu	18	8.35	16:0_18:1_20:2	902.81	$7.81 \pm 1.45^{a}$	$2.61 \pm 0.16^{\circ}$	pu	$3.74 \pm 0.18^{b}$	
8	7.76	18:1_18:2_19:2	912.79	nd	$5.93 \pm 0.29$	pu	pu	19	8.35	16:0_18:1_18:1	876.79	$3.88 \pm 0.27^{\rm b}$	nd	$7.39\pm1.33^{\rm a}$	$2.92\pm0.11^{\circ}$	
6	7.79	16:1_18:2_20:3	896.77	$12.44 \pm 0.41^{\circ}$	$5.54\pm0.07^{d}$	$15.76 \pm 0.34^{\rm b}$	$34.58 \pm 1.90^{a}$	20	8.62	18:0_18:2_30:5	1062.94	$3.41 \pm 0.38^{a}$	pu	$3.52 \pm 0.35^{a}$	pu	
10	7.90	15:0_18:2_18:2	858.75	nd	pu	pu	$2.6 \pm 0.17$	21	8.77	18:0_22:3_26:3	1064.95	$2.12 \pm 0.16^{a}$	nd	$2.6\pm0.21^a$	pu	
11	7.98	$18:1_18:2_18:2$	898.78	$13.09 \pm 0.61^{a}$	$6.71 \pm 0.90^{d}$	$12.12 \pm 0.13^{\rm b}$	$8.3 \pm 0.39^{\circ}$									
	DAG			$8.93 \pm 0.19^{\rm b}$	$7.64 \pm 0.55^{b}$	$8.32\pm0.35^{\rm b}$	$17.59\pm2.33^a$									
1	4.83	DG 15:1_18:2	594.51	$30.08 \pm 2.72^{\rm b}$	$46.55 \pm 2.90^{a}$	$42.68 \pm 2.16^{a}$	$42.05 \pm 5.53^{a}$	9	6.41	DG 18:1_18:2	636.55	$16.65 \pm 0.50^{a}$	$11.97 \pm 0.79^{\circ}$	$13.59 \pm 0.30^{b}$	$7.86 \pm 1.27^{d}$	
2	5.27	DG 15:1_18:1	596.52	$12.67 \pm 0.41^{\circ}$	$20.39\pm1.47^{\mathrm{a}}$	$15.44 \pm 0.56^{b}$	$4.52\pm1.63^{\rm d}$	~	6.55	DG 19:0_20:3	678.60	$5.98 \pm 0.16^{a}$	pu	$4.42\pm0.16^{\rm b}$	pu	
ო	6.13	DG 18:2_18:2	634.54	$8.03 \pm 0.37^{b}$	$5.12\pm0.32^{d}$	$6.60 \pm 0.48^{\circ}$	$31.41 \pm 2.76^{a}$	8	6.69	DG 18:1_18:1	638.57	$8.50 \pm 0.46^{a}$	$4.51\pm0.18^{\rm b}$	$8.44 \pm 0.11^{a}$	pu	
4	6.28	DG 21:1_18:3	676.58	$4.55 \pm 0.10$	pu	pu	pu	6	8.13	DG 18:2_21:2	676.60	$10.30 \pm 1.84^{a}$	$11.50 \pm 0.48^{a}$	$8.84 \pm 1.33^{ab}$	$6.58 \pm 0.56^{\rm b}$	
ŝ	6.40	DG 16:0_18:2	610.54	$3.23 \pm 0.14^{\rm b}$	pu	pu	$7.55 \pm 0.51^{a}$									
	MAG			$0.39 \pm 0.01^{\rm b}$	$0.50\pm0.00^{ab}$	$0.59 \pm 0.00^{a}$	$0.19\pm0.02^{\circ}$									
1	3.08	MG 16:0	348.33	nd	$13.75 \pm 0.43$	pu	pu	5	3.66	MG 22:3	426.36	$36.13 \pm 2.09^{b}$	$17.33 \pm 0.61^{\circ}$	$31.27 \pm 1.29^{b}$	$48.57 \pm 2.18^{a}$	Food
2	3.27	MG 18:1	374.33	$11.36 \pm 1.21^{a}$	$5.21 \pm 0.39^{b}$	$9.87 \pm 0.55^{a}$	pu	9	3.83	MG 21:2	414.35	pu	pu	$8.54 \pm 0.52$	pu	d Sci
ო	3.32	MG 16:0	348.33	nd	<b>4.96±0.96</b>	pu	pu	~	4.27	MG 22:2	428.37	$22.53 \pm 1.46^{a}$	$12.03 \pm 0.4^{\rm b}$	$25.63 \pm 0.29^{a}$	$17.34 \pm 3.94^{ab}$	ence
4	3.65	MG 21:2	414.34	nd	pu	pu	$18.52 \pm 0.91$	8	4.73	MG 24:2	456.40	$29.93 \pm 0.18^{\rm b}$	$46.75 \pm 0.86^{a}$	$24.51 \pm 0.09^{\circ}$	$15.55 \pm 1.59^{d}$	& N
	FFA			$67.92 \pm 3.23^{ab}$	$64.93 \pm 4.3^{\rm b}$	$70.26 \pm 0.88^{a}$	63.73±5.99 <sup>b</sup>									utriti
1	2.65	FA 18:3	277.22	$2.82 \pm 0.28^{b}$	$8.31 \pm 0.55^{a}$	$1.96\pm0.10^{\circ}$	$9.15 \pm 0.93^{a}$	9	3.53	FA 18:1	281.25	$37.65 \pm 0.48^{a}$	$35.63 \pm 1.31^{a}$	$38.33 \pm 0.85^{a}$	$18.44 \pm 1.25^{\rm b}$	ion C
2	2.87	FA 16:1	253.22	$1.88\pm0.17^{ab}$	$1.91\pm0.12^{\rm a}$	$1.76 \pm 0.09^{b}$	$1.03 \pm 0.27^{c}$	7	4.27	FA 18:0	283.26	$14.98 \pm 0.44^{\rm b}$	$7.94\pm0.22^{c}$	$16.71 \pm 0.49^{a}$	$2.56\pm0.13^{\rm d}$	pen Acc
ო	2.97	FA 18:2	279.23	$35.51 \pm 0.72^{\circ}$	$40.29 \pm 1.39^{\rm b}$	33.35±0.73 <sup>c</sup>	$54.41 \pm 1.75^{a}$	8	6.08	FA 24:0	367.35	$1.78 \pm 0.02^{a}$	$1.27 \pm 0.04^{b}$	$1.79 \pm 0.12^a$	$2.03\pm\!0.18^a$	ess
4	3.15	FA 15:0	241.22	nd	pu	pu	$1.23 \pm 0.06$	6	6.28	FA 25:0	381.37	pu	pu	pu	$1.7 \pm 0.31$	W
5	3.46	FA 16:0	255.23	$5.31 \pm 0.62^{b}$	$3.6\pm0.06^{\circ}$	$5.14 \pm 0.49^{b}$	$7.65 \pm 0.33^{a}$	10	6.48	FA 26:0	395.38	pu	$1.06 \pm 0.09^{b}$	$0.94\pm0.06^{\circ}$	$1.86\pm0.32^{\rm a}$	IL
	Ether Glycerides	es		$4.39 \pm 0.11^{\rm b}$	$13.76 \pm 0.29^{a}$	$3.44\pm0.13^{\circ}$	$2.07 \pm 0.09^{d}$									EY-
1	6.12	0-8:0_10:0_16:1	617.51	pu	pu	pu	$15.53 \pm 0.73$	œ	6.95	0-19:2_17:2	620.56	$7.83 \pm 0.54^{a}$	3.04±0.22 <sup>b</sup>	$7.46 \pm 0.55^{a}$	7.53±0.55a (Continues)	273

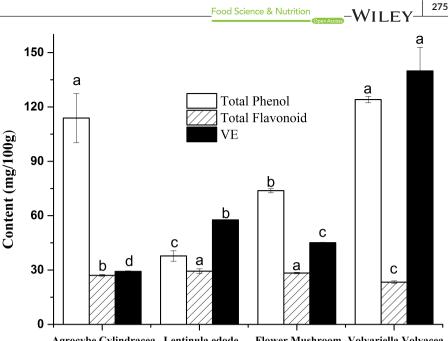
TABLE 3 Lipid molecular composition of four test mushroom oils.

2048717, 2024, 1, Downloaded from https://olinetibrary.wiley.com/toi/0.1027a3.3750 by Deutsche Zeatral Bibliothek Fore Medizin. Medizinische Abt, Wiley Online Library on [07/03/2024]. Se the Terms and Conditions (https://olinetibrary.wiley.com/toins) on Wiley Online Library for rules of use; O A articles are governed by the applicable Creative Commons. License

TAI	TABLE 3 (Cor	(Continued)													
Con	Content (%)/mean $\pm$ SD (n= 3)	±SD (n=3)													
Ŷ	RT	MS2Metabolite	m/z	AC	LE	FM	^^	٩	RT	<b>MS2Metabolite</b>	m/z	AC	LE	FM	>
7	6.52	O-8:0_18:2_18:3	749.62	$13.72 \pm 0.12^{\rm b}$	pu	$15.83 \pm 0.56^{a}$	pu	6	6.8	0-19:2_18:3	632.56	$15.15 \pm 1.19^{\rm b}$	$31.47 \pm 0.21^{a}$	9.63±0.69 <sup>c</sup>	$27.51 \pm 1.96^{a}$
ო	7.04	O-8:0_18:1_18:2	753.65	$6.91 \pm 0.34^{b}$	pu	$8.55\pm0.30^{a}$	pu	10	7.06	0-19:2_18:2	634.57	$22.29 \pm 2.23^{\rm b}$	$33.92 \pm 0.31^{a}$	$18.05 \pm 2.17^{\rm b}$	nd
4	6.65	0-19:2_16:3	604.53	pu	pu	pu	$11.93 \pm 0.61$	11	7.34	0-19:1_18:2	636.59	8.46±0.68 <sup>b</sup>	$14.52 \pm 0.07^{a}$	$9.27 \pm 1.34^{b}$	pu
5	6.8	0-19:2_16:2	606.54	pu	$1.8 \pm 0.05$	pu	pu	12	7.61	O-19:1_18:1	638.6	pu	$3.47\pm0.12^{a}$	$3.57 \pm 0.25^{a}$	pu
9	7.08	O-19:2_16:1	608.56	pu	6.22±0.07	pu	pu	13	7.27	0-22:6_21:2	710.61	$25.86 \pm 3.57^{a}$	$2.12\pm0.24^{b}$	$27.42 \pm 5.11^{a}$	$22.25 \pm 4.34^{a}$
7	7.35	O-19:1_16:1	610.57	pu	$3.4 \pm 0.08$	pu	nd	14	7.09	0-22:6_22:3	722.6	pu	pu	pu	$15.24 \pm 1.09$
) (q)	Glycolipid, phos	(b) Glycolipid, phospholipid, and sterol compositions of four mushroom lipids (%)	ompositio	ns of four mushra	om lipids (%)										
	Glycolipids			$0.91 \pm 0.06^{b}$	$0.54\pm0.03^{\circ}$	$0.91 \pm 0.08^{b}$	$1.41\pm0.06^{a}$								
-	2.39	MGDG 0-4:0_14:1	577.35	pu	pu	pu	$1.69 \pm 0.07$	2	6.20	MGDG 0-13:0_15:0	683.50	pu	$6.74 \pm 0.28^{\circ}$	$17.85 \pm 2.47^{a}$	7.83±1.05 <sup>b</sup>
7	4.42	SMGDG 0-8:0_18:0	711.43	pu	$7.75 \pm 0.59^{a}$	pu	$1.62 \pm 0.20^{b}$	9	6.75	DGDG O-8:0_22:1	871.57	$15.93 \pm 2.32^{a}$	pu	pu	pu
т	4.74	MGDG 0-9:0_18:2	701.45	pu	pu	$82.02 \pm 2.47^{a}$	$84.63 \pm 0.73^{a}$	7	7.70	DGDG O-27:0_26:0	1195.94		pu	pu	$1.98 \pm 0.44^{a}$
4	6.06	MGDG O-9:0_18:3	663.45	$84.13 \pm 2.30^{a}$	85.60±0.87 <sup>a</sup>	pu	2.20±0.27 <sup>b</sup>								
	Phospholipids	6		$1.64\pm0.23^{\rm bc}$	$1.88\pm0.05^{ m b}$	$2.01 \pm 0.02^{a}$	$1.40\pm0.23^{\circ}$								
1	1.82	PE-Cer 15:1;20/14:1	587.42	$20.21 \pm 1.33^{\circ}$	24.86±1.56 <sup>b</sup>	$25.02 \pm 1.98^{ab}$	$26.78 \pm 3.77^{a}$	5	5.97	Cer-NS 21:3;20/18:2 600.53	600.53	pu	pu	$15.9 \pm 0.37^{\rm b}$	$38.82 \pm 2.12^{a}$
7	3.33	SM 8:0;20/20:4	613.44	$13.37 \pm 0.8^{a}$	pu	pu	$11.82 \pm 3.02^{b}$	9	6.06	Cer-BS 19:1;20/16: 2;(30H)	562.48	pu	pu	$19.8 \pm 0.55^{a}$	pu
ო	4.06	PE-Cer 16:2;20/13:0	587.41	pu	pu	pu	9.09±3.46	2	6.24	Cer-BS 19:1;20/16: 2;(30H)	562.48	66.44±0.56 <sup>b</sup>	$75.12 \pm 1.46^{a}$	$39.31 \pm 2.29^{\circ}$	pu
4	5.72	SM 44:3;30	855.70	nd	pu	pu	$13.4\pm1.64$								
	<b>Sterols ester</b>			$1.58\pm0.23^{\rm a}$	$0.36 \pm 0.05^{\rm b}$	$1.52 \pm 0.29^{a}$	$1.57 \pm 0.25^{a}$								
1	8.30	BRSE 28:2/18:2	678.61	$60.47 \pm 2.47^{a}$	$60.38 \pm 0.33^{a}$	$58.94 \pm 1.82^{a}$	$34.07 \pm 3.32^{b}$	с	8.62	CASE 28:1/18:1	682.64	$4.64 \pm 0.28^{b}$	$5.86\pm0.02^{ab}$	$6.63 \pm 1.78^{a}$	$3.01 \pm 0.41^{\circ}$
7	8.43	CASE 28:1/18:2	680.63	$34.84 \pm 2.26^{b}$	$33.81 \pm 0.37^{\rm b}$	$34.46 \pm 0.53^{\rm b}$	$62.91 \pm 3.73^{a}$								
Noté Abb	e: Datas with c reviations: AC	Note: Datas with different superscript letter mean a significant difference (p < .05) betwwen two samples, while that with Abbreviations: AC, Agrocybe cylindracea; FM, Flower mushroom; LE, conventional Lentinula edodes; VV, Volvariella volvacea.	ot letter r <i>cea</i> ; FM,	nean a significa Flower mushroo	nt difference (, <i>m</i> ; LE, convent	p < .05) betww tional Lentinula	edodes; VV, V	es, wh olvarie	ile that Ila volvo	Note: Datas with different superscript letter mean a significant difference ( $p < .05$ ) betwwen two samples, while that with same letter signifys no significant difference. Abbreviations: AC, Agrocybe cylindracea; FM, Flower mushroom; LE, conventional Lentinula edodes; VV, Volvariella volvacea.	nifys no s	ignificant diffe	rence.		

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Agrocybe Cylindracea Lentinula edode Flower Mushroom Volvariella Volvacea Mushroom Oils

sterol-esters were found to exist in most mushroom oils except for *Volvariella volvacea* oil, which presented glycolipids, phospholipids, and sterol-esters at similar proportions.

Volvariella volvacea oil contained Cer-NS 21:3;2O/18:2 and CASE 28:1/18:2 as its main ingredients of phospholipids and sterols, which was different from other mushroom oils with the same main components of phospholipids and sterols, that is, Cer-BS 19:1;2O/16:2;(3OH) and BRSE 28:2/18:2. However, this situation changed when discussing the glycolipid content in mushroom oils, as MGDG O-9:0/18:3 was the main glycolipid of *Agrocybe cylind-racea* and conventional *L. edodes* oils, while MGDG O-9:0/18:2 was main glycolipid of *flower mushroom* and *Volvariella volvacea* oils. On the whole, *Volvariella volvacea* oil obviously had a higher content and molecular composition of polar lipids (5.39%, 14 molecules) than the other oils (*flower mushrooms*, approximately 4%, 9 molecules at most), consistent with the results described in Section 3.1.

### 3.4 | Trace components

# 3.4.1 | Total phenol, flavone, and tocopherol contents

Although phenolic compounds, flavones, tocopherols, and sterols were poorly soluble in oil, they are still regarded as effective antioxidants responsible for the storage stability of high-PUFA oil. Tocopherol, a fat-soluble vitamin, has often been studied owing to its antioxidant activity; however, in four tested mushroom oils,  $\alpha$ -tocopherol was identified as the only tocopherol analog. According to Figure 1, the four mushroom oils were discovered to have greater total phenol and tocopherol contents than flavone, which varied less from mushroom species. Distinguished from two mushroom oils

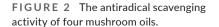
(conventional *L. edodes* and *flower mushroom* oils) containing flavone at 29.390 mg/100 g and 28.34 mg/100 g, *Volvariella volvacea* oil appeared to have the lowest flavone content (23.28 mg/100 g) but the highest amount of total phenol (124.081 mg/100 g) and tocopherol (139.86 mg/100 g) among the four tested oils. Therefore, based on the large amount of antioxidants (total phenols and tocopherol), *Volvariella volvacea* oil would be bound to exhibit stronger free radical scavenging ability.

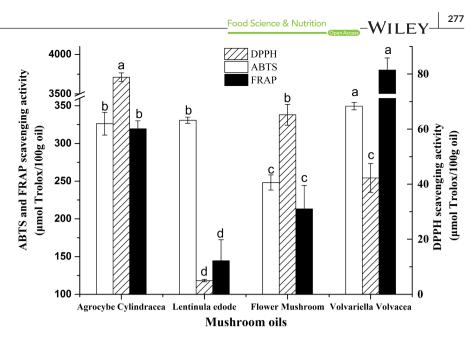
# 3.4.2 | Unsaponifiable matters

Oil unsaponifiable matters are some substances that cannot be saponified by strong basic solutions in oil, with the sterol component as the main component, oil unsaponifiable matter has often been studied, as sterol is often reported to present strong activities of lowering blood LDL-cholesterol (Products and Allergies, 2014). GC-MS was applied to detect the unsaponifiable matter in four mushroom oils, which were quantified by internal standard  $5\alpha$ -cholestane. According to the data in Table 4, 22 unsaponifiable matters containing 20 sterols and 2 nonsterols were identified in the four mushroom oils. The maximum amount of unsaponifiable matter existed in Volvariella volvacea oil (319.09 mg/kg), followed by Agrocybe cylindracea (183.59 mg/kg) and conventional L. edodes (190.60 mg/kg) oils. Egosterol and its analog were confirmed as the principal sterol components, as ergosterols in Volvariella volvacea, conventional L. edodes, Agrocybe cylindracea, and flower mushroom oils occupied 75.98%, 71.80%, 60.98%, and 56.26% of the unsaponifiable matter respectively, endowing Volvariella volvacea oil with optimal activity to improve osteoporosis (Morales et al., 2017). Moreover, stigmasterols and lanosterols were also found in four mushroom oils at amounts of 21.63-40.01 mg/kg and 2.70-7.56 mg/kg, and the existence of nonsterols such as

Ĭ		כטוווףטאונוטווא טו נווד מוואמאטווווומטוב ווומנובו טו וטמו נבאר ווומאווו טטווו טווא (וווצ/ גגן/		ו חו וחמו ובצר ווומ		115/ NS/.								
Cor	itent (mg	Content (mg/kg)/mean $\pm$ SD (n = 3)												I
°z	RT	Name	AC	LE	FM	٨٧	Ŷ	RT	Name	AC	LE	FM	^^	
		Ergosterol and its analog	$111.95 \pm 1.00^{\circ}$	$136.86 \pm 12.46^{\rm b}$	$48.08 \pm 2.42^{d}$	$242.45 \pm 2.51^{a}$								
7	11.4	Ergosta-5,7,9(11),22-tetraen- 3-ol, (3β,22E)-	pu	$9.85 \pm 0.91^{a}$	$0.93 \pm 0.14^{b}$	$9.29 \pm 0.04^{a}$	9	14.85	5.XiErgost-7-en-3β-ol, TMS derivative	$26.01 \pm 0.25^{b}$	$36.60 \pm 3.31^{a}$	pu	pu	
7	13.44	Ergosterol	$55.51 \pm 0.43^{\rm b}$	$59.20 \pm 5.46^{b}$	$0.64 \pm 0.00^{\circ}$	$117.67 \pm 1.04^{a}$	7	16.32	9,19-Cyclolanost-24-en-3-ol,3β, TMS derivative	$3.57 \pm 0.06^{a}$	$1.74 \pm 0.02^{b}$	$0.74 \pm 0.16^{\circ}$	$1.57 \pm 0.09^{b}$	
т	14.04	Ergosta-7,22-dien-3-ol, (3β,22E)-, TMS derivative	13.91±0.16 <sup>b</sup>	$12.51 \pm 1.25^{b}$	$13.85 \pm 0.70^{b}$	$21.59 \pm 0.21^{a}$	œ	17.51	9,19-Cycloergost-24(28)- en-3-ol, 4,14-dimethyl-, acetate, $(3\beta, 4\alpha, 5\alpha)$ -	2.50±0.08 <sup>b</sup>	pu	$2.26 \pm 0.48^{b}$	4.3±0.03 <sup>a</sup>	Open Access
4	14.35	Ergost-8(14)-en-3-ol, 3β-, TMS derivative	$2.21\pm0.06^{\circ}$	3.00±0.29 <sup>c</sup>	$23.41 \pm 1.23^{b}$	73.38±0.59ª	6	17.75	Ergost-25-ene-3,5,6,12-tetrol, (3β,5α,6β,12β)-	nd	pu	$0.13 \pm 0.18^{\rm b}$	$3.57 \pm 0.24^{a}$	
2	14.58	Ergosta-5,8-dien-3-ol, $3\beta$ -	$8.24\pm0.10^{\mathrm{ab}}$	$9.27\pm0.93^{ab}$	$6.12 \pm 0.24^{\rm b}$	$11.08\pm2.89^a$								
		Stigmasterol and its analog	$40.01 \pm 0.36^{a}$	$22.94 \pm 2.24^{\rm b}$	$21.63 \pm 1.05^{b}$	$37.27 \pm 0.07^{a}$								
Ţ	13.35	Stigmasterol, TMS derivative	$23.77 \pm 0.24^{a}$	pu	$14.41 \pm 0.80^{b}$	$6.67 \pm 0.14^{\circ}$	с	15.15	Stigmast-5-ene, 3β-(trimethylsiloxy)-, (24S)-	8.99±0.08 <sup>b</sup>	$15.01 \pm 1.50^{a}$	$7.21 \pm 0.33^{\circ}$	$13.71 \pm 0.12^{a}$	
7	14.74	Silane, trimethyl[[ββ-stigmasta- 7,24(28)-dien-3-yl]oxy]-	7.26±0.05 <sup>b</sup>	7.93±0.85 <sup>b</sup>	pu	$16.89 \pm 0.00^{a}$								
		Lanosterol and its analog	$4.35\pm0.10^{\rm b}$	$7.56\pm0.47^{a}$	$2.70\pm0.23^{\circ}$	$4.81\pm0.08^{\rm b}$								
4	15.91	Lanosterol, TMS derivative	$1.08 \pm 0.09^{b}$	$3.8 \pm 0.38^{a}$	pu	pu	7	16.67	3β-Lanosta-7,9(11),24-trien- 3-ol, TMS derivative	3.27±0.05 <sup>c</sup>	$3.76 \pm 0.32^{b}$	2.70±0.23 <sup>d</sup>	$4.81 \pm 0.08^{a}$	
		Other sterols	$19.43 \pm 0.19^{a}$	$7.79 \pm 1.34^{\rm b}$	$4.50\pm0.32^{\circ}$	$4.89 \pm 0.10^{\circ}$								
1	13.63	Desmosterol, TMS derivative	$2.23\pm0.04$	pu	nd	pu	4	15.34	Isofucosterol, O-TMS	$7.99 \pm 0.05^{a}$	$4.84 \pm 1.14^{\rm b}$	pu	pu	
2	14.26	4-Methylcholesta-8,24-dien- 3-ol, (3β,4α,5α)-, TMS derivative	$3.16 \pm 0.07^{b}$	2.95±0.30 <sup>b</sup>	2.20±0.09 <sup>c</sup>	$4.89 \pm 0.10^{a}$	Ŋ	15.33	Methyl (25RS)-3α-hydroxy-5β- cholestan-26-oate, TMS derivative	$2.55 \pm 0.02$	pu	pu	pu	
ო	15.03	$\beta$ -Sitosterol, TMS derivative	3.50±0.02	pu	pu	pu	9	17.17	Methyl 3-hydroxycholest-5-en- 26-oate, TMS derivative	pu	pu	2.30±0.23	pu	
		Non sterol	$7.85\pm0.12^{\circ}$	$15.44 \pm 0.91^{\rm b}$	$8.55 \pm 0.52^{c}$	$29.67 \pm 0.26^{a}$								
1	16.22	Lupeol trimethylsilyl ether	$4.64 \pm 0.05^{d}$	$8.87 \pm 0.34^{\rm b}$	$5.31\pm0.30^{\circ}$	$12.56 \pm 0.09^{a}$	2	18.65	Friedelan-3-one	$3.21 \pm 0.07^{d}$	$6.57 \pm 0.58^{b}$	$3.24 \pm 0.22^{d}$	$17.11 \pm 0.19^{a}$	
		Total content	$183.59 \pm 1.74^{\rm b}$	$190.60 \pm 17.52^{\rm b}$	85.46±4.37 <sup>c</sup>	$319.09 \pm 2.62^{a}$								
Noté Abb	:: Datas reviatio	Note: Datas with different superscript letter mean a significant difference (p < .05) betwwen two samples, while that with same letter signifys no significant difference. Abbreviations: AC, Agrocybe cylindracea; FM, Flower mushroom; LE, conventional Lentinula edodes; VV, Volvariella volvacea.	er mean a signifi M, Flower mushr	cant difference ( <sub>1</sub> oom; LE, convent	p < .05) betww ional Lentinula	en two samples edodes; VV, Vol	s, whil variel	le that w Ia volvac	/ith same letter signifys no sign ea.	nificant differe	nce.			

TABLE 4 Compositions of the unsaponifiable matter of four test mushroom oils (mg/kg).





lupeol and friedelan-3-one (7.85–29.67 mg/kg) benefited four tested oils with antimicrobial, anti-inflammatory, and anticonvulsant activities (Gallo & Sarachine, 2009; Ichiko et al., 2016; Jordan et al., 2020; Sousa et al., 2017).

# 3.5 | Antioxidant activity evaluation

The free radical scavenging capacity of the four mushroom oils was assessed by three indicators (i.e., values of DPPH, ABTS, and FRAP). According to Figure 2, the four tested oils all showed superior ABTS and FARP values (144.54-3801.70 µmol Trolox/100 g) superior to those of DPPH ( $4.99-78.84 \mu$ mol Trolox/100g). Relative to the best DPPH value (78.84 µmol Trolox/100g) in Agrocybe cylindracea oil, both the maximal ABTS (349.45 µmol Trolox/100g) and FRAP (3801.70µmol Trolox/100g) values only appeared at Volvariella volvacea oil. The antioxidant activities of two L. edodes oils were not significant, as flower mushroom oil had DPPH, ABTS, and FRAP values at 65.18, 248.23, and 213.22 µmol Trolox/100g, respectively, while conventional L. edodes oil presented poor DPPH and FRAP values. Overall, Volvariella volvacea oil was considered to have the optimal antioxidant activity, which mostly originated from its large amount of unsaponifiable substances, total phenols, and tocopherols.

# 4 | CONCLUSION

Mushroom oil, acting as a nontraditional oil with abundant polar lipids and lipid accompaniment, was shown to display biological activities such as lowering cholesterol levels, and antibacterial, antioxidant, anti-inflammatory, and anticonvulsant activities. In this paper, the optimal mushroom oil was selected by investigating the nutritional compositions and antioxidant activities of four mushroom oils. Based on superior indicators containing PUFAs, polar lipids, total phenols,  $\alpha$ -tocopherol, as well as ABTS and FRAP values, *Volvariella volvacea* oil was deemed the best oil among the four tested mushroom oils. Further investigations on related mushroom oils should focus on validating their biological activities as well as their development and application.

#### AUTHOR CONTRIBUTIONS

Jingjing Kang: Conceptualization (equal); investigation (equal); resources (equal); validation (equal); writing – original draft (equal). Yue Yue: Formal analysis (equal); writing – review and editing (equal). Shaofeng Wei: Supervision (equal). Huifang Chen: Writing – review and editing (equal). Peng Luo: Project administration (equal).

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#### DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the first author upon reasonable request.

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