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Nutritional value and volatiles of the edible mushroom *Leucocalocybe mongolica*

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RESEARCH ARTICLE

Abstract

Leucocalocybe mongolica (S. Imai) X.D. Yu & Y.J. Yao is a rare edible wild mushroom that is highly regarded in northeast Asia. Owing to its desirable flavour and health attributes, *L. mongolica* is collected unscrupulously by indigenous peoples and mycophiles. In addition, its habitat is under constant threat from human activities, and the wild production continues to decline as it cannot keep pace with the rate of harvest. To date, no cultivation techniques that can produce *L. mongolica* have been discovered; however, utilising fermentation technology offers a promising alternative approach. In this study, the nutrients and volatile components of the products arising from two fermentation techniques were evaluated. Significant differences were observed between the fruiting bodies and the fermented products of *L. mongolica* with respect to protein, fat, and fibre contents. The results of gas chromatography-mass spectrometry showed that 21 volatile components likely account for the flavour of basidiocarps. The two fermentation methods exhibited significant differences in terms of the enrichment of the different volatile compounds. Comparison of the active components before and after solid-state fermentation on *L. mongolica* showed that the content of flavonoids, polysaccharides, triterpenoids, sterols, and phenols after solid-state fermentation was enhanced compared with the unleavened substrate. Given these results, solid-state fermentation technology for *L. mongolica* appears to be a promising alternative to cultivation.

Keywords: GC-MS, active ingredients, liquid fermentation, solid-state fermentation

1. Introduction

Leucocalocybe mongolica (S. Imai) X.D. Yu & Y.J. Yao is a rare edible wild mushroom that is highly regarded in northeast Asia. This species was first described and submitted as '*Tricholoma mongolicum*' by the Japanese mycologist Sanshi Imai (Imai, 1937). In Chinese, its common name is '*Bai-mo*' or '*Kou-mo*'. In Mongolian, it is called *huragan mug*, which roughly translates to lamb-like mushroom (Tanesaka *et al.*, 1993). Following phylogenetic analysis, this species was reclassified as '*L. mongolica*', a unique member of the single-species genus *Leucocalocybe* (Yu *et al.*, 2011). The fruiting bodies, or basidiocarps, of *L. mongolica* have long been valued throughout East Asia as a delicacy because of their excellent food quality. Furthermore, this mushroom is also used by indigenous peoples as a traditional medicine for the treatment of botulism, wounds, detoxification, and the relief of inflammation (Wunir *et al.*, 2009). In addition, the literature frequently mentions that it is widely believed to have properties that fortify physical fitness, enhance immunity, and have an antitumor effect (Li *et al.*, 2015; Liu, 1984; Liu and Bau, 1980; Mao, 2000; Tanesaka *et al.*, 1993).

The role that mushrooms can play in human health is a substantial topic of interest. The main chemical constituents found in mushrooms include terpenoids, steroids, alkaloids, phenolic constituents, sphingolipids, pigments, and polysaccharides. Moreover, some active ingredients detected in the petroleum ether extract of *L. mongolica* basidiocarps have been found to have effects on tumour inhibition rates, immune organ indexes, and immune factor contents in H_{22}-bearing mice (Bau *et al.*, 2012; Tong *et al.*, 2010). The bioactivities of *L. mongolica* polysaccharides and lectins, which include an antiproliferative effect, antioxidant activity, a decrease in the risk of atherosclerosis, and antitumor activity, have also attracted keen interest from scientists (Bao *et al.*, 2014; Ge *et al.*, 2009; Wang *et al.*, 1996, 1997).

Owing to the highly desirable flavour and health attributes of *L. mongolica*, its fruiting bodies are collected excessively by local residents and vendors. Recent ecological surveys showed that *L. mongolica* is an endemic species with a limited distribution across the Mongolian Plateau. Furthermore, habitat loss and fragmentation have been observed to cause an accelerating decline in the population density of *L. mongolica*, posing an unprecedented threat for this species. The wild production cannot satisfy the growing demands, making commercial production a potentially lucrative avenue in the food industry. However, despite concerted efforts, there has still been no breakthrough in terms of cultivation techniques for *L. mongolica*. Fermentation technology holds great promise as an alternative approach, and optimisation of submerged fungal culture conditions has been performed experimentally for the production of extracellular and intracellular polysaccharides (Wu *et al.*, 2012).

Submerged liquid fermentation is often used to cultivate medicinal mushrooms. This approach has been suitable for upscaling and industrial development in the production of *Ganoderma* polysaccharide and ganoderic acid (Tang and Zhong, 2003), as well as *Lyophyllum* polysaccharide (Pokhrel and Ohga, 2007). However, solid-state mushroom fermentation is more cost-effective compared to submerged fermentation processes (Biesebeke *et al.*, 2002), and it is more sustainable because it makes full use of agricultural residues through the bioconversion of agricultural and forestry waste materials into valuable foods (Ohga and Kitamoto, 1997). Solid-state fermentation has been used in the production of some mushrooms, such as *Cordyceps sinensis*, *Termitomyces albuminosus*, *Antrodia camphorata*, and *Antrodia salmonea* (Liang *et al.*, 2009) as well as *Coprinus cinereus* (Mshandete, 2011). An important factor of solid-state fermentation technology is the substrate water potential, which plays a vital role in mycelial growth (Ohga, 1999). The study of fermentation technology on *L. mongolica* production is significant in terms of the development and utilisation of this rare and endangered mushroom resource.

2. Materials and methods

Acquisition of fermented product

The strain used in culture was isolated from a basidiocarp collected in the Chenbaerhu Banner in Nei Monggol Autonomous Region (China). The strain preservation number is MCCJLAU2016BK, and it was identified using the internal transcribed spacer (ITS) DNA barcode. Optimised formulas and conditions of liquid fermentation and solid-state fermentation were obtained in a previous study. The optimal liquid fermentation media recipe used included 35.13 g/l sucrose, 16.55 g/l soybean meal, and 33 g/l corn steep powder. The conditions used were 25 °C and initial pH 6.5 at 120 rpm with a liquid volume of 75 ml in a 250 ml flask using an inoculum size of 12.5% for a period of 13 days. The seed culture media recipe included 200 g/l (decoction) potato, 10 g/l glucose, 10 g/l saccharose, 2 g/l yeast extract, 2 g/l beef extract, 2 g/l peptone, 2 g/l KH_2PO_4, and 1 g/l $MgSO_4$.

As for solid-state fermentation, the optimal substrate was determined to be kernels. The compost medium was prepared with kernels (100 g) as the main substrate mixed with other ingredients, including beef extract (0.2 g) and a sufficient amount of water (material/water ratio = 10:1). Tissue culture containers were used for solid-state fermentation (container size: volume 300 ml, diameter 7 cm, height 10.5 cm). Sterilisation conditions were maintained at 121 °C for 60 min, and the substrate was allowed to cool naturally. The solid substrate was inoculated using 10 ml of inoculum. At approximately 32 days, the mycelium had colonised the entire solid substrate at 25 °C.

After full colonisation, the liquid fermentation media were centrifuged at 3,000 rpm for 30 min, and the fermented mycelium was collected and pelleted. The pellets were then dried to constant weight in an electric, air, heat-drum drying oven at 65 °C, followed by crushing and sieving. The sample produced from the liquid fermentation with this method is herein referred to as 'JS.' Samples for the evaluation of the solid-state fermentation were also obtained using the abovementioned technological process and referred to as 'GT.' Uninoculated substrate was processed in the same way as a negative control ('KB'), and the basidiocarps of *L. mongolica* were handled as positive control ('ZS'). The nutritional value and volatile constituents of samples ZS, JS, GT, and KB were determined, and the active components before and after solid-state fermentation of *L. mongolica* were evaluated.

Methods of evaluation

Determining nutrition content

The crude fat, crude fibre, and crude protein contents were tested according to Patil *et al.* (2010). The amino acid and total carbohydrate contents were determined in accordance with Hsu *et al.* (2002).

Determining volatile constituents

Gas chromatography-mass spectrometry (GC-MS) analysis was implemented using a gas chromatograph mass spectrometer, model Agilent 5975/6980N. The run parameters were set as follows:

- Chromatographic column: HP-35, 35% phenyl methyl siloxane, Agilent 19091G-133 (30 m × 0.25×0.25 μm) (Agilent Technologies Inc., Folsom, CA, USA).
- Heating procedure: initial temperature 50 °C and initial time 3.00 min, heated to 260 °C at 10 °C/min, 260 °C maintained for 25 min.
- Gas type: helium; pressure: 7.59 psi; split ratio: 1:1; split flow: 1.0 ml/min.
- Total flow: 5.0 ml/min
- General information contains SCAN parameters 20-700 (low mass: 20.0; high mass: 700.0)
- MS source: 230 °C, maximum 250 °C; energy: 69.922.

Detection method of the active ingredients

The sodium nitrite-aluminium nitrate colorimetric method was used to determine the total flavonoid content in accordance with Kong (2009). Sulfuric acid-phenol colorimetry was used to determine the total polysaccharide content in accordance with Liu *et al.* (2013). Vanillin acetic acid-perchloric acid colorimetry was used to determine the total triterpene content in accordance with Lin *et al.* (2007). Sulfonyl acetate reagent colorimetry was used to determine the total triterpene content in accordance with Wang *et al.* (2014). A Prussian Blue method was used to analyse the total phenols in accordance with Budini *et al.* (1980).

3. Results and discussion

As shown in Figure 1, mycelial pellets and mycoplasma of *L. mongolica* were obtained by liquid and solid-state fermentation. It was found that the nutritional content and volatile components of both fermented products were comparable to those of basidiocarps.

The basic nutritional content of the mushrooms is depicted in Table 1 and expressed in dry weight on a g/100 g basis. Additionally, 16 amino acid profiles were determined on a dry weight basis and are shown in Table 2 also in g/100 g. Significant differences were observed between the fruiting bodies and the fermented products of *L. mongolica* with respect to the protein, fat, and fibre contents.

Mushrooms are rich in nutrients and typically have a >30% higher protein content compared to ordinary vegetables, which is also true for *L. mongolica*. This is reflected in that the basidiocarps (ZS) had a conspicuously high protein content of 42.2% compared with 27.2% of the fermented mycelium by liquid fermentation (JS) and 17.9% of the solid-state fermentation (GT). With regard to total carbohydrates, the fermented products had a higher carbohydrate content (59.78% in GT and 34.95% in JS) compared to the fruiting body (ZS, 29.96%), whereas the control group

Figure 1. Mycelial pellets and mycoplasma of *L. mongolica*.

Table 1. Major nutritional composition.

Sample production type	Sample code	Crude fat (%)	Crude fibre (%)	Crude protein (%)	Total carbohydrates (%)
L. mongolica basidiocarps	ZS	10.25	1.02	42.2	29.96
Liquid fermentation	JS	11.64	1.32	27.2	34.95
Solid-state fermentation	GT	12.17	0.95	10.9	59.78
Uninoculated solid-state substrate	KB	13.3	0.88	9.8	70.14

Table 2. Amino acid profiles of the four samples.[1]

Amino acid profile	ZS (%)	JS (%)	GT (%)	KB (%)
Aspartic acid	3.438	1.4425	0.5506	0.4208
Threonine	1.4745	0.8799	0.3149	0.2513
Serine	1.5143	0.8100	0.3744	0.2723
Glutamate	5.5607	1.7748	1.3740	0.9876
Glycine	1.3375	0.9920	0.3285	0.2602
Alanine	2.0824	1.1401	0.5700	0.3952
Valine	0.8712	0.4640	0.3486	0.1797
Methionine	0.2903	0.1956	0.0911	0.0622
Isoleucine	0.9929	0.6483	0.2613	0.1742
Leucine	1.7683	1.1161	0.9580	0.6019
Tyrosine	0.5036	0.2079	0.1126	0.0781
Phenylalanine	1.0935	0.7521	0.4102	0.2878
Lysine	1.432	0.6175	0.2370	0.1389
Histidine	0.5113	0.2946	0.1726	0.1115
Arginine	1.5733	0.6356	0.3277	0.2004
Proline	1.3578	0.9574	0.6676	0.5804
Total	25.8	12.9	7.09	5.00

[1] GT = samples of the solid-state fermentation ; JS = sample produced from the liquid fermentation ; KB = uninoculated substrate (negative control); ZS = basidiocarps of *L. mongolica* (positive control).

of unfermented substrate had the highest carbohydrate content (KB, 70.14%). The crude fibre content ranged from 0.88 (KB) to 1.32 (JS), and solid-state fermentation (GT) exhibited a more similar crude fibre content to the fruiting body (ZS) than the liquid fermentation (JS). As for crude fat, its content ranged from 10.25 (ZS) to 13.3 (KB), and both of the fermented products were at the intermediate level.

The three principal amino acids and their levels in the basidiocarps were 5.5607% glutamate, 3.438% asparagine, and 2.0824% alanine, and the three principal amino acids in the fermented mycelium by liquid fermentation showed a similar makeup. However, the three principal amino acids and their levels in the solid-state fermentation were 1.3740% glutamate, 0.9580% leucine, and 0.6676% proline, and the results of the analysis of the uninoculated substrate (KB) showed a similar trend. The total level of amino acids was significantly lower (5%) in the unfermented control substance compared with the basidiocarps (25.8%).

Figure S1-S4 show the GC-MS total ion chromatograms of volatile components for the ZS, JS, GT, and KB samples. Table S1 to S4 list the volatile components that were identified with more than 80% similarity. 41 volatile components were detected in ZS, 21 of which are likely to be flavour compounds of basidiocarps, which are 3-methylbutyraldehyde, pentanal, 3-methyl-1-butanol, 3-hydroxy-2-butanone, 3-methylbutanoic acid, hexyl alcohol, 2-methylpyrazine, 2-heptanone, heptaldehyde, 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, formic acid, heptyl ester, 1-octen-3-ol, benzaldehyde, 4,5-dihydro-5-methyl-2(3H)-furanone, 1-nonanal, 2-acetyl pyrrole, octanoic acid, 2-undecanone, 4-hydroxynonanoic acid gamma-lactone, and ethyl-9,12-octadecadienoate. The characteristic flavour of *L. mongolica* may be related to these complex volatile components consisting of proteins, nucleic acids, and some other flavour compounds. As for the fermented products, 59 volatile components were detected in GT and 51 in JS. The uninoculated control only contained 40 volatile components.

The Venn diagram (Figure 2) illustrates the interrelation of volatile components in the four samples. The 10 common constituents of the four samples were benzaldehyde, 4-hydroxynonanoic acid gamma-lactone, dodecane, 1-nonanal, 2-pentylfuran, (R)-4,4a,5,6,7,8-hexahydro-4a,7,7-trimethylnaphthalen-2(3H)-one, hexadecanoic acid, tridecane, tetradecane, and ethyl-9,12-octadecadienoate. JS and GT shared 31 common constituents, followed by KB and GT with 21 and ZS and KB with 12. As for volatile components unique to each sample, ZS had 19, JS had 17, KB had 15, and GT had the most with 22.

Twelve essential volatile compounds were distributed in four samples as illustrated in Figure 3. Interestingly, (E)-2-octenal, 2-butyl-2-octenal, and 1,1-dimethyl-3,4-bis(1-methylethenyl)cyclohexane were detected in the fermented products, JS and GT. These three compounds may be metabolic products of the mycelial growth stage on *L. mongolica* and, therefore, are not present in the fruiting body. 3-Methylbutyraldehyde was detected in JS and ZS. The content in JS at 2.84% was lower than 4.14% in ZS; however, the production of this important flavour compound is an important characteristic. Heptaldehyde and 1-octen-3-ol were detected in GT and ZS. The content

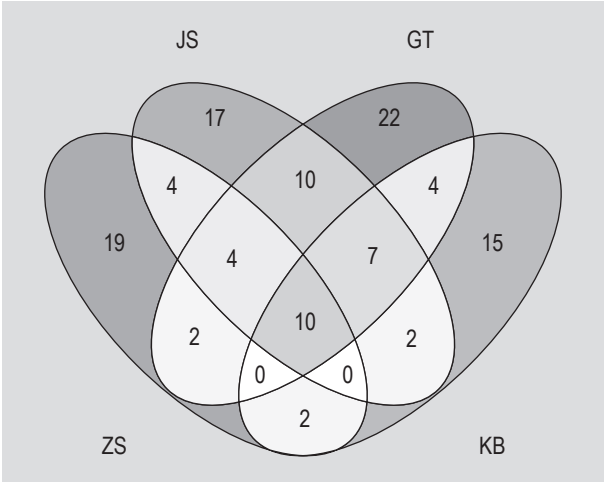

Figure 2. Venn diagram of the volatile components of four samples: ZS, JS, GT, and KB.

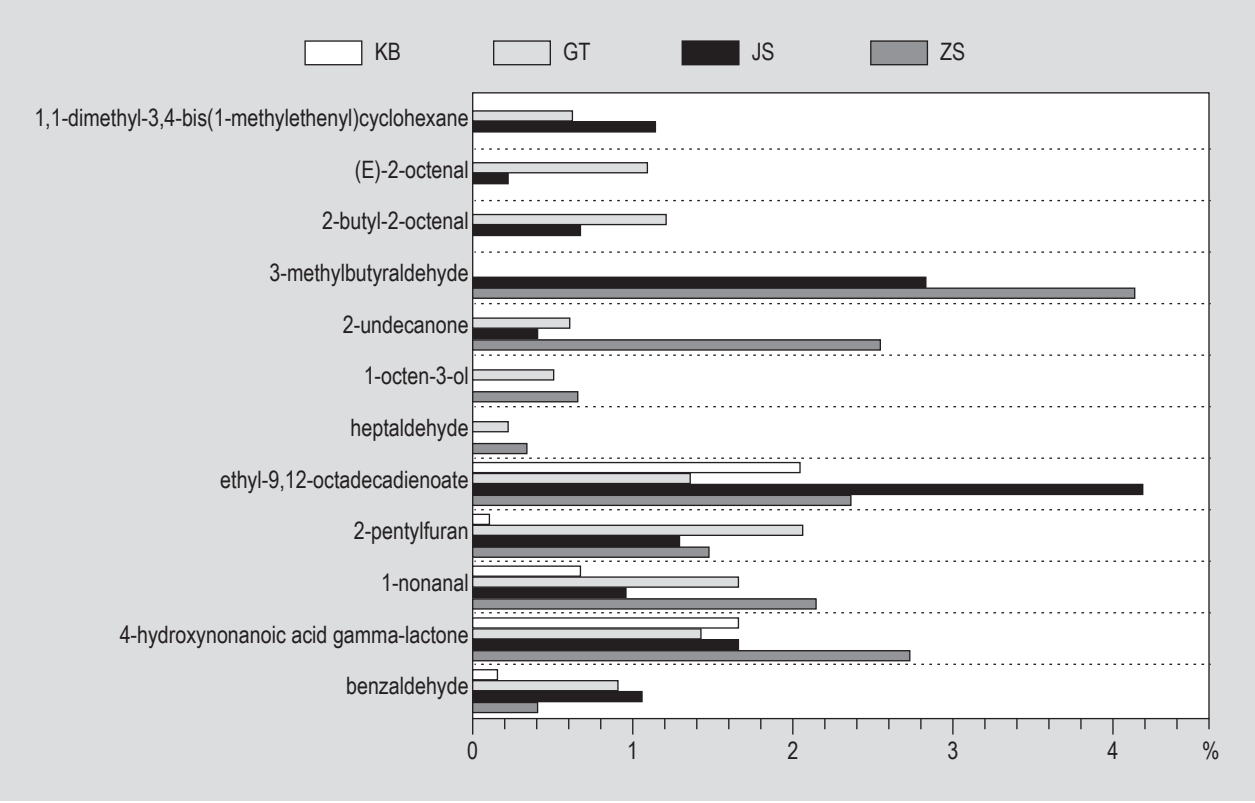

Figure 3. Distribution of important compounds.

of these two compounds of GT is similar to that of ZS. The similarity in content, especially of 1-octen-3-ol, is promising with regard to production as it is a widely used fragrance and flavour component in mushrooms. Benzaldehyde, 4-hydroxynonanoic acid gamma-lactone, ethyl-9,12-octadecadienoate, 1-nonanal, and 2-pentylfuran were detected in all four samples. 2-Undecanone was detected in three samples excluding the uninoculated control. Both methods of fermentation exhibited significant differences in terms of the enrichment of different volatile compounds. Liquid fermentation enriched benzaldehyde, 4-hydroxynonanoic acid gamma-lactone, and ethyl-9,12-octadecadienoate, whereas solid-state fermentation enriched 1-nonanal, 2-pentylfuran, and 2-undecanone. However, maximising target products and optimising production methods at larger scales commonly occur in commercial production.

The percentages of five active components before and after solid-state fermentation of *L. mongolica* are shown in Figure 4. Evaluations showed that the flavonoid, polysaccharide, triterpenoid, sterol, and phenol contents after solid-state fermentation were enhanced compared with the unleavened substrate. The polysaccharide content of GT was 7.473%, which is more than that of KB (4.154%). Polysaccharides are now a topic of great interest in the pharmaceutical and health food industries.

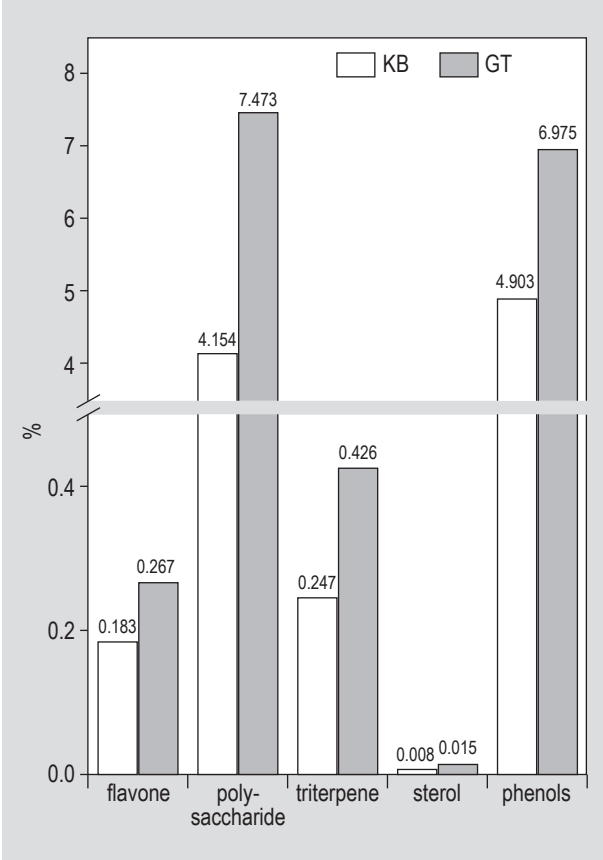

Figure 4. Comparison of the contents of the five components.

Solid-state fermentation takes full advantage of agricultural and forestry by-products. Through this reuse and deriving value from by-products, it can accelerate the depth of a sustainable economy and make better use of resources. Solid-state fermentation technology for cultivating *L. mongolica* merits further research.

Supplementary material

The Supplementary Material can be found online at https://doi.org/10.3920/QAS2019.1585.

Figure S1. Total ion current of volatile on the basidiocarps of *L. mongolica* (sample ZS).

Figure S2. Total ion current of volatile on the liquid fermented mycelium of *L. mongolica* (sample JS).

Figure S3. Total ion current of volatile on the solid-state fermented mycoplasma of *L. mongolica* (sample GT).

Figure S4. Total ion current of volatile on the solid-state fermentative substrate of *L. mongolica* as a blank control group (sample KB).

Table S1. GC-MS analysis results of volatile on the basidiocarps of *L. mongolica* (sample ZS).

Table S2. GC-MS analysis results of volatile on the liquid fermented mycelium of *L. mongolica* (sample JS).

Table S3. GC-MS analysis results of volatile on the solid-state fermented mycoplasma of *L. mongolica* (sample GT).

Table S4. GC-MS analysis results of volatile on the solid-state fermentative substrate of *L. mongolica* as a blank control group (sample KB).

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