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Volatile components, total phenolic compounds, and antioxidant capacities of worm-infected *Gomphidius rutilus*

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ABSTRACT

This study evaluated the effects of worm infection on the volatile components, total phenolic compounds, and antioxidant capacities of *Gomphidius rutilus*. *G. rutilus* without worms (GW), *G. rutilus* infected by a small amount of worms (GS; infected area <50%), and *G. rutilus* infected by a large amount of worms (GL; infected area>50%) were investigated. The volatile components of *G. rutilus* were analyzed by simultaneous distillation–extraction (SDE) and headspace solid-phase microextraction (HS-SPME) using gas chromatography–mass spectrometry (GC–MS). A total of 17 and 19 types of volatile compounds were detected, including ketones, alcohols, benzene, alkenes, aldehydes, esters, acids, and alkanes. Alcohols comprised the most abundant compound in GL, GS, and GW. The relative content of 1-octen-3-ol was the highest in all mushrooms. The concentration of eight-carbon (C8) compounds relative to the total volatile compounds varied widely, ranging from 40% (GW) to 64.34% (GS) and 84.42% (GS) and to 91.59% (GL), respectively, among the three samples. The antioxidant capability and the total phenolic contents of *G. rutilus* were evaluated in this study. The highest total phenolic content (TPC) of 192.23 mg GAE/g was found in GL, which differed significantly ($P < 0.05$) from the latter two samples, whereas the lowest value of 156.11 mg GAE/g was found in GW. ABTS radical cation scavenging activity, FRAP ferric reducing antioxidant capacity (FRAP) radical scavenging activity, and oxygen radical absorbance capacity (ORAC) were investigated to screen the antioxidant properties of extracts. The contents of total phenolic compounds and their antioxidant capacities in vitro showed significant correlations ($P < 0.01$). Among the three types of samples, the phenolic compounds of GL exhibited the highest antioxidant capacity, showing the values of 0.089 mM TE/g for ABTS, 0.949 mM Fe^{2+} E/g for FRAP, and 1.952 M TE/g for ORAC. However, regarding the total antioxidant capacity, GS exhibited the highest antioxidant capacity, showing the values of 0.002648 mM TE/g for ABTS, 0.004437 mM Fe^{2+} E/g for FRAP, and 0.256 μM TE/g for ORAC. In conclusion, HS-SPME was more suitable for the extraction of volatile aroma components from *G. rutilus*. GL had the most abundant aroma components. GL had the highest TPC and antioxidant capacity compared with those of GS and GW, whereas GS showed the opposite results. Interestingly, GS was found to have the highest total antioxidant capacity in vitro. Based on these measured indicators, worm infection had no negative effect on the quality of *G. rutilus*. Therefore, worm-infected *G. rutilus* can also be consumed by humans.

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1. Introduction

Mushrooms are widely found in nature, and it has been estimated that there are about 140,000 mushroom species on earth, of which only 10% of them may be found [1]. The majority of them are edible and have been used as food and medicine throughout

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the world [2]. Due to their unique flavor and taste, mushrooms are commonly consumed as raw materials, condiments, and high-grade natural substances worldwide, especially in some oriental countries [3]. Edible fungus has become an important component of human diet because of its high nutritional value, which can be attributed to its high content of proteins, fatty acids, vitamins, and minerals but low content of calories and fat [4]. In addition, mushrooms contain a variety of bioactive molecules such as phenolic compounds, organic acids, terpenes, polysaccharides, and steroids [5,6]. Edible mushroom has been considered as a novel source of dietary fiber and beneficial to human health [7]. Mushroom and its processed products have been widely accepted as health nutraceuticals [8–10], pharmaceuticals [11–13], and cosmeceuticals [14].

The characteristic flavor of mushrooms has been considered to be due to the presence of volatile and nonvolatile components. The aroma of mushrooms is attributed to fruiting bodies, which have been claimed to produce pleasant, odorous substances [15]. Extensive evidence has demonstrated that terpenes, aromatic alcohols, aldehydes, ketones, eight-carbon compounds, and their derivatives are responsible for the characteristic flavor of mushrooms [16]. It is known that linoleic acid is the precursor of 1-octen-3-ol, which is considered as an alcohol present in fungi, with a unique earthy taste and sweetness [17]. The unique taste of mushroom has been ascribed to the nonvolatile components, including free amino acids and 5′-nucleotides [18].

Phenolic compounds are one of the most important groups of secondary metabolites that are derivatives of the pentose phosphate, shikimate, and phenyl propanol pathways. Based on their structure, which possesses an aromatic ring bearing one or more hydroxyl groups, phenolic compounds can range from simple molecules to complex high molecular weight polymers [19]. It has been reported that the phenols present in mushroom fruit bodies significantly contribute to the antioxidant activity [4]. The mechanism of their antioxidant activity involves several methods. Especially, they can act as reductants by donating electrons and protect cells against damage by neutralizing reactive oxygen species and chelating those elements (e.g., Fe, Cu) that can generate reactive oxygen species [20].

Gomphidius rutilus is a traditional Chinese wild edible and medicinal fungus, belonging to the subphylum Basidiomycotina. It is often found beneath pine trees. The mushroom is widely distributed in China, including Liaoning, Jilin, Heilongjiang, Hebei, Shanxi, Hunan, Sichuan, and Tibet [21]. The present study evaluated the polysaccharides from *G. rutilus* and their antioxidant activities and immune activities in vitro [22–24]. A novel fungal immunomodulatory protein from *G. rutilus* was identified, cloned, and analyzed [25]. Similar to the majority of ectomycorrhizal fungi, the phenomenon by which the fruiting bodies of this fungus are infected with worms was often observed. However, *G. rutilus* infected with worms did not exhibit any poisonous effect. Instead, worm infection might be related to the volatile components, total phenolic compounds, and antioxidant capacities of the mushroom—a question that remains unexplored. We had recently demonstrated that worm infection affects the texture profile and the nutritional and flavor components of *G. rutilus* [26]. The aim of the present study was to examine the effect of the degree of worm infection on the volatile components, total phenolic compounds, and antioxidant capacities.

2. Materials and methods

2.1. Samples and sample preparation

Fresh fruiting bodies of *G. rutilus* mushrooms were collected from a local producer in Chaoyang, Liaoning Province, China. The harvested mushrooms were expediently transferred to the Food Analysis Laboratory at Shenyang Agricultural University on the second day of collection. All the wild mushrooms were identically selected in terms of the degree of worm infection. The samples were identified as follows: *G. rutilus* without worms (GW), *G. rutilus* with a small amount of worms (GS; infected area <50%), and *G. rutilus* with a large amount of worms (GL; infected area >50%). Subsequently, the samples were kept in a freezer at −40 °C. All mushrooms from the same types were homogenized for analysis to minimize variability among individuals.

2.2. Extraction of volatile aroma components

2.2.1. Simultaneous distillation and extraction (SDE)

SDE analysis was performed as described by Du etc [27]. with few modifications. A total of 500 g of fresh mushrooms was immersed in a flask containing 1000 mL of distilled water, and 40 mL of dichloromethane was added as an organic solvent in another flask. The sample was left for 15 min to maximize the enzymatic production of the flavor compounds, as reported by Hong et al. [28]. Both flasks were placed in a Likens–Nickerson apparatus and heated up to their boiling points. When the two flasks started to reflux, the distillation extraction was continued for 2 h to allow the collection of the volatile components in the organic solvent. After the procedure, the extract was collected at room temperature. The flavor extract was dried overnight over sodium sulfate (anhydrous) and approximately concentrated to 2 mL at 35 °C in a water bath. The extract was stored at −4 °C temporarily before analysis.

2.2.2. Headspace solid-phase microextraction (HS-SPME)

HS-SPME was performed according to the method described by Ouzouni, Koller, Badeka, and Riganakos [29], with slight modifications. Approximately 20 g of fresh mushroom sample and 3 g of NaCl were homogenized. The homogenate was filtered through Whatman No. 1 filter paper. The filtrate was placed in a 15-mL glass vial and sealed with a plastic screwed cap equipped with a Teflon-coated, needle-pierceable septum (Supelco, Bellefonte, PA, USA). The SPME fiber was coated with 50/30 μm DVB/CAR/PDMS on polydimethylsiloxane. The vial was placed in a 40 °C water bath and equilibrated for 10 min. After equilibration, the needle of the SPME holder was inserted into the vial through the septum and the fiber was exposed to the headspace of the sample for 40 min to adsorb the volatile components.

2.2.3. Gas chromatography–mass spectrometry analysis

GC–MS analysis was performed as described by Du etc. [27], with some modifications. The volatile compounds obtained by SPME and SDE were analyzed by GC–MS using an Agilent 5975C mass selective detector coupled to an Agilent 7890 A GC (Agilent, Santa Clara, CA), equipped with an HP-5MS capillary column (60 m × 250 μm × 0.50 μm). Helium was used as the carrier gas, and the flow rate was 1.0 mL min^{-1}. The flow rate of the helium carrier gas was 1.0 mL min^{-1}. The injector temperature was 250 °C in the splitless mode. The GC oven temperature gradient was maintained as follows: the initial temperature of the column was 40 °C (held for 2 min), increased to 180 °C (held for 2 min) at 3 °C min^{-1}, and finally increased to 260 °C (held for 1 min) at 10 °C min^{-1}. The transfer line temperature was 280 °C. The ion source temperature was 230 °C, and the MS was scanned at 70 eV over 50–450 mass range.

The compounds were tentatively identified using the NIST11.L mass spectra library. Each compound was further confirmed by comparing its mass spectra, linear retention index, and retention times with those obtained for the reference standards.

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