Mushroom Biomass Waste Is a Source of the Antioxidants Ergothioneine and Glutathione

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Abstract: Low-grade foodstuffs and unharvested materials from farms contribute a sizable amount of the waste that is disposed to landfills. Mushroom farms also contribute to this problem, as unmarketable fruiting bodies or parts of them are discarded in the waste stream. To limit the proportion of mushroom biomass waste that is deposited to landfills, we assessed whether culls of oyster and shiitake mushrooms and white button mushroom stem waste contain the antioxidants ergothioneine and glutathione. Enzyme-coupled spectrophotometric assays were used to assess the concentrations of glutathione (GSH) and its oxidized form glutathione disulfide in mushroom biomass waste. Ergothioneine analysis was performed with a high-performance liquid chromatography analysis. Most of the biomass waste contained ergothioneine and GSH concentrations that were on par with each one of the fresh mushrooms. Conversely, white button mushroom stem waste contained 77% less GSH than market-ready mushrooms. Finally, as a proof-of-concept cation exchange chromatography was used to capture ergothioneine from oyster mushroom culls. This strategy has the potential to produce gram quantities of high value ergothioneine per tonne of mushroom biomass waste. These findings provide a strategy for the valorization of mushroom biomass waste and its diversion from landfills.

Keywords: antioxidant; biomass waste; ergothioneine; glutathione; glutathione disulfide; mushrooms

1. Introduction

Mushrooms contain dietary fiber, minerals, and vitamins (e.g., niacin) including the vitamin D2 precursor ergosterol [1,2]. Moreover, fresh mushrooms supply the human diet with the antioxidants glutathione and ergothioneine [3]. In nature, glutathione occurs in reduced (i.e., GSH) and oxidized (i.e., glutathione disulfide, GSSG) forms. In most organisms, reactive oxygen species (ROS) such as hydrogen peroxide are detoxified to less reactive molecules (e.g., water), a step that is metabolically coupled to the conversion of GSH to GSSG by peroxidases within the glutathione–ascorbate recycling pathway [4]. During postharvest storage and handling practices, this pathway limits oxidative stress-related events that promote the cellular damage associated with physiological disorders of fruit (e.g., apples, tomatoes) and the yellowing of green vegetables [5,6]. GSH is present in many organisms including fresh plant and meat products. GSH concentrations in foodstuffs range from 16 to 855 nmol g fresh weight (FW)\(^{-1}\), with some of the highest levels measured in pork, spinach, broccoli, green bell pepper, avocado, and asparagus [7,8]. Similarly, a wide range of total glutathione (i.e., sum of GSH and GSSG) concentrations are evident in fresh culinary mushrooms, with as much as 1.1 µmol g FW\(^{-1}\) in maitake, and as little as 111 nmol g FW\(^{-1}\) in crimini mushrooms [3]. It is important to note that this study did not assess the proportion of GSH and GSSG in the mushrooms investigated therein. Thus, the finite amounts of GSH and GSSG remain unknown in the culinary mushrooms that are grown in North America.

Ergothioneine is an antioxidant that is proposed to limit ROS-mediated processes that are associated with various cancers, inflammation, and neurodegenerative diseases...
humans [9]. Ergothioneine is present in commercial culinary mushrooms, with some of the highest levels found in oyster and shiitake mushrooms; small amounts of this antioxidant are detected in other dietary sources of foods [3,10]. Ergothioneine biosynthesis occurs in some bacteria and in fungi including mushrooms [11,12], but not in plants and animals.

In 2021, 44.2 million metric tons of edible mushrooms (including truffles) were produced worldwide, with 93% of the supply derived from China [13]. Canada is among the top ten global producers, with $654 million dollars (CDN) of mushroom product sales in 2021 [14]. Most Canadian producers cultivate *Agaricus* mushrooms (e.g., white button), but specialty types (e.g., shiitake and oyster mushrooms) are also produced year-round [15]. It is estimated that as much as 20% of the fruiting body biomass from the commercial production of mushrooms is deposited to landfills [16]. This includes the bottom portion of the stem (i.e., stipe) that remains attached to the growth substrate after harvest, and low grades (i.e., culls) that fail to meet the acceptable commercial standards of the fresh mushroom market [17]. Over the past decade, by-products of mushroom biomass waste have been developed. For example, flour prepared from oyster mushroom stem waste improves the texture of chicken patties [18]. Similarly, powders derived from enoki mushroom stem waste increase the water retention capacity of processed goat meat products [19]. It is speculated that mushroom biomass waste could serve as an inexpensive source of nutraceuticals [20], but this has been scarcely explored. In fact, recent research has discovered that *Agaricus* mushroom biomass waste is a source of polysaccharides and has antioxidant activity [21]. In this study, we investigated antioxidant profiles in the stem waste of white button mushrooms and culls of oyster and shiitake mushrooms that are discarded post-production in commercial operations. Our research provides the first evidence that mushroom biomass waste is a source of GSH and ergothioneine, with low-grade oyster mushrooms recovered from the waste stream being the optimal source of both compounds. In addition, we developed a feasible technology platform for the capture of ergothioneine from oyster mushroom culls. This is a key step towards minimizing food waste from farms, and provides a source of antioxidants for use in industrial products (e.g., nutraceuticals).

2. Materials and Methods

2.1. Mushroom and Chemical Materials

Fresh and waste mushroom fruiting bodies were attained from three separate commercial mushrooms producers located within a 150 km radius of the University of Guelph (43.52774° N, 80.22927° W). White button mushrooms (*Agaricus bisporus*) and their stem waste were obtained from two separate farms on 18 November 2021. A third batch of these white button mushroom materials were obtained from a local farm on 21 June 2022. Upon harvest at each farm, fresh mushroom and stem wastes were separated and placed in commercial transport boxes. The bottom surface of each box was covered by plastic sheeting prior to its placement on ice for immediate transport to the University of Guelph. Thereafter, the stem waste (free of substrate) and the whole mushrooms were separately diced and flash-frozen under liquid N$_2$ prior to cryogenic storage. Similarly, oyster mushrooms (*Pleurotus ostreatus*) of the grey variety and shiitake mushrooms (*Lentinula edodes*) and their respective culls were provided immediately after harvest from a farmer on each of the following dates: 29 November 2021; 7 June 2022; and 22 July 2022. Mushroom culls represented those discarded by the farmers due to defects and deformations incurred during cultivation (Figure 1). These materials were maintained on ice during transport to the University of Guelph, and then processed and flash-frozen as outlined above. The frozen mushroom materials were separately powdered with a mortar and pestle under liquid N$_2$ and returned to cryogenic storage for downstream antioxidant analyses.
Frozen powders of fresh mushrooms and their biomass waste were separately analyzed for total glutathione and GSSG using a GR-coupled spectrophotometric assay [22]. Briefly, 1 g of frozen mushroom powder was combined with 10 mL of 0.2 M hydrochloric acid, agitated for 5 min, and then centrifuged at 13,000 × g for 10 min at 4 °C. A 5 mL aliquot of the supernatant was adjusted to pH 5.5 with 500 µL of 0.2 M NaH₂PO₄ (pH 5.6) and approximately 4 mL of 0.2 M NaOH. A small aliquot of baker’s yeast GR containing ammonium sulfate was centrifuged, and the pellet was gently resuspended in a solution of 0.2 M NaH₂PO₄ (pH 7.5) containing 20 mM ethylenediaminetetraacetic acid. The final working solution contained 20 units of GR activity. One unit of GR converts 1 µmol of GSH to 1 µmol of GSH per min. This solution and all other GR assay reagents were covered with aluminum foil to prevent photo-oxidation. To determine the amount of total glutathione in the mushroom extract, each assay within a microplate included 40 µL of the neutralized mushroom extract (or dilution) and 170 µL of the assay mixture. The assay mix contained 118 mM NaH₂PO₄ (pH 7.5), 5.9 mM ethylenediaminetetraacetic acid, 590 µM nicotinamide adenine dinucleotide phosphate (reduced form), and 710 µM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). Each assay was initiated by the addition of 10 µL of the GR working solution, and the absorbance at 412 nm was continuously monitored with a BioTek Epoch 2 microplate spectrophotometer (Fisher Scientific) for up to 99 s thereafter. For the GR recycling assay, GSH within a sample reduces DTNB to the yellow-colored product.

Figure 1. Market-ready (fresh) and unmarketable (culls) of shiitake and grey oyster mushrooms. Representative images were taken on the day of harvest. Scale bar in each photo = 5 cm. Culls represent whole mushrooms recovered from the waste bin on the day of harvest, which were discarded due to visible defects. Not shown here are the fresh and stem waste portions of the white button mushrooms used in this study.

Unless otherwise mentioned, all reagents used for metabolite analysis, including baker’s yeast glutathione reductase (GR), were acquired from Sigma-Aldrich Canada (Oakville, ON, Canada). Acids and solvents for metabolite extraction and high-performance liquid chromatography (HPLC) analyses were from Fisher Scientific (Mississauga, ON, Canada). HPLC analysis materials were from Agilent (Mississauga, ON, Canada).

2.2. Glutathione Metabolite Analysis

Frozen powders of fresh mushrooms and their biomass waste were separately analyzed for total glutathione and GSSG using a GR-coupled spectrophotometric assay [22].
5-thio-2-nitrobenzoic acid which is detected at 412 nm. GSSG in the extract is converted to GSH by GR, which also reduces DTNB. The rate of 5-thio-2-nitrobenzoic acid formation provides a measure of the total glutathione in the extract when compared to rates measured for a range of GSH standards (0.05 to 1 nmol per assay). To assess the quantity of GSSG in each mushroom extract, a 200 µL aliquot of the neutralized extract was combined with 1 µL of 2-vinylpyridine in an aluminum foil-wrapped tube, and then mixed on a nutator for 60 min. Thereafter, the mixture was centrifuged at 14,800 × g for 5 min, and the supernatant collected. If required, the supernatant was clarified via a second centrifugation step. A 40 µL aliquot of the supernatant was used in a GR recycling assay as described above, but the absorbance change at 412 nm was compared to assays performed with a known range of GSSG standards (10 to 400 pmol). The amounts of total glutathione and GSSG within each extract were each corrected for the percentage recovery of a respective GSH (90, 100, 115 or 250 nmol) or GSSG (30, 50 or 70 nmol) spike that was added to duplicates of representative mushroom samples.

2.3. Ergothioneine Analysis

Frozen mushroom powders corresponding to each of the three experimental replicates were separately analyzed for ergothioneine using HPLC-diode array detection (HPLC-DAD). Ergothioneine extractions were performed according to a previously published protocol [23] with some modifications. Briefly, 500 mg of frozen mushroom powder was combined with 10 mL of pre-chilled 70% (v/v) ethanol containing 10 mM dithiothreitol and 100 µM betaine. The homogenate was agitated for 20 min. Thereafter, 2 mL of 70% (v/v) ethanol containing 1% (w/v) sodium dodecyl sulfate was added to the homogenate and mixed on a nutator for 10 min. The homogenate was clarified by centrifugation at 1500 × g for 20 min. The supernatant was decanted and passed through a 0.45 µm Restek polytetrafluoroethylene syringe filter (Fisher Scientific Canada). A 3 mL aliquot of the filtrate was dried under vacuum in a rotary centrifuge, and the residue was resuspended in 150 µL Milli-Q water. A final 1 min centrifugation at 14,800 × g was included to ensure the absence of particulate in the filtrate prior to HPLC analysis.

The amount of ergothioneine in each mushroom extract was assessed using a hydrophilic interaction liquid chromatography (HILIC) method [24,25]. Briefly, a 6 µL aliquot of the filtered and clarified mushroom extract was injected onto a Kinetex® HILIC column (5 µm, 100 Å, 150 × 4.6 mm) (Phenomenex Inc., Torrance, CA, USA) attached to an Agilent 1200 HPLC-DAD system. The column was pre-equilibrated with solvent A (acetonitrile: 20 mM ammonium acetate, LC-MS grade (85:15, v/v)). Metabolites were eluted from the HILIC column using an increasing gradient of solvent B (Milli-Q water: 20 mM ammonium acetate, HPLC grade (85:15, v/v)) at 1 mL min⁻¹. The gradient was as follows: 0–7 min (0% B), 7–15 min (0% B–15.6% B), 15–25 min (15.6% B–33.3% B), 25–27 min (33.3% B), 27–30 min (33.3% B–0% B). Thereafter, the column was re-equilibrated at 0% B for 20 min with a linear increase in the flow rate from 1 mL min⁻¹ to 1.25 mL min⁻¹. The eluting metabolites were detected at 264 nm, and retention times and peak areas were compared to a range of authentic ergothioneine standards (as low as 20 pmol and as high as 19.6 nmol) that were analyzed daily. The extraction of ergothioneine in each mushroom sample was corrected for the percentage recovery of an ergothioneine spike (360 nmol or 1080 nmol) that was added to a duplicate sample for each type of mushroom.

2.4. Ergothioneine Capture from Mushroom Biomass Waste

Frozen oyster mushroom biomass waste powder (2 g) was combined with 40 mL of pre-chilled 70% (v/v) ethanol. The homogenate was agitated, clarified by centrifugation, and the whole 40 mL was filtered and dried under vacuum, as described in Section 2.3. The dried residue was dissolved in 4.2 mL of Milli-Q water, and then 4 mL of the resuspension was acidified with 108 µL of 1 M HCl. The enrichment of ergothioneine was adapted from a published cation exchange column chromatography method for the enrichment of amino acids from corn extracts [26]. Briefly, 8 mL of Amberlite™ IRC120 H (hydrogen
form) was activated as per the manufacturer’s instructions (Sigma-Aldrich) and transferred to a gravity flow column (1.4 cm × 5 cm, internal diameter × length) fitted with glass wool at its base. The acidified oyster mushroom cull extract was applied to the Amberlite resin, which had been pre-equilibrated with Milli-Q water. The column flow-through was collected into a single fraction. Thereafter, the Amberlite resin was washed with 28 mL of Milli-Q water, and the wash effluent was collected. Ergothioneine was eluted from the cation exchange column with 28 mL of 6 M NaOH, and 4 mL fractions were collected. All chromatography fractions were neutralized with 10 M HCl. Unless otherwise mentioned, 10 µL of each fraction was analyzed via HPLC-DAD, as described under Section 2.3.

2.5. Statistical Analysis

Statistical analyses of metabolite data were conducted with R version 4.3.0 [27]. Prior to analysis, data were assessed for normality using the Shapiro–Wilk test and quantile–quantile plot, and homoscedasticity was assessed using Bartlett’s test and boxplots of residuals. When required, data were log transformed to satisfy the assumption of homogeneity of variance. Thereafter, a two-way ANOVA followed by Tukey’s HSD test were performed to determine significant differences among fresh and waste mushrooms; means were considered statistically different at $p \leq 0.05$.

3. Results and Discussion

3.1. Analysis of Glutathione Metabolites in Fresh and Waste Mushrooms

The fresh and biomass waste mushrooms were analyzed for their total glutathione (sum of GSH and GSSG) alongside the individual GSSG and GSH profiles. Using a spectrophotometric-based GR recycling assay coupled with the reduction of DTNB (Figure 2). The total glutathione concentration in fresh white button mushrooms was 203 nmol g FW$^{-1}$. A statistical analysis revealed 75% smaller concentrations were evident in the stem waste of this mushroom. Interestingly, the total glutathione concentrations in both the fresh and biomass waste of grey oyster and shiitake mushrooms were comparable to that of fresh white button mushrooms. Apart from white button mushroom stem waste, the total glutathione concentration (i.e., on a mg g FW$^{-1}$ equivalence basis) ranged from 0.062 to 0.108 mg g FW$^{-1}$, matching a previous report [3]. It is worth mentioning that prior study did not establish the respective proportions of GSH and GSSG in fresh mushrooms.

The GSH concentration ranged from 190 to 389 nmol g FW$^{-1}$ for most of the fresh mushrooms and biomass waste that were assessed in this study (Figure 2). GSH accounted for most of the total glutathione pool. The GSH concentration in white button mushroom stem waste was 77% less than that of fresh mushrooms, and as much as 89% smaller than the GSH concentration detected in oyster mushroom waste. This is the first report of GSH in mushroom biomass waste, as well as for North American fresh mushrooms. Except for a study analyzing edible mushrooms cultivated in Türkiye [28], there is a scarcity of information on their finite levels of GSSG, and on the glutathione redox ratio (GSH/GSSG). We found that GSSG represented a marginal proportion (i.e., 3% to 7%) of the total glutathione detected in most of the fresh and waste mushrooms. By contrast, GSSG accounted for 14% of the total glutathione in the stem waste of white button mushrooms. To assess the antioxidant potential of the mushroom materials, we calculated the ratio of GSH to GSSG, which ranged between 13.8 and 28.5 for most mushrooms, including their biomass waste. An exception was the stem waste of white button mushrooms which contained a GSH to GSSG ratio that was at least 62% lower than all other mushrooms. To some extent, our findings match the GSH to GSSG ratios reported for a few lesser-known edible mushrooms, (e.g., Brittlegill mushroom) [28], but this was not the case for other mushrooms. For example, the GSH to GSSG ratio in the stem waste of white button mushrooms is 2-fold to 18-fold more than that of other edible mushrooms that are regionally grown in Türkiye, including white button mushrooms [28]. The relatively low GSH/GSSG in white button mushroom stem waste could be due in part to some degree of oxidative stress during cultivation or harvesting. This is plausible given that there is an increase in the conversion
of GSH to GSSG in abiotic-stressed plants, including with postharvest handling [22,29]. Indeed, it is known that the total glutathione pool of abiotically stressed plants contain 10% or more GSSG than non-stressed plants [4]. Moreover, it has been proposed that the recycling of ergothioneine from its oxidized forms requires reductant in the form of GSH, culminating in GSSG formation [30]. In mammalian systems, ergothioneine disulfide and 5-oxo-ergothioneine are converted to ergothioneine by GR and thioredoxin reductase [31]. It remains to be determined whether the lower glutathione redox in white button mushroom stem waste coincides with a greater degree of ergothioneine recycling than that which occurs in the fresh fruiting body.

Figure 2. Glutathione metabolites and glutathione redox status of fresh mushrooms and biomass waste of white button, shiitake, and grey oyster mushrooms. All glutathione metabolite concentrations were calculated on a per g fresh weight (FW) basis. Total glutathione represents the sum of GSH and GSSG. The concentration of GSH for each mushroom was calculated as the difference between total glutathione and GSSG. The bottom plot represents the ratio of the concentration of GSH to the concentration of GSSG (i.e., glutathione redox) in each mushroom type. Within each plot, each datum represents the mean ± standard error of three cultivation replicates, and no statistical difference is represented by means sharing the same letter ($\alpha \leq 0.05$).
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3.2. Analysis of Ergothioneine in Fresh and Waste Mushrooms

Fresh harvested mushrooms of the three species and their culls/stem waste discarded after cultivation were separately assessed for their concentrations of ergothioneine following ethanolic extraction and detection via HPLC-DAD. HPLC-DAD analysis revealed a peak within all mushroom extracts that co-eluted with that of an authentic ergothioneine standard. The ergothioneine concentration in fresh harvested white button mushrooms was approximately 330 nmol g FW\(^{-1}\) (Figure 3). Similar levels of this antioxidant were apparent in extracts prepared from fresh shiitake mushrooms, but as much as 205% greater in fresh grey oyster mushrooms. On an equivalence basis, the ergothioneine concentrations for all three types of fresh mushrooms ranged from 0.075 to 0.231 mg g FW\(^{-1}\). These levels range from being on par to 2.2 times in excess of the ergothioneine concentrations previously reported for these same fresh mushrooms [3,32], and 20% less to as much as 144% greater than the ergothioneine of dried mushrooms [33], assuming a 90% loss of moisture with drying. The variation between studies could be due in part to the manner in which the mushrooms were handled prior to analysis. Our analysis used mushroom powders that were initially flash-frozen with liquid N\(_2\) and then transferred to \(-80^\circ\text{C}\), whereas the Kalaras et al. study used mushrooms that were held at \(-20^\circ\text{C}\) [3]. Some of the mushrooms analyzed therein contained ergothioneine concentrations that were 30% to 78% less than those detected in freeze-dried mushrooms of the same culinary types, as described in another study [23]. In fact, it is well known that some metabolites are easily degraded within samples that are frozen at \(-20^\circ\text{C}\), whereas chemical profiles are better preserved with flash-freezing followed by ultra-low temperature storage or freeze-drying [34]. Alternatively, the differences in ergothioneine content across studies including our own could be associated with varying cultivation-related practices. For example, an increase in the moisture content of the cultivation substrate enhances the ergothioneine content of king oyster mushrooms [35]. The cultivation substrate also influences ergothioneine content, as growth on grape pomace yields golden oyster mushrooms that contain 23% less ergothioneine than those cultivated on wheat straw [36].

The stem waste of white button mushrooms and the culls of the other two mushroom types were also analyzed for the presence of ergothioneine. Here, we provide evidence that ethanolic extracts prepared from white button mushroom stem waste contained ergothioneine concentrations that matched those of freshly harvested mushrooms. This is surprising given that ergothioneine and total glutathione concentrations within the edible portion of the stem of shiitake, portabella and yellow oyster mushrooms are lower than the levels in their fruiting body caps [3]. In fungi, ergothioneine biosynthesis requires the metabolic precursors histidine, cysteine, and S-adenosylmethionine [11,12], but an argument can be made that ergothioneine content in mushrooms could be due in part to GSH-mediated regeneration from oxidized ergothioneine [30,31]. This may provide a rationale for why ergothioneine was detected in 37% to 389% greater concentrations than GSH in the three mushroom types investigated in this study, including the biomass waste. The greatest difference between these two metabolite concentrations was evident in the stem waste of white button mushrooms. Moreover, GSH-mediated ergothioneine recycling may somewhat explain why ergothioneine in oyster mushrooms was 120 to 450% greater than the other two mushroom types. Apart from recycling, it remains to be determined whether ergothioneine biosynthesis enzyme (i.e., Egt1 and Egt2 [11]) activities are more prevalent in oyster mushrooms than shiitake and white button mushrooms.
were calculated on a per g fresh weight (FW) basis. Within each plot, each datum represents the mean ± standard error of three cultivation replicates, and no statistical difference is represented by means sharing the same letter (α ≤ 0.05).

High concentrations of ergothioneine were detected within extracts prepared from the culls of shiitake and grey oyster mushrooms, which matched those of their market-ready produce counterparts. On the whole, the ergothioneine detected in the waste biomass from our study is two to four orders of magnitude greater than the ergothioneine of boiled extracts prepared from the processing wastes of enoki and branching oyster mushrooms [32]. In fact, boiling reduces ergothioneine content in enoki and some oyster mushrooms [37,38]. Other sources of ergothioneine include microbes. A recent study found that the food-grade microbe Aspergillus oryzae produces 11.5 mg of ergothioneine per kg of culture media, although a 20 times greater yield is accomplished when A. oryzae is engineered with ergothioneine biosynthesis genes from Neurospora crassa [39]. Much greater ergothioneine production is possible in bioengineered Escherichia coli [40], but this bacterium does not meet food-grade standards. By comparison, extrapolation of the data for oyster mushroom culls demonstrates this biomass waste contains 274 mg ergothioneine per kg. These culls represent an inexpensive and ready source of ergothioneine for industrial purposes.

3.3. Capture of Ergothioneine from Oyster Mushroom Culls

The second aim of our study was to develop a feasible strategy for the capture of ergothioneine from mushroom biomass waste. We used oyster mushroom culls for small-scale ergothioneine purification, as this waste material had the most ergothioneine. Borodina et al. [41] proposed that ion exchange chromatography could be used to isolate ergothioneine from mushrooms, although this has never been tested. Here, Amberlite IRC120 H (hydrogen form) (i.e., cation exchange) column chromatography was used to capture ergothioneine from ethanolic extracts of oyster mushroom culls (Figure 4). The procedure was adapted from a strategy used for the separation of amino acids derived from corn kernels [26]. A survey of the spectral properties of the peaks in the HPLC chromatogram of the oyster mushroom cull extract revealed that many of these metabolites had absorption maxima within the UV light range that are typical of most amino acids [42]. Ethanolic extracts of mushrooms are known to contain amino acids [43].

Figure 3. Ergothioneine concentrations in fresh mushrooms and waste generated from the cultivation of white button, shiitake, and grey oyster mushrooms. All ergothioneine concentrations were calculated on a per g fresh weight (FW) basis. Within each plot, each datum represents the mean ± standard error of three cultivation replicates, and no statistical difference is represented by means sharing the same letter (α ≤ 0.05).
Figure 4. HPLC-DAD analysis of an oyster mushroom cull extract and ergothioneine captured from Amberlite IRC120 H column chromatography. Chromatograms represent the following: a representative ergothioneine standard (258 pmol); a ten times dilution of the oyster mushroom cull extract after it was taken to dryness and resuspended in Milli-Q water; and a representative fraction (i.e., fraction 2) from the Amberlite IRC120 H eluate after a neutralization step. The volume of the neutralized eluate recovered from the cation exchange column was 4.4 times greater than that of concentrated biomass waste extract that was loaded onto the column.
The HPLC-DAD analysis determined that the initial three elution fractions contained a peak with an absorption maximum of 264 nm that matched the retention time (13.5 min) of an authentic ergothioneine standard. HPLC analysis determined that 50% or more of the metabolite peaks that were evident in the chromatogram of the biomass waste extract were not present in the eluate collected from the Amberlite IRC120 H column (Figure 4). Ergothioneine was absent in the flow-through and wash fractions, as well as fractions 4 to 7 of the eluate. Across three separate experiments, the cation exchange chromatography procedure captured 6 to 12% of the ergothioneine present in the original oyster mushroom cull extract. On average, 78 ± 9.4 nmol of ergothioneine were captured from oyster mushrooms culls containing 956 ± 19 nmol of ergothioneine per g FW⁻¹. The smaller ergothioneine content in the ethanolic extract relative to that shown in Figure 3 is likely due to the exclusion of additives in the extraction solution (e.g., dithiothreitol). The finite amount of ergothioneine in the cation exchange eluate is likely a consequence of its exposure to alkaline conditions thereafter, given that 6 M NaOH was used as the eluent. This rationale is based on the observation that ergothioneine predominates as its bioactive thione form at physiological pHs, whereas it is converted to its unstable thiol form at pH > 11 [44]. Although the ergothioneine capture platform is promising, future research should investigate the removal of the contaminants that co-occur with ergothioneine in the Amberlite IRC120 H eluate, as well as alternate elution solutions for this capture step. The fact that the final ergothioneine preparation contained other metabolites is not uncommon. The enrichment of glycine betaine (a chemical with structural similarity to ergothioneine) from plants via cation exchange yields a final preparation that is contaminated with 20% of the amino acids that were originally present in the extract, including proline [45]. There are many potential industrial uses of this waste-derived antioxidant. For example, due to its anti-aging properties, ergothioneine is an ingredient in some cosmetic products [46], with levels approximating 0.2 mg per g of cosmetic product [47]. There is also the possibility of targeting the use of the ergothioneine and GSH from mushroom waste as food preservation agents. Exogenously applied ergothioneine prevents the browning of beef, fish, and seafood products, as well as sliced mushrooms [48]. Similarly, the application of exogenous GSH alleviates postharvest chilling injury in bell pepper fruit [49]. The platform developed here can be used to prepare ergothioneine-based nutraceutical and/or postharvest preservation products.

4. Conclusions

The biomass waste products collected from Canadian mushroom farms were shown to have high concentrations of ergothioneine and GSH that rivaled the content of these antioxidants in fresh mushrooms, specifically oyster, shiitake, and white button mushrooms. Furthermore, these mushrooms contained little GSSG, indicating that there is strong glutathione redox potential within mushroom biomass waste. Oyster mushroom culls were the richest source of ergothioneine. The purification of ergothioneine from oyster mushrooms culls represents a unique economic opportunity given the value of commercially available ergothioneine. Assuming that the complete purification of ergothioneine would further reduce the Amberlite IRC120 H column yield to 1% or less, as little as 3.64 kg of this biomass waste would be required to provide a milligram of high-purity ergothioneine. Given this information, it is tempting to speculate a metric ton of mushroom biomass waste could generate 275 mg of purified ergothioneine with a projected value of $3300 to $14,300 (US dollars). This is based on the current cost of $12 to $52 (US dollars) per milligram for high-purity ergothioneine available from commercial sources. The ergothioneine-capturing technology established in this study provides the foundation for repurposing mushroom biomass waste that would otherwise be deposited to landfills. The valorization of this mushroom waste also provides an opportunity for secondary industries looking to develop antioxidant-based products such as nutraceuticals.
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