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SHORT COMMUNICATION

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Stability of psilocybin and its four analogs in the biomass of the psychotropic mushroom *Psilocybe cubensis*

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Abstract

Psilocybin, psilocin, baeocystin, norbaeocystin, and aeruginascin are tryptamines structurally similar to the neurotransmitter serotonin. Psilocybin and its pharmacologically active metabolite psilocin in particular are known for their psychoactive effects. These substances typically occur in most species of the genus *Psilocybe* (Fungi, Strophariaceae). Even the sclerotia of some of these fungi known as "magic truffles" are of growing interest in microdosing due to them improving cognitive function studies. In addition to microdosing studies, psilocybin has also been applied in clinical studies, but only its pure form has been administrated so far. Moreover, the determination of tryptamine alkaloids is used in forensic analysis.

In this study, freshly cultivated fruit bodies of *Psilocybe cubensis* were used for monitoring stability (including storage and processing conditions of fruiting bodies). Furthermore, mycelium and the individual parts of the fruiting bodies (caps, stipes, and basidiospores) were also examined. The concentration of tryptamines in final extracts was analyzed using ultra-high-performance liquid chromatography coupled with mass spectrometry. No tryptamines were detected in the basidiospores, and only psilocin was present at 0.47 wt.% in the mycelium. The stipes contained approximately half the amount of tryptamine alkaloids (0.52 wt.%) than the caps (1.03 wt.%); however, these results were not statistically significant, as the concentration of tryptamines in individual fruiting bodies is highly variable. The storage conditions showed that the highest degradation of tryptamines was seen in fresh mushrooms stored at −80°C, and the lowest decay was seen in dried biomass stored in the dark at room temperature.

KEYWORDS

LC-MS, mushrooms, psilocybin, stability, tryptamines

1 | INTRODUCTION

Psychoactive mushrooms have been used as entheogens during transcendental ceremonies by Mexican inhabitants, mainly by shamans, for thousands of years. Mushrooms used for religious and spiritual rituals are called "magic mushrooms."[1–3] There are many mushroom species that contain high levels of psychotropic tryptamines, including the genera *Pluteus*, *Panaeolus*, *Inocybe*, *Gymnopilus*, *Galerina*, *Pholiotina*, and especially *Psilocybe*.[4–7] In the 1950s, these magic mushrooms were introduced to Europe thanks to Gordon Wasson, who brought them from Mexico from the shaman Maria Sabina of the Mazatec tribe.[8,9] Gordon Wasson passed these Mexican mushrooms on to Roger Heim, who identified them as *Psilocybe mexicana*, and then passed them on to Albert Hofmann, who in 1958 isolated

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psilocybin and psilocin from them.[10,11] Psilocybin and its hydrolyzed metabolite psilocin are well-known psychoactive tryptamines of mushrooms, which are controlled substances.[5,12,13] Nevertheless, other fungal tryptamine analogs have not been explored in detail. Structural analogs of psilocybin and psilocin are baeocystin, norbaeocystin, aeruginascin,[14–16] and norpsilocin, which was identified in 2017.[17]

Currently, psilocin and psilocybin are used in neuropsychopharmacological studies because of their therapeutic potential in the treatment of depression, obsessive–compulsive disorder, anxiety, and cluster headaches.[18–20] In clinical studies, mainly synthetic forms of psilocybin and psilocin have been administrated to subjects. However, studies in which people were given the natural form of these tryptamines known as "magic truffles" (sclerotia of some *Psilocybe* species) have also been published.[21,22]

The knowledge of tryptamine stability in mushrooms is very important for sample processing and long-term storage. It is already known that aqueous standard solutions of psilocin and psilocybin are photosensitive and thermolabile and undergo the oxidation upon exposure to air and light.[23–25] However, the stability of tryptamine alkaloids in mushrooms and the effect of storage and drying conditions have not been comprehensively published.[26,27]

The most common methods for the quantification of psychoactive tryptamines are based mainly on liquid (LC) or gas chromatography (GC) coupled with mass spectrometry (MS). The advantages of these methods are accuracy, repeatability, selectivity, robustness, and the possibility to reach low detection limits and multiple analyte analyses in a short time. However, gas chromatography is not recommended for the quantification of tryptamines because of their poor volatility and heat lability.[28–30] In our study, ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-MS/MS) was employed to investigate the stability of five tryptamine alkaloids in the biomass of cultivated fruiting bodies of the psychoactive species *Psilocybe cubensis*.[31–33]

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Analytical standards of psilocybin and psilocin were purchased from Cayman Chemical, USA. The analytical standards of aeruginascin, baeocystin, and norbaeocystin were synthesized in-house at the UCT Prague by Dr. Radek Jurok of the Department of Organic Chemistry. Details of the synthesis and characterization of these tryptamines are described in the supporting information.

Psilocin, psilocybin, and aeruginascin were dissolved in methanol, and baeocystin and norbaeocystin were dissolved in 50% (v/v) methanol, respectively. Eluent additives for LC-MS—acetic acid, formic acid, and ammonium formate—were obtained from Honeywell, Fluka™, USA. Methanol LC-MS CHROMASOL™ was obtained from Honeywell, Reidel-de Haën™, USA. Ultrapure water, 18.2 MΩ·cm, was produced by the Smart2Pure 12 UV system (Thermo Fisher Scientific, Barnstead, Germany).

2.2 | Cultivation of mushrooms and their drying and storage

P. cubensis mushrooms were cultivated in the laboratory at the UCT Prague to obtain a large compact matrix for stability experiments according to the procedure in the supporting information where cultivation is also discussed.

To verify which mushroom storage is most suitable for maintaining the tryptamine content, five fresh fruiting bodies of *P. cubensis* (height of approximately 20 cm) were cut lengthwise into equivalent portions. Subsequently, these parts were stored under various conditions that are given in Table 1. The cultured mushrooms were then dried in the dark at room temperature. Approximately 10 dried fruiting bodies were homogeneously powdered using a mortar and pestle. The powder was divided into five different conditions according to Table 1.

2.3 | Blank matrices and preparation of samples and their extracts

The microscopic fungus *Penicillium candidum* was used as a blank matrix for the mycelium and fresh or and dried *Agaricus bisporus* fruiting bodies were used as a blank matrix for the *P. cubensis* mushrooms. The fruiting bodies were extracted using 0.5% (v/v) acetic acid in methanol after weighing. They were subsequently vortexed, centrifuged, and re-extracted according to the details included in the supporting information, which also includes a description of the formation and plotting of the blank matrices.

2.4 | Instrumentation and conditions of UHPLC-MS/MS

A UHPLC 1290 Infinity assembly (Agilent Technologies, USA) with a Zorbax Eclipse Plus C18 column, 100 × 2.1 mm, 1.8 μm and with a Zorbax Eclipse Plus C18, 5 × 2.1 mm, 1.8 μm pre-column (Agilent

TABLE 1 Processing of fresh fruiting bodies and storage conditions of dried fungal homogenized powder

Sample identification	Processing conditions of fresh fruiting bodies	Storage conditions of dried fungal powder
1	Dark, 20°C	Light, 20°C
2	Light, 20°C	Dark, 20°C
3	Lyophilizer, −50°C	Fridge, 4°C
4	Freezer, −20°C	Freezer, −20°C
5	Freezer, −80°C	Freezer, −80°C

Technologies, USA) at 40°C was used for the chromatographic separation; 10 mmol·L^{-1} ammonium formate with 0.1% (v/v) formic acid (mobile phase A, MPA) and 10 mmol·L^{-1} ammonium formate with 0.1% (v/v) formic acid in methanol (mobile phase B, MPB) were used as the mobile phases. The flow rate was 0.25 ml·min^{-1}, and the injection volume of the sample was 3 μl. The total run time of the separation was 7 min. The chromatographic method held the initial composition 10% MPB ramping to 35% MPB in 3.5 min and then increased to 100% MPB at 4 min and held for 0.5 min. It then followed a linear gradient to 90% MFA at 5 min and held 2 min to the starting condition.

A triple quadrupole 6460 spectrometer (Agilent Technologies, USA) was used for the detection and quantification of tryptamines in the mushrooms. Electrospray ionization in the positive mode (ESI$^+$) was used for all of the analytes. The parameters of the ion source were as follows: drying gas temperature (nitrogen) 340°C, drying gas flow 10 L·min^{-1}, nebulizer (nitrogen) 25 psig, sheath gas temperature (nitrogen) 400°C, sheath gas flow 12 L·min^{-1}, and capillary voltage 2300 V.

The analysis was conducted in the dynamic multiple reaction monitoring (dMRM) acquisition mode. The two most intensive transitions were used for all of the analytes except for psilocin, for which we used three transitions. The characteristics of the dMRM method are summarized in Table S1 with the chromatograms of aeruginascin, baeocystin, norbaeocystin, psilocin, and psilocybin (Figure S3).

2.5 | Method validation

The developed method was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) standard practices for method validation in forensic toxicology,[34] Tutorial review on validation of liquid chromatography-mass spectrometry methods: Part I,[35] and the European Medicines Agency (EMA) guideline on bioanalytical method validation.[36] The method was validated for specificity, linearity, limits of detection (LOD), limits of quantitation (LOQ), precision, accuracy, recovery, matrix effect, and stability in the supporting information together with its results.

3 | RESULTS AND DISCUSSION

3.1 | Optimization of the extraction procedure

All of the concentration levels are given in wt.% of dry matter. The preparation of the mushroom samples and the extraction method were inspired by other studies.[22,37–41] Based on the fact that mushroom indole alkaloids are compounds with high polarity, we used polar solvents.[16] Due to the high concentration of psilocybin, it was always evaluated in 1000× diluted samples, so the matrix effect was kept negligible. Other analytes were evaluated from 100× or 10× diluted samples. If their concentration was within the calibration curve of both dilutions, the results were evaluated using their average.

3.1.1 | Effect of homogenization

The experiments verified whether it is better to extract fruiting bodies in pieces or homogenized. The experiments were performed on fresh and dried mushrooms according to the procedure in the supporting information.

We found that the dried fungal fruiting bodies had a better yield from a fungal powder than from whole pieces. Conversely, in the case of fresh fungal fruiting bodies, the extraction of larger unprocessed pieces of fresh fruiting bodies was found to be more effective than chopping these mushrooms into small pieces. The fresh fruiting bodies quickly turned blue as they were cut into smaller pieces, which may be due to the psilocybin hydrolyzing to psilocin, which then oxidizes to quinoid dye.[24,25]

In summary, the yields of the analytes from the dried mushroom powder in comparison with the unprocessed dried mushrooms were as follows: 16% increase in psilocybin, 13% increase in psilocin, 74% increase in baeocystin, and 40% increase in aeruginascin. The concentration of norbaeocystin was below the LOQ. On the contrary, the yields of the analytes from unprocessed fresh mushrooms in comparison with chopped fresh mushrooms were as follows: 33% increase in psilocybin, 37% increase in psilocin, 1.5% increase in baeocystin, and 12% increase in aeruginascin. The concentration of norbaeocystin was below the LOQ. Therefore, the unprocessed fresh mushrooms contained approximately 30% more tryptamines than the chopped fresh mushrooms.

3.1.2 | Extraction solvents

Methanol with formic acid (0.1%, 0.2%, 0.5%, and 1.0%, v/v) or acetic acid (0.1%, 0.2%, 0.5%, and 1.0%, v/v), 50% (v/v) methanol, deionised water, ethanol, 75% (v/v) ethanol, isopropanol, and 25 mmol·L^{-1} acetate buffer (pH 4.5) were tested for the optimization of extraction solvents. More details on the process are included in the supporting information. Methanol with 0.5% (v/v) acetic acid was chosen as the optimal extraction solvent. The results were evaluated only qualitatively using by Agilent software MassHunter Qualitative Analysis, according to which the solvents with the largest area under the peak were selected. Methanol is often used as a suitable extraction solvent in many scientific works.[14,42,43] It corresponds with the hypothesis that acidification may be suitable for increase the solubility of basic tryptamine alkaloids, mainly in a phosphorylated form.[44]

3.1.3 | Repeated extraction

The next step after choosing the extraction solvent was to evaluate whether the efficiency of the whole process was sufficient. We decided to use a subsequent extraction of the matrix with another solvent. To evaluate the efficiency of the extraction, subsequent extractions of the matrix were performed. Approximately 20% of the analytes were found in the second extract, but no further

re-extractions improved recovery. Methanol was chosen as the best solvent for a re-extraction of the matrix. The re-extraction solvents were selected in the same way as the extraction solvents, that is, using Agilent software MassHunter Qualitative Analysis. During the re-extraction of the residual pellet, acidification of the solvent was probably no longer necessary.

3.1.4 | Effect of mechanical agitation and extraction time

Part of the experiment was to evaluate whether vortexing increases the yield of tryptamines from the matrix. Ten tubes of pulverized mushrooms were macerated in 1 ml of solvent, five were vortexed at 13 × *g* for 2 h, and five were not mechanically agitated. Higher concentrations of all of the analytes were found in the vortexed samples, with a 9% increase in psilocin, a 17% increase in aeruginascin, 21% increase in psilocybin, 26% increase in baeocystin, and 40% increase in norbaeocystin.

The duration of mechanical agitation, ranging from 20 to 360 min, gave almost identical concentrations of all of the analytes (difference <1%).

3.2 | Effect of temperature change

Degradation of tryptamines was observed at 25°C, 50°C, 75°C, 100°C, 125°C, and 150°C for 30 min according to the procedure attached in the supporting information.

The decay in the concentration of phosphorylated tryptamines is significantly noticeable from 100°C. Up to this temperature, the concentrations of phosphorylated tryptamines reduced gradually; for example, at 150°C, the yields significantly decreased by approximately 80% (Figure 1). An exception to these results was psilocin, where the concentration increased at higher temperatures. The loss of phosphorylated analytes and at the same time the increased amount of psilocin may be explained by the dephosphorylation of tryptamines to their hydrolyzed forms, which are easily subjected to oxidative degradation. The temperature of 25°C was chosen for the extraction as it reached the highest yield of analytes, which is in line with the previously published work.[9]

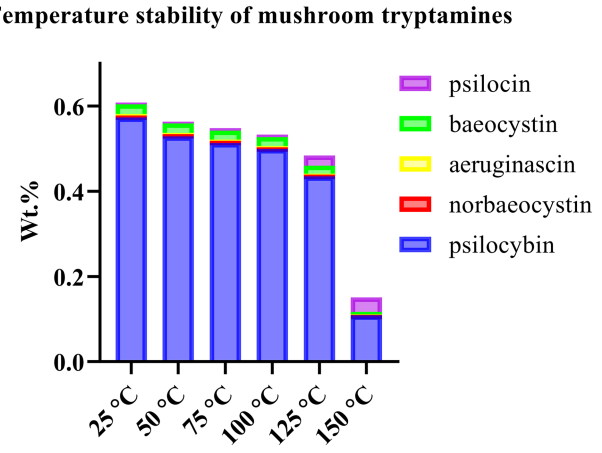

FIGURE 1 Temperature stability of mushroom tryptamines from fungal homogenous powder that was heated in five replicates for 30 min [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Basidiospores and mycelium

Spore prints (biomass of spores released from the hymenium) from three individual caps of *P. cubensis* were analyzed, but no tryptamines were detected.

Mycelium samples of *P. cubensis* from two Petri dishes were prepared in six replicates. The mycelia were dried after analysis for 6 h in vacuum evaporators to determine the dry weight and water content. The water in the mycelium was approximately 89 wt.%. The mycelium samples contained only psilocin with an average concentration of 0.15 wt.% of dry matter. Mycelial samples in previously published studies have contained highly variable concentrations of psilocin (0.0–0.2 wt.%) and psilocybin (0.0–2.0 wt.%). The results of our analysis are in the range of the published values.[45–47] We also tried to evaluate whether psilocin permeates through agar, and we found that approximately 10 times lower concentrations of psilocin (0.01 wt.%) were quantified in the agar on which the mycelium grew. Although this is contrary to previous results, it is not exceptional due to the variability of the alkaloid content in the mycelia themselves.[31]

3.4 | Fresh versus dried fruiting bodies

These experiments aimed to determine the water content in fresh mushrooms and to analyze the concentration of tryptamines in fresh and dried fruiting bodies, which were dried in the dark at room temperature. According to the previous results from the homogenization effects, the dried mushrooms were analyzed as mushroom powder and fresh mushrooms in whole pieces for the highest possible yield. In the experiment with fresh mushrooms, an equivalent part of each fruiting body was used to determine the water content, and the other part was used to analyze the concentration of tryptamines. These experiments were performed on 10 fresh cultivated fruiting bodies of *P. cubensis*. In conclusion, the water content in the fresh mushrooms was approximately 90%, which is in agreement with another mushroom study,[48] and the concentrations of tryptamines were as follows: 0.54 wt.% of psilocybin, 0.02 wt.% of norbaeocystin, 0.01 wt.% of aeruginascin, 0.05 wt.% of baeocystin, and 0.25 wt.% of psilocin. Within the experiments with homogeneous dried fungal powder, the concentrations of tryptamines were as follows: 0.62 wt.% of psilocybin, 0.02 wt.% of norbaeocystin, 0.01 wt.% of aeruginascin, 0.06 wt.% of baeocystin, and 0.16 wt.% of psilocin. The presence of baeocystin in *P. cubensis* the fruiting bodies was demonstrated more than 30 years ago, but the content of aeruginascin and norbaeocystin has not been studied yet in this fungal species.[31,40,49] The measured data correlate with previously

published works, where the concentrations of alkaloids were in the range of 0.00–0.60 wt.% for psilocin, 0.17–0.63 wt.% for psilocybin, and 0.02 for baeocystin, for which we measured more.[18,31,40,46] All of the concentration levels are given in wt.% of dry matter.

In summary, the average total content of tryptamines in fresh and dried mushrooms was approximately 0.87 wt.% in both cases. This is confirmed by studies that claim that the tryptamine content in fungal biomass is not reduced by drying in the dark at room temperature.[14,26] Generally, there were higher concentrations of all analytes except for psilocin in dried mushroom powder. We found a 27% increase in norbaeocystin, a 12% increase in aeruginascin, a 26% increase in baeocystin, and an 18% increase in psilocybin in comparison to the fresh mushrooms. On the contrary, there was a 34% increase in psilocin in the fresh mushrooms. It is supposed that the higher concentrations of hydrolyzed forms of tryptamines are present in fresh mushrooms, but the phosphorylated forms are mainly found in dried fungal biomass. Tryptamines in fresh fruiting bodies may act enzymatically by kinases before the fruiting body is dried, as in the case of phosphorylating psilocin to psilocybin.[50] It may be also because, according to Alexander Shulgin, psilocin is already unstable in solutions, and fresh fruiting bodies still contain approximately 90% water, which means that it forms a similar environment to the solution[51] while psilocybin is more stable than psilocin.[4]

3.5 | Caps versus stipes of fungal fruiting bodies

The fruiting bodies were separated into caps and stipes to determine the tryptamine concentrations in these mushroom parts. These experiments were performed on dried fungal fruiting bodies in six replicates. On average, the caps contained 0.01 wt.% of aeruginascin, 0.07 wt.% of baeocystin, 0.88 wt.% of psilocybin, 0.01 wt.% of norbaeocystin, and 0.06 wt.% of psilocin. In stipes, we found <0.01 wt.% aeruginascin, 0.03 wt.% baeocystin, 0.47 wt.% psilocybin, <0.01 wt.% norbaeocystin, and 0.01 wt.% psilocin.

There was approximately 50% less baeocystin, psilocybin, and norbaeocystin in the stipes than in the caps. The stipes contained 32% less aeruginascin and 85% less psilocin than the caps. The total content of tryptamine alkaloids in the stipes was approximately 50% less than in the caps. These results are slightly different from an older study, which states that the psilocin content is higher in the stipes than in the caps in *P. cubensis*, but a similar distribution of psilocybin (higher levels in the caps than in the stipes) was observed in *Psilocybe samuiensis*.[52] Our results correspond with the published work.[26]

The parametric two-sample unpaired *t* test with Welch correction in the R program found that the results were not statistically significant, as the *p* value was 0.3756. This may be due to the large difference between the alkaloid concentrations in the individual fruiting bodies. This means that although the average content of tryptamines in caps is higher than in stipes, due to the SD, where there is high variability between individual fruiting bodies, it cannot be said that this statement applies to all fruiting bodies. The variability of tryptamines in individual fruiting bodies has been described in other published works.[52,53]

3.6 | Processing of fresh fruiting bodies

The samples were measured in duplicates using UHPLC-MS/MS after 3 months of storage (Figure 2). To verify which type of mushroom processing, including storage and drying, is most suitable for maintaining tryptamine content, five fresh fruiting bodies of *P. cubensis* (height approximately 20 cm) were cut lengthwise into equal portions as described in Section 2.2. These groups were processed and stored under different conditions, which are summarized in Table 1.

1. The mushrooms that were stored in the light at room temperature contained very similar concentrations of norbaeocystin and aeruginascin, but a 12% decrease in baeocystin, 9% decrease in psilocybin, and even 46% decrease in psilocin were noted when compared with the fungal samples that were stored in the dark at the same temperature. This is a confirmation of rapid psilocin degradation in the light because of high photosensitivity.[23,54]

2. The highest concentration of tryptamines was found in samples that were stored in the dark at 20°C (1.30 wt.% psilocybin, 0.01 wt.%

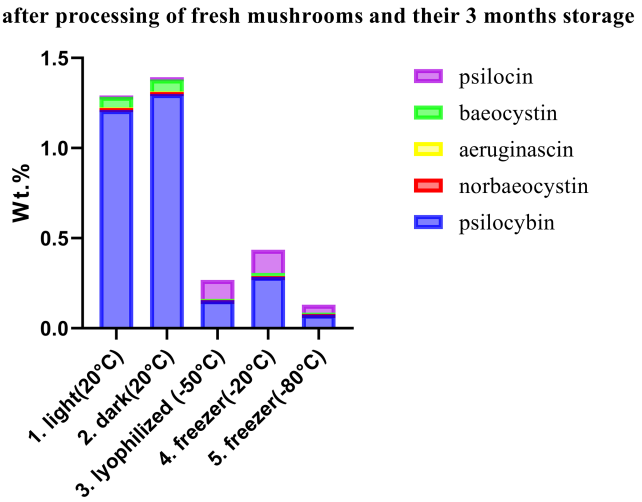

FIGURE 2 Tryptamine content after processing of fresh fruiting bodies and their storage after 3 months [Colour figure can be viewed at wileyonlinelibrary.com]

norbaeocystin, <0.01 wt.% aeruginascin, 0.07 wt.% baeocystin, and 0.01 wt.% psilocin). This is confirmation as discussed in Section 3.4 that drying the mushrooms in the dark at room temperature does not have a reducing effect on the concentration of indole alkaloids.

3. The mushrooms were stored in a freezer (−20°C) before loading into a lyophilizer. It was found that the concentrations of all analytes were reduced in the lyophilized samples except for psilocin. The most significant decay was in psilocybin, where there was an 88% reduction in concentration, from 1.30 wt.% to 0.16 wt.%. Gartz states that lyophilized mushroom samples decompose rapidly when stored at room temperature after lyophilization because these fungi have a porous structure.[9] After lyophilization, our lyophilized mushrooms were stored in a freezer at −20°C, and the tryptamines also rapidly degraded.[9] This is in contrast to Beug and Bigwood, who state that lyophilization of fungi does not decrease the concentration levels of tryptamine alkaloids (specifically psilocybin and psilocin).[27] This difference may be due to a disruption of the cellular structure of the fungus, which occurred due to the splitting of the mushroom into analogous parts that were used for various types of processing. Disruption of the cell structure may lead to faster degradation of tryptamines due to hydrolysis to psilocin and subsequent oxidation to quinoid dyes.[55,56] This hypothesis was confirmed by the fact that almost immediately after cutting, the fresh fruiting body turned blue.

4. and 5. When storing mushrooms in the freezer (−20°C and −80°C), significantly lower concentrations of all of the analytes were detected except for psilocin. The most pronounced decay occurred at −80°C, where 94% less psilocybin was measured than for samples stored in the dark at room temperature.

The degradation of psilocybin may be due to the gradual biotransformation of the sliced specimens since psilocybin is dephosphorylated enzymatically by phosphatase to psilocin, which is unstable because of it being readily subjected to oxidation to blue quinoid dye.[55,56] Significant blue staining was observed for the chopped fresh mushroom samples only a few seconds after the fruiting bodies were cut.

For all of the samples stored in the freezer, the concentration of phosphorylated analytes (the main psilocybin) was reduced. Furthermore, a trend in increased concentrations of psilocin was observed over samples stored at room temperature. For future research, we suggest that the concentration of quinoid dyes as products of tryptamine metabolism should also be monitored. Furthermore, we hypothesize that the mushrooms should be frozen with liquid nitrogen to avoid cell disruption.

3.7 | Stability of tryptamines in dried fungal powder

The original concentration of freshly homogenized *P. cubensis* mushroom powder was 0.01 wt.% norbaeocystin, <0.01 wt.% aeruginascin, 0.07 wt.% baeocystin, 1.51 wt.% psilocybin, and 0.04 wt.% psilocin. The fungal powder was used for monitoring of the tryptamines stability. These powder was divided into different zip bags and stored under various conditions as shown in Table 1. Aliquots were measured several times at different intervals: 1 week, 1 month, 2 months, and 15 months. The dried mushroom powder was chosen as a suitable model, as the whole fruiting bodies could not be divided into many homogeneous pieces for the experiments used (five storage conditions in different periods).

The most pronounced changes in the decay in concentration over time were observed with psilocybin, which is the main tryptamine alkaloid in fruiting bodies.

3.7.1 | After 1 week

The concentration of psilocybin reduced from 1.51 wt.% to 1.31 wt.%. The greatest loss of psilocybin was found in a sample stored in the light at 20°C, where the psilocybin value dropped to 0.96 wt.%. This trend was also observed for norbaeocystin, aeruginascin, baeocystin, and psilocin, where the measured concentration gradually decreased. From the results shown in Figure 3, it is apparent that tryptamines are not very stable in the homogenized mushroom powder.

3.7.2 | After 1 month

The degradation to approximately 50% of the initial concentration of all tryptamines occurred on storage under all of the test conditions. The most significant decrease in concentration to 0.72 wt.% appeared in the sample that was stored in the light at 20°C. This effect is probably due to the photooxidation of alkaloids, similarly as described in Section 3.6. The lowest concentration reduction to 0.85 wt.% was found in the dark at 20°C.

3.7.3 | After 2 months

This phenomenon was also observed when the most significant degradation to 0.67 wt.% was measured in a sample that was stored in the light at 20°C. In contrast, the smallest loss of psilocybin occurred when the sample was stored in the dark at 20°C, where the psilocybin was 0.82 wt.%.

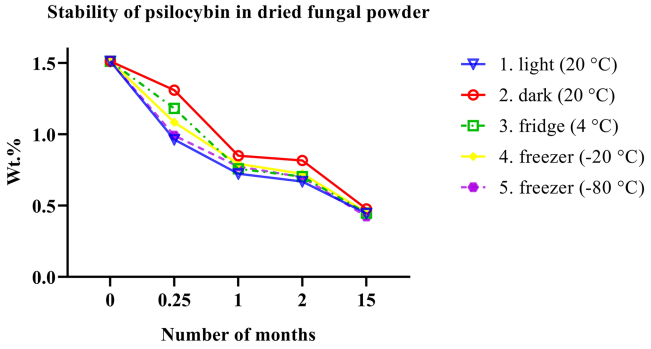

FIGURE 3 Stability of psilocybin as a major tryptamine in fungal powder after 15 months [Colour figure can be viewed at wileyonlinelibrary.com]

3.7.4 | After 15 months

A very similar concentration of all tryptamines was found after 15 months under all of the storage conditions. The psilocybin concentration varied by a maximum of 0.04% in these samples. The lowest degradation of psilocybin was found in a sample that was stored in the dark at 20°C. The concentration of all analytes gradually decreased (Figure 3), which corresponds to studies that claim the same but do not state under what conditions.[14,26]

For the minor alkaloids (aeruginascin, baeocystin, norbaeocystin, and psilocin), the changes in concentrations were negligible after the first week. After 1 month of storage, a slight decrease in the concentration of all of the remaining alkaloids was seen at a similar time under all of the defined conditions. After 2 months of storage, all of the alkaloids were reduced, except for psilocin, which decomposed more only in the light at 20°C. After 15 months of storage, no further changes occurred, except for psilocin, whose concentration increased (probably due to the degradation of psilocybin). The measured data are summarized in Table S2, which is found in the supporting information.

4 | CONCLUSION

An extraction procedure and a UHPLC-MS/MS analytical method for the analysis of psilocybin, psilocin, baeocystin, norbaeocystin, and aeruginascin in fungal biomass were developed and validated. All of these analytes were present in *P. cubensis*. Concentrations of aeruginascin are low and have not been reported before. It was found that the degradation of indole alkaloids occurs when fresh fungal fruiting bodies are mechanically damaged (sliced). Conversely, better extraction yields for dried fungal fruiting bodies were achieved after the mushrooms were homogenized to a powder form. Higher amounts of phosphorylated tryptamines (e.g., psilocybin) were found in the dried fungal fruiting bodies, while higher amounts of the dephosphorylated forms of indoles (e.g., psilocin) were found in the fresh fruiting bodies.

To prevent the degradation of fungal tryptamines during sample processing, the most suitable conditions were to dry the fruiting bodies in the dark at room temperature. The total content of tryptamines was strikingly reduced when they were stored under different conditions. Mushrooms that were stored in the freezer (−80°C) lost almost 90% of tryptamines. Regardless of the storage conditions, rapid degradation of all analytes was observed when the dried fungal powder was stored. To improve the storage of dried mushrooms, they should be stored in an inert gas environment.

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