Mushroom Debacle: Can Store-Bought Mushrooms Match Natural Mushrooms in Protein Content?

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Foreword

Do not eat wild mushrooms! Edible and poisonous wild mushrooms are often very similar in appearance. Poisonous mushrooms contain toxic substances that, if ingested, can produce a wide range of effects, from mild gastrointestinal discomfort to death (Wennig et al., 2020).

Abstract

Mushrooms are the reproductive spore-bearing structures produced by fungi. There are both inedible and edible mushrooms, and different types of mushrooms have different chemical compositions. We engineered an experiment to test a large variety of mushroom types for protein concentration. The experiment involved 12 wild and 9 store-bought mushrooms, as well as Ninhydrin solution, a reagent that forms a purple compound in the presence of amino acids. We applied Ninhydrin solution to slices of mushrooms, and heated them to expedite the reaction. We analyzed the protein concentrations in the mushrooms by comparing the shades of purple on the mushrooms to a purple hue rubric. We found that the store-bought mushrooms generally turned darker shades of purple than the wild mushrooms; some of the wild mushrooms did not turn purple at all. Additionally, mushrooms that were more fresh and softer seemed to observe a purple colour at a faster rate and developed a darker purple shade after heating. These results mean that commercially cultivated mushroom species generally had higher protein concentrations than those found in the wild, demonstrating that overall protein availability affects the eligibility of a mushroom to be considered food.

Introduction

Mushrooms or toadstools are the general term for the reproductive spore-bearing structures produced by fungi. The term "mushroom" is generally used to refer to the edible species; Toadstools refer to mushrooms that are poisonous to humans (Bromhall, 2022).

Mushrooms are a common aspect of the culinary arts. Mushrooms such as the common white mushroom (*Agaricus bisporus*), the shiitake (*Lentinula edodes*), and the oyster mushroom (*Pleurotus ostreatus*) are grown in mushroom farms and sold at grocery stores (Valverde et al., 2015). These mushrooms are prized for their nutrition, as well as their enjoyable taste and texture. According to Valverde et al., there are thousands of mushroom species, but only about 25 are regularly consumed as food, and are commercially cultivated.

Some mushrooms have psychoactive properties, and are used for spiritual or recreational purposes. Psilocybin mushrooms, also known as magic mushrooms, contain psilocybin, which turns into psilocin (a psychedelic substance) once ingested, and produces hallucinatory effects comparable to Lysergic Acid Diethylamide (LSD; Government of Canada, 2015). Psilocybin mushrooms have been used recreationally and for religious purposes for centuries, with many users considering their psilocybin enhanced experiences some of the most personally meaningful and spiritually significant experiences of their lives (Griffiths et al., 2008).

There are also mushrooms used for medicinal and therapeutic purposes. Some mushroom extracts are used to create antibiotics, anti-cancer drugs, fungicides, and immune system stimulants, and are often used to treat infection, lung diseases, and cancer (PDQ Integrative, Alternative, and Complementary Therapies Editorial Board, 2022). A notable example is the Reishi mushroom (*Ganoderma lucidum*), a mushroom that has been a staple of Eastern medicine

for thousands of years (Benzie and Wachtel-Galor, 2011). According to Benzie and Wachtel-Galor, Reishi mushrooms can be brewed in teas or taken as powders, and have various health benefits, such as the control of blood sugar levels, stimulation of the immune system, liver protection, and more.

Some mushrooms are poisonous, containing toxic substances that, if ingested, can produce a wide range of effects, from mild gastrointestinal discomfort to death. Mushroom poisoning is usually the result of misidentification of a poisonous mushroom as an edible one (Wennig et al., 2020). Additionally, mushrooms have a proclivity for absorbing heavy metals, such as radioactive elements. The result of this is edible mushrooms becoming irradiated, and are no longer safe for human consumption (Turhan et al., 2007).

Mushrooms have a large variety of uses, suggesting large differences in chemical composition between different species. While the nutritional value and chemical composition of edible cultivated mushroom species have been studied, the same cannot be said for wild mushrooms. With this knowledge gap in mind, we tested a hypothesis that the average protein concentration between wild mushroom species found around Metro Vancouver woodlands will be different from cultivated mushroom species found in grocery stores in a statistically significant manner. Our null hypothesis states there is not a statistically significant difference between the mean protein content of the wild mushroom species and the store-bought mushrooms. We predict that the mean protein content will be different between wild mushroom species and store-bought species, and that the store-bought mushrooms will have a higher protein content.

Methods

Mushroom Collection

For the data collection, we collected nine mushrooms from grocery stores in Metro Vancouver and fourteen mushrooms from various parks in Metro Vancouver four days prior to our experiment. A variety of different species of both store-bought and wild mushrooms were collected. Both store-bought and wild mushrooms were placed in paper bags and kept refrigerated until lab day to maintain quality and prevent rotting.

Procedure: Protein measurement

In order to test for the amount of primary amino acids synthesised in the wild and store bought mushrooms, we performed ninhydrin tests. Ninhydrin is a chemical compound that produces different shades of violet color when it reacts with the primary amino acids. Specifically, ninhydrin is a class 2 category chemical that must be handled in a fume chamber (*ACS Chemical Reactions*, 2017). First, each mushroom group was cut vertically into a thin layer and labelled on a paper towel. Then, a sliced mushroom was placed on a watch glass and a before photo was taken (Figure 1). Next, a micropipette was used to add 200 μLof the ninhydrin solution evenly on the whole area, covering the mushroom. Next, using a metal tong, the mushroom was placed on the non-luminous region of the alcohol lamp for one minute (Figure 2). Then, using tongs, the mushroom was placed back on the watch glass, and after sixty seconds, the after photo was taken from the same distance and location as the before photo to minimize visual errors (Figure 1). In our experiment, we designated solid cat food as the positive control, as the high protein composition was stated on the manufacturer's label. Additionally, a solid rock was assigned as the negative control. Lastly, the mushroom was disposed of in the waste container. These steps were repeated for all the store-bought and wild mushrooms, along with the positive control (solid cat food) and the negative control (rock).

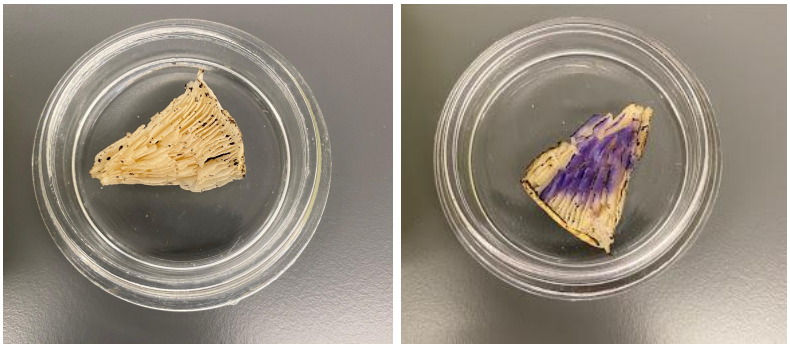

Figure 1. Before (left) and after (right), *Amanita Pantherinoides* (wild mushroom) reacts with ninhydrin.

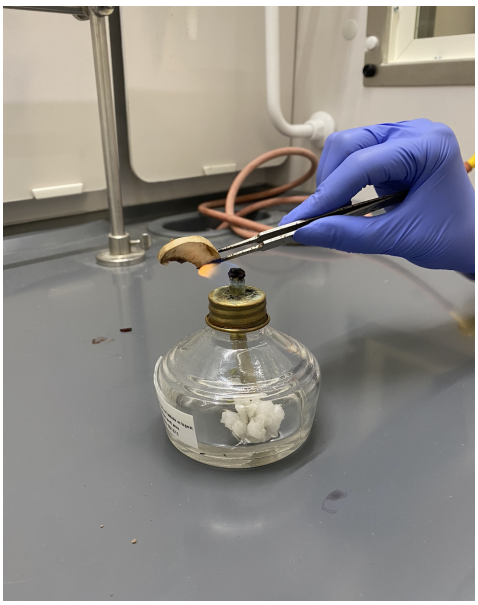

Figure 2. Mushroom placed on the non-luminous area of the alcohol lamp using a metal tong.

Data Analysis

The photos of the wild and store-bought mushrooms that were taken during the experiments were uploaded onto imagecolorpicker.com where we used the deepest purple color in the image to determine the specific RGB values for each mushroom. After finding the RGB values, we compared those values to the RGB values of our purple hue rubric (Figure 3). Thus, we categorized each mushroom from 1 to 5 using our purple hue rubric. The data was analyzed using an unpaired t-test to see whether there was a significant difference between the protein

content of wild and store-bought mushrooms. Additionally, the mean of the purple hue rubric values for the wild and store-bought mushrooms was calculated.

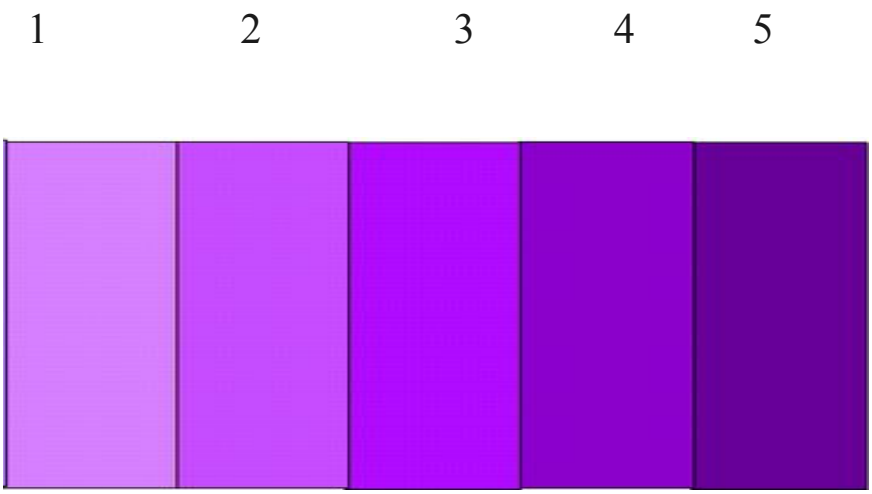

Figure 3. The Purple hue rubric.

Results

For a sample calculation of mushroom purple score, the wild mushroom *Amanita pantherinoides* (Figure 1) produced the deepest shade of purple at the center of the mushroom in the image specifically, at an RGB value of R44 G16 B22. This RGB value is closest to the purple hue rubric RGB value R127 G23 B195 and therefore has a purple hue rubric value of 4.

The average mean purple hue rubric value was 2.429 for the wild group and 4.056 for the store bought group. Our results from the unpaired t-test found that there is a statistical difference in the purple mean rubric value between the wild and store-bought mushrooms ($t=2.329$, $p\text{-value}=0.0299$). Therefore, we reject the null hypothesis, which assumes that the mean purple hue rubric value for the wild and store bought mushrooms is equal.

Figure 4- The mean purple hue rubric value for the wild mushrooms and the store-bought mushrooms. The error bars represent 95% confidence intervals.The sample size is N=14 and N=9 for the wild mushrooms and the store-bought mushrooms respectively. The mean purple hue rubric value is 2.429 for the wild mushrooms and 4.056 for the store-bought mushrooms.

Similarities in both the wild and store-bought mushrooms were that in both groups, the most dark violet purple color was observed at the corners and edges of a significant number of the mushrooms (Figure 5 and Figure 6). In the store-bought mushrooms, *Pleurotus ostreatus* and *Flammulina filiformis* mushrooms had dark shades of purple, especially around the edges of the mushrooms, and contained the highest purple hue rubric values of 5 (Figure 5). However, *Agaricus bisporus* had a pale light mauve color and contained a low purple hue rubric value of 2 (Figure 6). In the wild mushrooms, *Amanita Pantherinoides* (Figure 1) had one of the darkest shades of purple present and contained purple hue rubric values of 4. We found that in the wild mushrooms, two out of the fourteen mushrooms found had no visible purple color present and obtained purple hue rubric values of 0 (Figure 7). Overall, mushrooms that were more fresh and

softer seemed to observe a purple colour at a faster rate and developed a darker purple shade after heating.

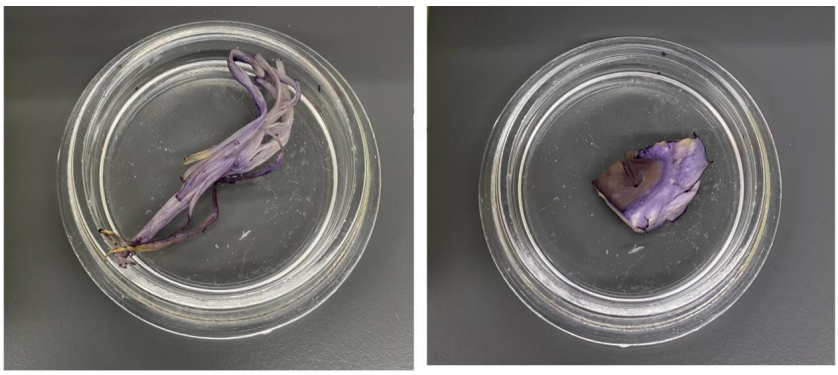

Figure 5. *Flammulina filiformis* (left) and *Pleurotus ostreatus* (right) mushrooms after reaction with ninhydrin.

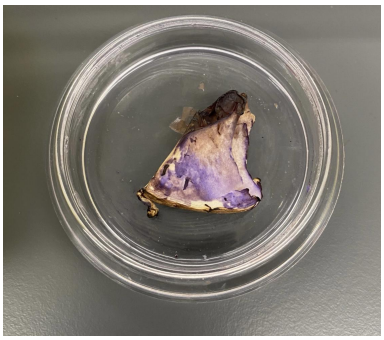

Figure 6. *Agaricus bisporus* mushroom after reaction with ninhydrin.

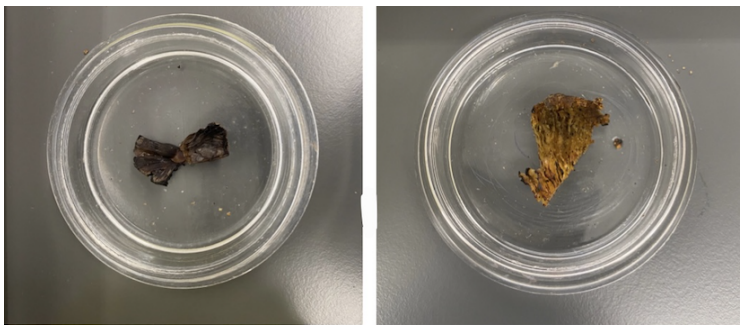

Figure 7. Wild mushrooms after reaction with ninhydrin.

Discussion

The result of our experiment indicated that there is a statistically significant difference in the amino acid composition of wild mushrooms compared to store-bought mushrooms. The probability value of 0.0299 is smaller than the critical value of 0.05 that we previously set for the experiment, and hence strongly suggests that the difference is statistically significant. The values obtained in our t-test support the alternative hypothesis, leading us to reject the null hypothesis. From an observational point of view, we predicted that store-bought mushrooms would exhibit a darker purple color. The results indicated a clear difference in the amount of purple colour emitted from the store-bought mushrooms compared to the wild mushrooms collected from the woodlands. Mushrooms are diverse species, and the distinct color observed during the experiment could be explained by the difference in the genus families of the treatment groups. Different species of mushrooms are composed of different structures of amino acids. The ninhydrin solution used in our experiment produced different shades of purple when bound to different amino groups of the peptides present in mushrooms (Friedman et al., 2004). This could be a source of error because the different shades of purple produced by wild and store-bought mushrooms may not be due to the different conditions in which they were grown, but rather could be directly related to the specific type of mushroom and its amino acid content.

The differences in mean protein content between the observed cultivated store-bought mushrooms and wild mushrooms, presented in Figure 4, can be supported by previous research on the free amino acid content of cultivated and wild-caught mushrooms. This research suggested a notably higher free amino acid load in cultivated mushrooms in comparison to the same species of mushrooms grown in the wild (Kalac, 2013). This can be explained by the unstable conditions of the wildly grown mushrooms vs the controlled conditions in which the

mushrooms are cultivated. This difference in protein content is further supported by the results of the t-test, indicating a statistically significant difference between the protein content of the wild and cultivated store bought mushrooms.

Although wild mushrooms are often not seen as a food source for humans, consumption of certain wild mushrooms with higher protein content could be beneficial to other species in the wild. A study by Trujillo et al, links the longevity of fruit flies to the presence of mushrooms in their diets likely due to the protein and antioxidant contents of mushrooms (Trujillo et al., 2021).

Additionally, lectins are found at high levels in mushrooms. Lectins act as bioactive storage proteins in mushrooms and are typically found in caps, stalks, and mycelia- which are thought to have a potential role in the immunity of mushrooms similar to plants (Hassan et al., 2015). Lectins have recently been investigated for their potential in a wide range of biological activities, such as antiproliferative and antitumor activities toward tumor cells, due to their exploitable specific binding abilities to carbohydrates (Singh et al., 2014). Even though they are present in many mushrooms, lectins are not present in all mushrooms and have been found to be present in 50% of edible mushrooms (Singh et al., 2014). The availability and presence of lectin in certain mushrooms as compared to others may play an important role in differing protein contents. While it is possible that other mushrooms that do not contain lectins use a different protein for the same function, the amounts of protein needed to attain the same function may differ. This could give rise to differing mushroom protein levels between wild and store mushrooms since farmers and stores could specifically aim to collect and grow mushrooms with higher bioactive proteins under conditions to make them better for human consumption. Although research in potential pharmacological uses of lectin from mushrooms are in early stages, potential other proteins in mushrooms not containing lectin could be investigated.

Conclusion

To conclude, our analysis demonstrated that mushrooms purchased from grocery stores in the Greater Vancouver area and consumed by people have a statistically higher amino acid load than wild mushrooms collected from the woodlands of the area. This aligns with previously published research which highlights that store-bought mushrooms are cultivated and selected for higher protein content (Kalac, 2013), as well as with research showing that mushrooms consumed by humans are specifically selected for their bioactive proteins and overall protein availability (Singh et al., 2014). In the future, further investigations can be undertaken to observe the exact differences in amino acids and the potential roles these differences could have in mushroom function between store-bought and wild mushrooms. To improve the experimental design, mushrooms can be blended and the purple colours formed in the reaction can be observed in solution form. This avoids experimenter bias that may arise from selecting purple spots on mushroom slices. Furthermore, the different shades of purple formed may be directly related to the peptide structure of each mushroom. This means that the results may not accurately answer the scientific question. To improve the accuracy of the results and focus on growth location as the only independent variable, the same species of mushroom should be investigated in both wild-type and controlled environments.

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