# Food for Thought:
# Protein content variation in commercial *Agaricus bisporus* mushrooms

University of British Columbia
BIOL 342 201: Integrative Biology Laboratory
Hooria Bilal, Jaia Manhas, Minji Seo & Payton Angus

## Abstract

Edible mushrooms have many nutritional benefits because they contain essential proteins and vitamins. However, various factors can influence their protein content including growth environment, packaging, and processing conditions. We collected nine samples of *Agaricus bisporus* mushroom species from three stores across metro Vancouver and conducted ninhydrin testing to compare their protein contents. Ninhydrin is a chemical compound that produces varying shades of violet when in contact with primary amino acids. We compared the shades of violet obtained from our mushroom samples to determine their protein amounts. Statistical testing revealed that the three differently sourced and stored mushrooms used in this experiment had no significant difference in protein concentration. This discovery was confirmed by a one-factor ANOVA test that produced a p-value of 0.3847. This result is unexpected as previous literature suggests that there should be a statistical difference in protein content between the mushrooms. Further research into the protein content of mushrooms should be conducted and the investigation of different factors like packaging and storage should be prioritized.


## Introduction

The edible mushroom species commonly referred to as the button mushroom, *Agaricus bisporus*, is one of the most commercially cultivated mushrooms in the US (Li & Hu, 2014). Edible mushrooms are rich in nutrients, proteins, fats, vitamins, and antioxidants, and are known to have many nutritional and medicinal benefits (Atila et al., 2017). Atila et al. (2017) state that *A. bisporus* contains many nutrients beneficial for human health, including proteins that are composed of the nine essential amino acids that the human body cannot produce on its own. This makes these mushrooms a great and cost-effective alternative to meat.

However, various factors can affect the protein content of mushrooms such as the substrates in which they grew, the stage of development, and conditions before and after harvesting (Atila et al., 2017). Atila et al. (2017) noted the protein content of *A. bisporus* varied from 11.01 to 29.14% when grown in different substrates. Different conservation methods can also influence the chemical composition of mushrooms, consequently impacting their nutritional value (Vetter, 2003). Further, different growth environments or storage conditions of mushrooms can lead to variations in amino acid concentrations (Braaksma et al., 1996). Vetter (2003) noted that this occurs because mushroom proteinase activity increases during storage, hence reducing protein content due to chemical breakdown. Malinowski et al. (2021) conducted research observing protein content in three different mushroom species and analyzed the impact of soil pH on the bioaccumulation of macronutrients within these mushrooms. Results from this study conclude that the organic matter in the soil itself seemed to have little effect on the content of macronutrients for particular mushroom species.

A study conducted in the previous year analyzed and compared the protein content of different wild mushroom species and store-bought mushrooms using ninhydrin tests (Arman et al., 2021). Ninhydrin is a chemical compound that detects the primary amino acids present in a sample by forming different shades of purple (Friedman, 2004). Using a similar procedure outlined in Arman et al. (2021), we conducted an observational study to determine if the protein content of the mushroom species, *A. bisporus*, will differ from various sources. Given the myriad of factors influencing protein content in mushrooms, in our study, we decided to select a single species to obtain more comparable results as to how protein content varies. Our null hypothesis is that the variation in protein content between mushrooms collected from different sources will not be statistically significant. Alternatively, if the protein content in mushrooms does depend on

their growth and storage conditions, we predict that *A. bisporus* mushrooms bought from different stores will significantly differ in protein content.

## Methods

*Sample Collection*

Nine samples of *A. bisporus* mushrooms were bought from three stores across Metro Vancouver. Specifically, three mushroom samples each were purchased from a Farmer's Market in Richmond, Farmer's Market in Vancouver, and a grocery store in Vancouver. Six of the samples had been packaged in plastic wrap by the seller, while three had been packaged in a brown paper bag by the seller. Six of the samples were refrigerated while three were frozen. The mushrooms were obtained on February 26th, 2023, three days before experimental testing on March 1st, 2023.

**Table 1.** Grower, quantity, store location, packaging type, and storage type and length for the nine *A. bisporous* mushroom samples.

| **Grower** | **Quantity** | **Store obtained** | **Packaging** | **Storage** |
|---|---|---|---|---|
| A (Abbotsford) | 3 | Farmers Market (Richmond) | Plastic wrap | Refrigerator (5 days) |
| B (Unknown) | 3 | Grocery Store (Vancouver) | Plastic wrap | Freezer (2 days) |
| C (Unknown) | 3 | Farmers Market (Vancouver) | Brown paper bag | Refrigerator (5 days) |

*Procedure: Protein Measurement*

To observe how different factors influence the protein content of *A. bisporus*, we performed ninhydrin testing. Ninhydrin is a Class 2 chemical that should be handled in a fume chamber (ACS Chemical Reactions, 2017). After being applied to the sample in question, ninhydrin produces a shade of violet upon reacting with the primary amino acids present. In

samples with lesser amounts of primary amino acids, the shade of violet is faint; in samples with more amounts of primary amino acids, the shade of violet is more vibrant. The variation in shades of violet of different samples creates a scale representing a variation in the amounts of protein present.

Before commencing our experimental testing, we assigned apple as our low protein/negative control and extra firm tofu as our high protein/positive control. Since it is known that apple has a protein content of 0.3 grams per 100 grams, while the tofu we used has a protein content of 14 grams per 100 grams, these food items will serve as appropriate low and high bounds of our protein scale. The first step of our experimental testing was cutting all nine mushroom samples, three apple samples, and three tofu samples into 3 cm x 2.5 cm x 1 cm cross-sections. These measurements were chosen because they provided the largest area we could obtain from our smallest mushrooms while avoiding any brown colouration in each cross-section. Next, all cross-sectional samples were placed in a watch glass, before testing, to take ‘before’ pictures (Figure 1). These pictures were taken in the same location, lighting, and distance to minimize visual errors. Then, 70 μL of ninhydrin solution was drawn by a micropipette in the middle of each sample. Using sterilized metal tongs, samples were held over an alcohol lamp flame for one minute in the fume hood (Figure 2). Next, the samples were placed back on the watch glass to take ‘after’ pictures (Figure 1) under the same conditions as previously. Lastly, the samples were disposed of in the waste container.

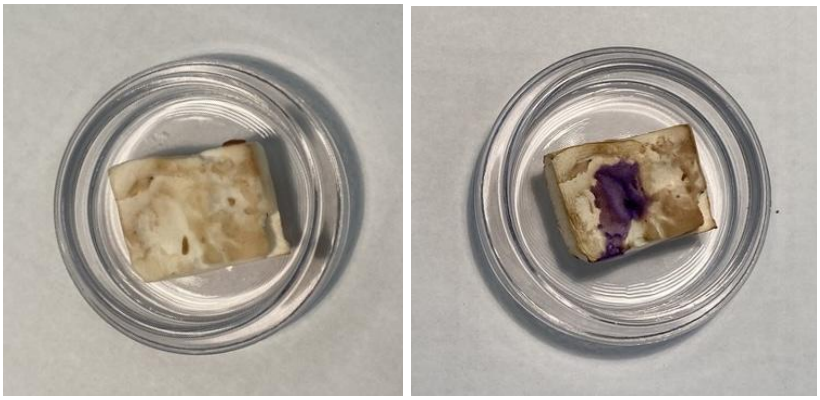

**Figure 1.** ‘Before’ and ‘after’ (left and right images respectively) of sample one from Store A during ninhydrin testing.


**Figure 2**. A mushroom sample was held over an alcohol lamp flame in the fume hood.

*Data Analysis*

To analyze the data collected, photos of the controls and mushrooms after the heated ninhydrin reaction were uploaded to Image Color Picker (imagecolorpicker.com) to obtain their RGB colour codes. From there the RGB colour codes were compared to a ninhydrin reaction protein concentration scale. The resulting protein concentration for each mushroom sample was used in a one-factor ANOVA statistical analysis. Data and average RGB colour codes for each mushroom sample and control were also placed along a scale for physical comparison.

## Results

Samples from Store B seemed to be the soggiest and wet compared to the other samples, with A following suit, and C being the least watery. After ninhydrin was placed on the samples of mushrooms and then samples were placed in the flame, within around 30 seconds each sample started to turn purple, with sample C turning purple in the shortest amount of time. Tofu samples were incredibly watery and had to be patted down multiple times. Apple samples were more off-white (veering towards yellow) than any of the other samples tested and had very small specs of purple appear instead of the other samples which had blobs of purple. The mushroom samples were relatively consistent in comparison with each other and no trends or particular patterns were observed through the statistical data.

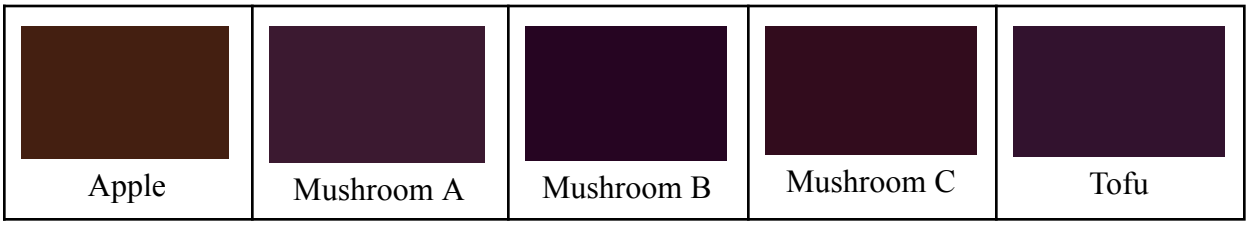


**Figure 3.** Visual depictions of the average RGB colour codes of the controls apple and tofu, and samples of *A. bisporus* from three different stores (*Colors RGB and RGBA*, 2021).

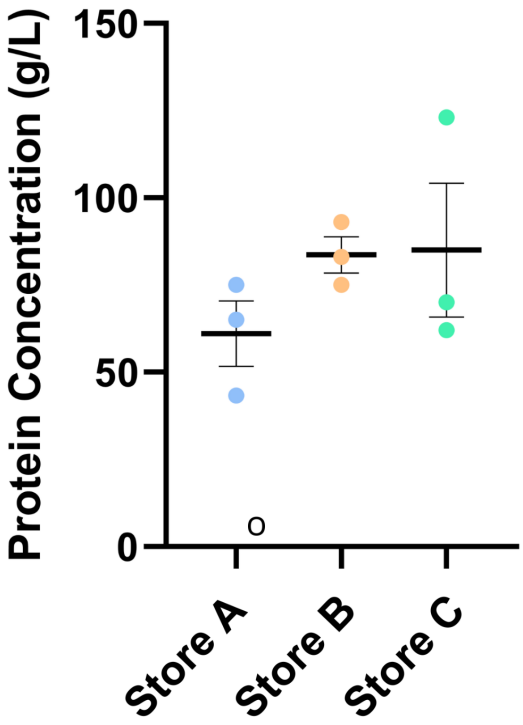


**Figure 4.** Protein concentrations (g/L) of *A. bisporus* mushroom samples from three different stores with standard error of the mean. An analysis of variance shows that the average protein concentration of samples from Store A ($M = 61$, $SEM = 9.36$, n = 3), Store B ($M = 83$, $SEM = 5.21$, n = 3), and Store C ($M = 85$, $SEM = 19.14$, n = 3) had no significant difference, $F(2, 6) = 1.125$, $p = 0.3847$.

## Discussion

From our one-factor ANOVA test, we obtained a p-value of 0.3847. Because the p-value is greater than 0.05, we can not reject the ANOVA null hypothesis that mean values of protein concentration of mushrooms from different sources are the same. We can not reject the ANOVA null hypothesis and therefore we reject our initial prediction that the protein content of our mushrooms significantly differs between sources.

Despite the conclusion of the ANOVA, Figure 4 shows the presence of slight variations between mean mushroom protein concentration (though not significant, still present). Specifically, Store A samples appeared to have the lowest average protein content out of the three stores. One possible reason for this result could be due to packaging. Data from studies

show that dried mushrooms have higher retention of protein but Store A samples were stored in a plastic wrap which increased water retention and prevented drying (Reid et al., 2016). In contrast, Store C samples were stored in paper bags which could explain the slight protein discrepancy between those differently sourced mushrooms.

The results, however, come as a surprise as they contradict what has been established by previous literature; particularly, the results of Store B samples showing no significant difference in protein content compared to other mushrooms. This result is interesting as previous studies have concluded that using freezing as a preservation method ultimately decreases the nutritional value and overall protein content within mushrooms (Jaworska et al., 2011). It is important to note, however, that this study was conducted over 12 months whereas the frozen Store B samples in our experiment were kept in the freezer for 2 days. Additionally, the results from Reid et. al, showed that after a similar freezing experiment of 14 days, the effects of freezing on protein content were very minimal.

Further surprising results come from protein content not significantly differing between Store B and C despite the difference in packaging. As mentioned earlier plastic packaging maintains water content which will have lower protein content than mushrooms that are drier, such as Store C samples stored in paper bags. During the heating of the ninhydrin-soaked mushrooms, it was noted that Store C samples started showing a purple colour much quicker than the previous samples. Store B in particular took a while for the purple dye to appear because the samples were quite wet due to its thawing. It was initially thought that the abundance of water interfering with the colour change from the ninhydrin solution would affect the results.

As mentioned above, one reason our data disagrees with previous literature might be the difference in storage time. More possible errors that could have affected our results include

human error when choosing which piece of the mushroom to garner the RGB numbers from. Each sample had ranging shades and concentrations of purple, which could significantly change the RGB results depending on where each sample was chosen. Additionally, the conversion/quantification of RGB numbers into protein concentration was not as precise and consistent as hoped for the most accurate results and data collection and analysis. Without a computational program to confirm the conversion, and instead using approximations from subjective viewing, the protein concentrations were recorded. This potential for inaccuracies would mean that the results of the ANOVA test are skewed randomly and slightly.

Another potential source of error involves inconsistency with the ninhydrin reactions themselves. The samples covered with ninhydrin were held under a flame to speed up reactions, with pictures taken nearly immediately after using the flame. However, each mushroom contained a drop of ninhydrin that did not in fact cover the whole mushroom, and in addition to the initial clear colour of ninhydrin, this made precise flame-ninhydrin interactions difficult. Varying angles and times in the flame could ultimately mean some of the samples underwent a complete reaction while others needed more time or may have differed in colour which would correlate to a difference in the calculated protein concentration.

## Conclusion

Despite the different sources and storage conditions that the mushrooms were purchased from and subject to, the protein content of the mushrooms was not found to be statistically different. Mushrooms from Stores A, B, and C had average protein contents of 61 g/L, 83 g/L, and 85 g/L which appear quite different, but concluded insignificantly different due to an ANOVA p-value greater than 0.05. These results are in direct contrast with the prediction that the mushroom samples would differ in protein content. However, it is important to note that

conditions such as storage and packaging type could have significantly affected the findings, although not evident given this experiment's short timeline and small scale. Overall, mushrooms are a common alternative to meat and given that they offer consumers some protein, knowing their exact nutritional values remains important when considering where to purchase mushrooms.

## Acknowledgments

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## Appendix

**Table 2.** RGB colour codes for three samples of each of our low and high protein controls, tofu and apple (*Color Picker Online | HEX Color Picker | HTML Color Picker*, 2022).

| Control | S1 RGB Code | S2 RGB Code | S3 RGB Code | Avg RGB Code |
|---|---|---|---|---|
| Apple | rgb(59,23,17) | rgb(62,26,35) | rgb(82,45,0) | rgb(68,31,17) |
| Tofu | rgb(58,19,41) | rgb(41,16,63) | rgb(50,19,34) | rgb(50,18,46) |

**Table 3.** RGB colour codes for the nine mushroom samples of *A. bisporus* (*Color Picker Online | HEX Color Picker | HTML Color Picker*, 2022).

| Store | M1 RGB Code | M2 RGB Code | M3 RGB Code | Avg RGB Code |
|---|---|---|---|---|
| A | rgb(35,9,39) | rgb(62,21,38) | rgb(79,45,69) | rgb(59,25,48) |
| B | rgb(29,1,30) | rgb(28, 8, 35) | rgb(56,7,37) | rgb(38,5,34) |
| C | rgb(70,22,44) | rgb(47,13,37) | rgb(34,2,7) | rgb(50,12,29) |

**Table 4.** The protein content of samples of *A. bisporus* mushrooms based on ninhydrin reaction RGB colour codes corresponding to protein concentration (g/L) scale.

| Store | Sample 1 | Sample 2 | Sample 3 | Average |
|---|---|---|---|---|
| A | 75 | 65 | 43.3 | 61 |
| B | 93 | 83 | 75 | 83 |
| C | 62 | 70 | 123 | 85 |

**Table 5.** One-factor ANOVA test of three differently sourced *A. bisporus* mushrooms (*ANOVA Calculator - One Way ANOVA and Tukey HSD Test, 2023*).

| Source | DF | Sum of Squares (SS) | Mean Squares (MS) | F Statistic | P-value |
|---|---|---|---|---|---|
| Groups (between) | 2 | 1082.2422 | 541.1211 | 1.125 | 0.3847 |
| Error (within) | 6 | 2885.9267 | 480.9878 | | |
| Total | 8 | 3968.1689 | 496.0211 | | |