Comparative Analysis of Protein Content in Animal-based Chicken, Plant-based Chicken, and Tofu: An Experimental Study

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Abstract

This study aimed to compare and analyze the protein content in plant-based meat and animal-based meat. For this experiment, we selected three protein sources, including animal-based chicken nuggets, plant-based nuggets, and plant-based tofu, for the analysis. The research sought to determine whether plant-based meat alternatives would have similar or lower protein content than animal-based chicken nuggets. In order to conduct the experiment, we first took three samples from each of our protein sources, and all samples were left in the dryer for 2 days to extract all the moisture. The samples were then ground into a fine powder, and water and ninhydrin were added before being placed in a water bath at 90 degrees Celsius for 20 minutes. The resulting colour change was recorded, and RGB values were extracted for the analysis. The study findings suggest no significant difference in protein content between the different samples.

Introduction

Protein is a key essential component of a healthy diet, serving as a building block for the body's muscles, tissues, and enzymes. An average adult's recommended daily protein intake is approximately 0.8 grams per kilogram of body weight. With so many different diets and lifestyles, knowing how much protein each diet consumes is vital to ensure healthy body growth. In recent years, there has been a surge in the popularity of vegetarian diets due to concerns about health, animal welfare, and environmental sustainability. Due to this trend, there has also been an increase in the availability and popularity of plant-based meat alternatives such as

soy-based tofu and veggie burgers. Tofu is a trendy plant-based protein alternative as it is a rich protein, iron, and calcium source. However, research suggests that plant-based proteins generally provide less complete protein nutrition because of lower digestibility and source-specific deficiencies in essential amino acids compared to animal-based proteins (Day et al., 2021). To better understand the nutritional content of meat alternatives compared to animal-based meat, this study analyzed the protein content of popular plant-based alternatives, including animal-based chicken nuggets, plant-based chicken nuggets, and plant-based tofu. The null hypothesis for this experiment is that animal-based chicken would have the same protein content compared to the plant-based alternatives. The alternative hypothesis for this experiment is that animal-based chicken would have different protein content than the plant-based alternatives. Our predicted result was that animal-based chicken and plant-based chicken would have different protein content. At the outset of our experiment, we were under the impression that people who follow vegetarian or vegan lifestyles must consume more plant-based protein products to compensate for a lower protein content per product. The findings of this research have implications for individuals considering plant-based diets and the potential challenges they might face in meeting their daily recommended protein intake requirements. By analyzing the protein content of popular plant-based alternatives, this study aids the growing body of research exploring the nutritional properties of plant-based diets.

Methods

Sample collection

For the experiment, we opted for three pieces of each sample (individual chicken/vegan nuggets, cat kibble, and tofu slices). We purchased each sample in different packaging to avoid pseudoreplication. The animal-based chicken nuggets were from a well-known fast food chain, the plant-based chicken nuggets were from a grocery store, and the tofu was from a grocery

store. We used a rock collected on the UBC campus as a negative control and dry cat food as a positive control.

Figure 1. Rock used as control (before and after ninhydrin application)

Procedure: Sample preparation

Each sample had varying water content inside, and we aimed to standardize the samples based on weight. To reduce the variability in water content, we subjected all the experimental units to a drying oven at 90°C for two days. After two days, we placed each sample in separate plastic ziplock bags and stored them inside a fridge to minimize moisture absorption from the air.

Procedure: Data collection

We ground the cat food, animal-based chicken nuggets, plant-based chicken nuggets, and tofu slices into a fine powder using a pestle and mortar. We did not grind the rock since we used it as a negative control and could determine that there was no protein content without grinding it as per our original procedure, where we did not plan to grind our samples. To prevent cross-contamination, we rinsed the pestle and mortar thoroughly with tap water and dried the equipment before proceeding to the following sample. Since the chicken nuggets were coated

with bread, we removed the outer layer before grinding to increase the weight of the protein-containing portions since breaded portions would have close to zero protein content. Next, using a metal spatula, we measured approximately 0.400g of the sample onto a weight paper and then transferred the measured sample into a test tube. After measuring with a micropipette for the liquid components, each test tube contained 1000uL of distilled water, 200uL ninhydrin, and 0.400g of the sample. The distilled water served as a buffer. Once all the components had been added to the tube, the contents were evenly mixed, and the liquid portion was visible using a vortex at speed five. Before placing the tubes into the water bath, we took photographs of the test tube at equal distances for color comparisons that were made later using camera settings at Kelvin 4000 (Figure 2 below as an example). All 12 tubes were then placed at the same time into a water bath, which was heated to 90^{o}C for 20 minutes to activate the ninhydrin in the solution. Similarly, once the 20 minutes of heating was completed, all the tubes were taken out simultaneously. Subsequently, photographs of each test tube were taken immediately after using the same camera settings and distance as the pre-water bath photos in Figure 2.

Figure 2. Before and after photos of tofu-ninhydrin solution after 20 minutes of water bath heating at 90 degrees Celsius.

Procedure: Data Analysis

First, we used photo editing software to obtain the RGB values from the photos. We measured the RGB values where the protein-containing parts visually merged with the liquid component. Second, we compared the RGB values to a ninhydrin scale produced by another group to approximate the protein content in our samples. We compared the RGB values from our experiment to the RGB values for each protein concentration on the ninhydrin scale (completed by L03 B4; see Appendix for reference) using delta E CIE2000. We used the closest corresponding RGB (smallest delta E CIE2000) value on the ninhydrin scale to estimate the protein content of our samples. Then, we multiplied the estimated protein concentration (g/L) in each tube by the volume of solution (0.0012 L) used to obtain the protein mass and divided the result by the mass (0.400 g) of the sample used to calculate the amount of protein per gram of the sample. Finally, the relative protein content between each sample was analyzed with an ANOVA on GraphPad Prism 9 to determine whether there was a statistically significant difference.

Results

The mean protein content of each protein type is represented in Figure 3 below. In each of the 4 groups, 3 replicates were tested. The results show that each of the 4 protein groups had varying mean protein contents, with 13.08g for tofu, 12.24g for plant-based chicken, 12.27g for animal-based chicken, and 10.29g for cat food. All groups had positive non-zero mean protein contents. However, the cat food group, which was used as a positive control and was expected to have the highest average protein content, instead had the lowest average protein content, a surprising result. A one-way ANOVA test was conducted to determine if there was a significant difference in average protein content between the 4 groups tested. The p-value of 0.1388

indicates no significant difference in average protein content between the 4 tested protein groups.

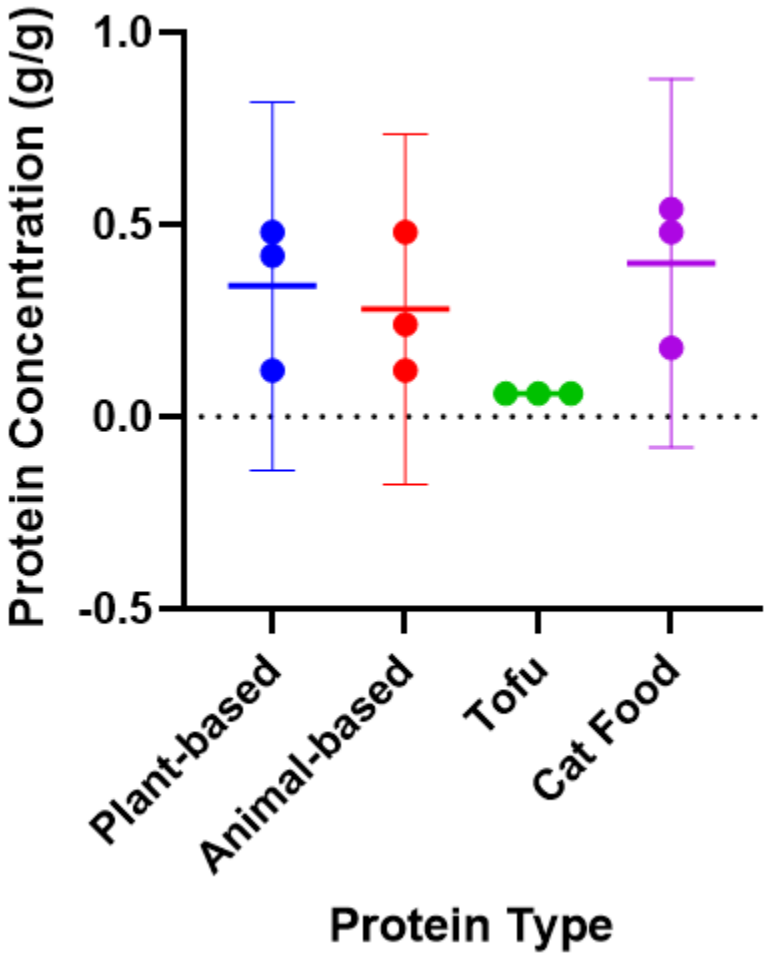

Figure 3. Protein Concentration (g/g) vs. Protein Types (Plant-based Chicken, Animal-based Chicken, Plant-based Tofu, Animal-based Cat food). Each of the dots represents the individual protein concentrations measured for each sample of the 4 protein types (n=3) and the line in each group

represents the median value from each sample. The horizontal line in each of the groups represents the mean protein concentration for each protein type. The bars going above and below each data point represent the 95% confidence interval for the mean protein concentration per group. The p-value we obtained via a one-way ANOVA test is 0.1388.

Discussion

The ANOVA test shows no significant difference in protein content between all tested protein types since we obtained a p-value of 0.1388. Therefore, we cannot reject the null hypothesis that there is no significant difference in protein between chicken nuggets, vegetarian nuggets, and tofu. This conclusion aligns with research showing that vegetarian diets can have more or less the same amount of protein. However, the differences lie in the type of amino acids provided by each protein source and other factors that influence protein absorption (Lee et al., 2020; Bohrer, 2019).

Although the ANOVA test suggests no significant difference in protein content between the samples, it is essential to note that the procedure used in the experiment differs from the planned initial procedure. After drying all the samples (Figure 4 below), we planned to apply the ninhydrin to the sample and activate it with an alcohol lamp under a fume hood after absorbing it. However, upon drying, we encountered two issues. Firstly, some samples became too dark to accurately measure the shade of purple that the ninhydrin would produce. After the drying process, some samples appeared to have been overcooked and burnt, resulting in some samples becoming a near-black colour (Figure 5 below). The second issue was that all the oven-dried samples could no longer absorb liquids within a reasonable time. We found that only after approximately one hour, a tiny portion of the 200uL ninhydrin pipetted onto the sample had been absorbed, which we deemed unreasonable given time constraints. After discovering these issues, we discussed potential alternatives, and a suggested idea was to grind up the samples

and place them into test tubes along with the ninhydrin and some water to act as a buffer. This idea worked brilliantly and allowed us to view the beautiful shades of purple produced by the ninhydrin while still using our oven-dried samples.

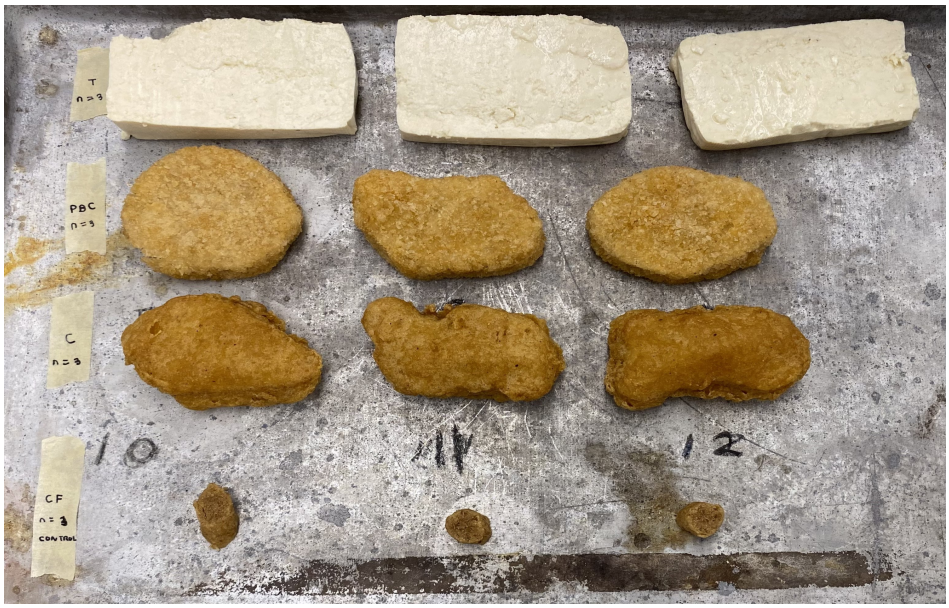

Figure 4. The 4 groups of samples prior to oven-drying at 90 degrees C in the initial procedure

Figure 5. The 4 groups of samples after oven-drying at 90 degrees C in the initial procedure.

The potential limitations of this study are its quantitative nature and the small amount of data collected. The ninhydrin test is a qualitative analysis; therefore, we cannot accurately predict the amount of protein from the colour of the reaction. In order to see the purple hues of the ninhydrin, light-colored samples were chosen and made into a solution. However, the sample's color and the ninhydrin's dilution still influence the resulting solution's color. Additionally, the protein may not have formed a homogeneous mixture affecting the ninhydrin reaction to the protein. Furthermore, the RGB values of the solutions were taken from the boundary layer between solid protein and liquid, which was a choice made under the assumption that it is the layer where most of the ninhydrin and protein came into contact. Therefore, the estimation of the protein concentration using the ninhydrin scale needs to be more accurate because the scale was produced using equal parts of water and ninhydrin with a colorless protein to obtain their RGB values. Comparing the RGB values from our photos to the

RGB values on the scale does not accurately estimate the protein concentration and is more about relative protein concentration.

Conclusion

In conclusion, our study suggests no significant difference in protein content between animal-based chicken nuggets, plant-based vegan nuggets, and plant-based tofu. This conclusion supports previous research that vegan alternatives can provide comparable amounts of protein to animal-based diets. However, there remain differences in the types of amino acids the alternative provides (Lee et al., 2020). However, the limitations of our study, including the small sample size and the quantitative nature of the analysis, affect the reliability of our results. Therefore, further research with a more accurate and precise methodology should be considered to confirm our findings.

Additionally, it would be beneficial to investigate the difference in amino acid concentration types between any future samples to gain a more comprehensive understanding of their nutritional value. Despite the limitations, our study provides insight into the protein content of popular foods and highlights the possibility of plant-based alternatives as a viable source of protein. Novel plant-based meats are often considered a healthy alternative. However, there is a need for more research on the nutritional value and health impacts of ultra-processed plant-based products to create better policies around labeling and public health. These results are essential since a negligible difference in protein content between animal-based and plant-based protein products, like chicken nuggets, means that differences in diet do not cause as significant of a gap in nutritional requirements as people may expect.

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Literature Cited:

1. Day, L., Cakebread, J. A., & Loveday, S. M. (2021, December 18). *Food proteins from animals and plants: Differences in the nutritional and functional properties*. Trends in Food Science & Technology. Retrieved March 18, 2023, from https://www.sciencedirect.com/science/article/abs/pii/S0924224421006774
2. Lee, H. J., Yong, H. I., Kim, M., Choi, Y.-S., & Jo, C. (2020, October). *Status of meat alternatives and their potential role in the future meat market - a review*. Asian-Australasian journal of animal sciences. Retrieved January 29, 2023, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7463075/
3. Bohrer, B. M. (2019). An investigation of the formulation and nutritional composition of modern meat analogue products. *Food Science and Human Wellness*, *8*(4), 320–329. https://doi.org/10.1016/j.fshw.2019.11.006

Appendix:

Table 1. Ninhydrin Scale (taken from L03 B4, another group in BIOL342)

Protein Concentration (g/L)	Mean R Value	Mean G Value	Mean B Value

0	**222**	**222**	**213**
20	**107**	**85**	**156**
40	**66**	**44**	**107**
60	**34**	**15**	**70**
80	**19**	**6**	**53**
100	**12**	**1**	**36**
120	**6**	**1**	**27**
140	**6**	**0**	**25**
160	**7**	**1**	**15**
180	**6**	**0**	**16**
200	**5**	**0**	**10**