

Different protein powders and their dissolvability in water

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Abstract

The purpose of this experiment is to compare the dissolvability between different types of commercial protein powder. The types used in the study are Whey, Vegan, and Isolate. Because there is less fat in isolate powder, we predicted that it would have the fastest dissolving time, as fats are hydrophobic, and proteins are soluble. We conducted this test by centrifuging 2 samples of the same protein, one being mixed for 20 seconds, and one being mixed for 5 minutes. We then added ninhydrin reagent to these samples, which produce a blue color, in which we can compare to previous works to determine the protein concentration, and ultimately the amount of protein dissolved. Unfortunately, both the whey and isolate samples used contained too much protein, and as a result, we are unable to determine the amount of protein dissolved. There was success in the vegan sample, but without other proteins to compare to, we cannot make a conclusion about which protein has the highest dissolvability.

Introduction

By combining modern imaging technology with established chemical assays, our research aims to provide a comprehensive understanding of how Whey, Isolate, and Vegan protein powders behave upon dissolution. This investigation holds promise not only for the fitness and nutrition communities but also for researchers and

manufacturers looking to optimize protein supplement formulations for enhanced bioavailability and effectiveness.

Although the body-building industry was starting to garner attention in the 1970's it wasn't until the early 1990's that the popularity of nutritional supplements started to take shape, protein powders have become integral for individuals seeking to optimize their dietary intake, enhance athletic performance, or address specific health goals. With the diverse range of protein sources available, such as whey, isolate, and vegan options, it is crucial to understand not only the nutritional content but also the dissolution rates of these powders. The dissolution rate, or the speed at which a protein powder disperses in a solution, directly influences the speed of its absorption into the body and other potential physiological impacts. In this study, we investigate the dissolution kinetics of three protein sources. Whey powder, which is a processed dairy product, Vegan powder, which combines many vegetarian proteins such as pea protein, hemp seed protein, and Sacha Inchi Seed protein, and Isolate Powder, which is a purified, highly concentrated whey protein that eliminates most of the fat that resides in whey protein. While traditional methods for assessing dissolution rates involve intricate laboratory techniques, our study introduces a novel methodology by employing color analysis values extracted from photographs taken during the reaction between the protein powders and ninhydrin reagent.

Ninhydrin, a chemical commonly used to detect amino acids, provides a visual indication of the rate at which proteins break down in a solution. Ninhydrin reacts with

the α -amino group of primary amino acids producing a new compound, which has a deep blue color, usually referred to as 'Ruhemann's purple'. There is also excretion of CO_2 and NH_3 . The color analysis values of these reactions serve as quantitative metrics, allowing for a nuanced and efficient evaluation of dissolution kinetics.

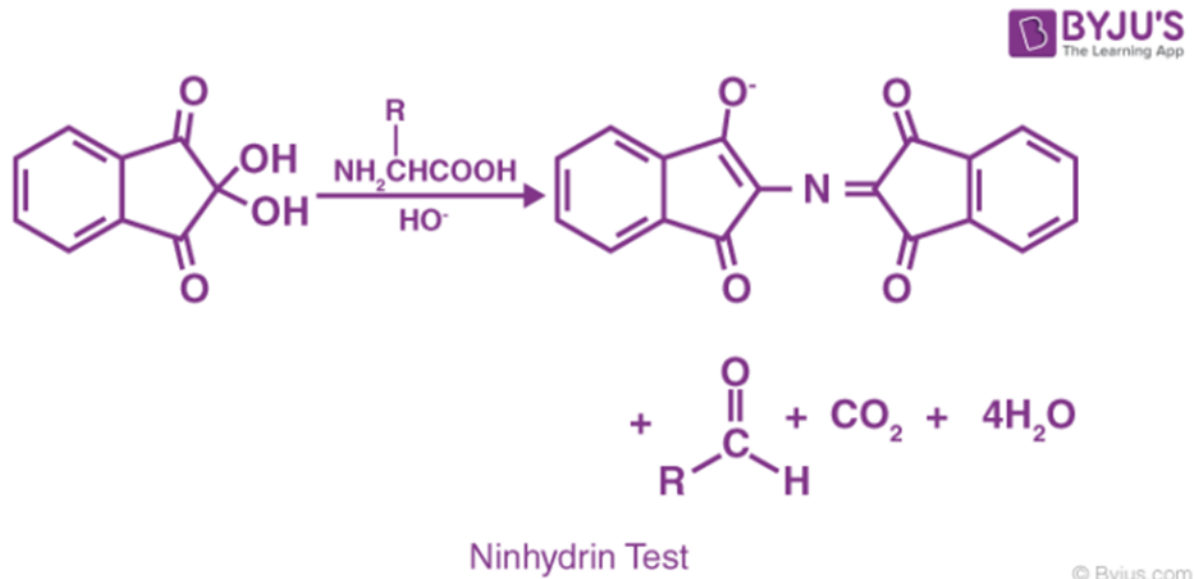


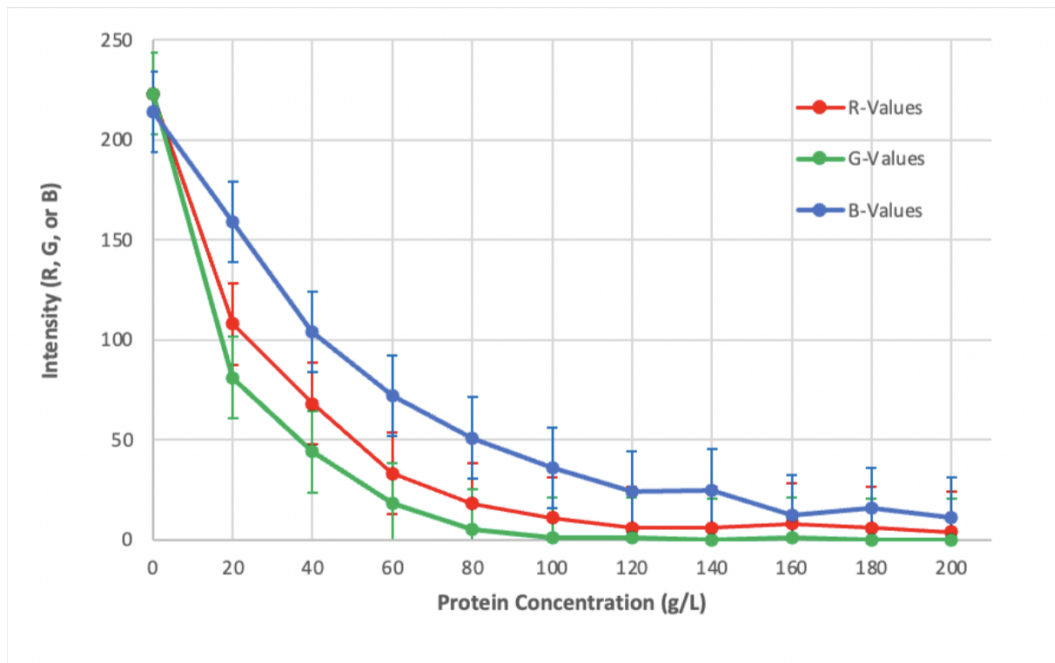
Figure 1: Ninhydrin reaction with amino acid - <https://cdn1.byjus.com/wp-content/uploads/2020/09/Ninhydrin-Test-1.png>

Whey protein, derived from milk, has long been a staple in the fitness and nutrition industry due to its rich amino acid profile and rapid absorption. Isolate, a more purified form of whey, is renowned for its higher protein content and lower levels of fats and carbohydrates. On the other hand, Vegan protein powders, sourced from plant-based ingredients, cater to individuals with dietary restrictions, ethical considerations, or those seeking alternative protein options.

This investigation holds promise not only for the fitness and nutrition communities but also for researchers and manufacturers looking to optimize protein supplement formulations for enhanced dissolution and effectiveness.

Hypothesis/Preliminary Research

Our study utilized findings produced in the paper 'Development and Protocol for Use of a Ninhydrin Scale in the Rapid Measurement of Protein Content of Liquid Samples' by (Bhiladvala et al.). Their paper focused on mapping RGB values to amino acids reacted with ninhydrin. The experiment consisted of performing ninhydrin tests on solutions of variable protein concentrations, and taking the color of the ninhydrin



solution and mapping it to a graph (For example, a solution with a protein concentration of 40g/L should produce a color with values of 70 red, 45 green, and 105 blue, creating

a value of 70,45,105). This graph was produced to show the relative protein concentrations at each quantitative RGB data set.

We can combine our RGB value findings for each photograph with the result of their mapping to determine the protein concentrations before and after the addition of the ninhydrin reagent, thus we will be able to calculate the best and fastest dissolving amino acids out of the three protein powders.

Based on research we believe that Isolate will exhibit the highest dissolution rate, as measured by the nitrogen solubility index using ninhydrin reagent, compared to Whey concentrate and Vegan. Isolate is expected to dissolve more rapidly due to its higher purity and lower content of non-protein compounds, which may interfere with solubility.

Methods

1. Measure out 3 different samples of the protein-specific weight for powder #1
 - a. 2.37g for Isolate
 - b. 2.72g for Whey
 - c. 2.84g for Vegan
 - d. These measurements were made so that there is an even amount of protein content in each measurement.
2. Add each sample to a separate beaker filled with 20mL of water.
3. Add a stir bar to each beaker, and place on a magnetic stirrer for 20 seconds.
4. Take a 100uL sample of each beaker, and move to an eppendorf tube.

5. With the same beakers, place back on the magnetic stirrer for 5 more minutes.
6. Take another 200uL sample, and move to another eppendorf tube
7. Repeat steps 1-6 with the other 2 types of powders.
8. This will produce 18 eppendorf tubes. Move these to a centrifuge, and centrifuge them all for 2 minutes.
9. This will separate the undissolved powder from the solution with the dissolved powder. Extract 100uL of this solution, and move to a new eppendorf tube, repeat for the other 17 tubes.
10. Add 100uL of 0.5% concentration ninhydrin to the new tubes. Add to an eppendorf temperature control device, and heat at 95°C for 15 minutes.
11. The tubes will now be a blue mixture. Take 2 tubes of the same sample (ie: Whey protein sample #1), and use a 20uL pipette to lay them next to each other on a depression slide. Repeat this for all 9 samples.
12. Under similar lighting conditions (in this case, 620 lux), take a photo of the depression slide, making sure the angle and lighting is the same for both droplets.
13. Record the RGB value for the droplets, and then compare them to the pre-made chart to compare RGB with protein concentration.
14. Given the protein concentration of the mixtures, calculate the difference for the between and after concentrations. The one with the largest difference will be the powder with the fastest dissolvability.

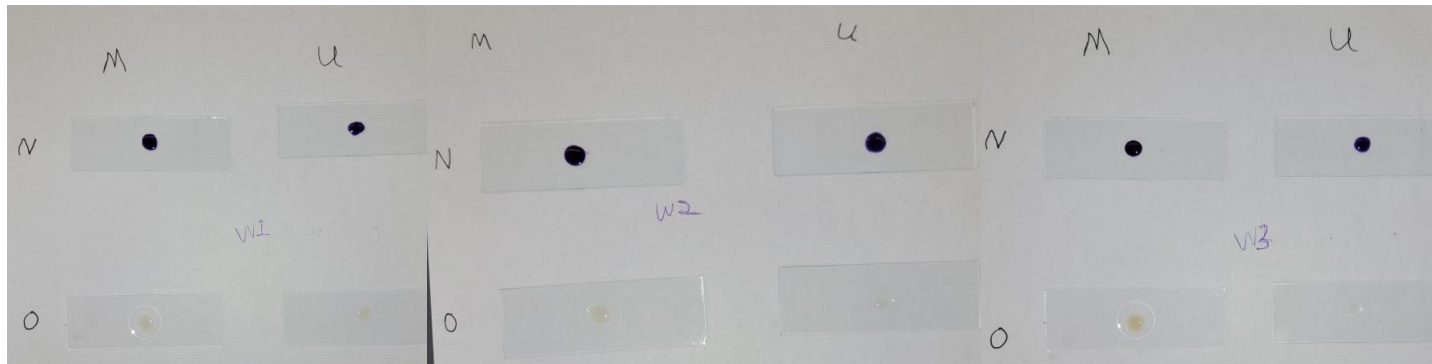
Analysis

The columns are marked M for Mixed and U for unmixed.
The rows are marked N for ninhydrin and O for original.

Whey Sample 1

Whey Sample 2

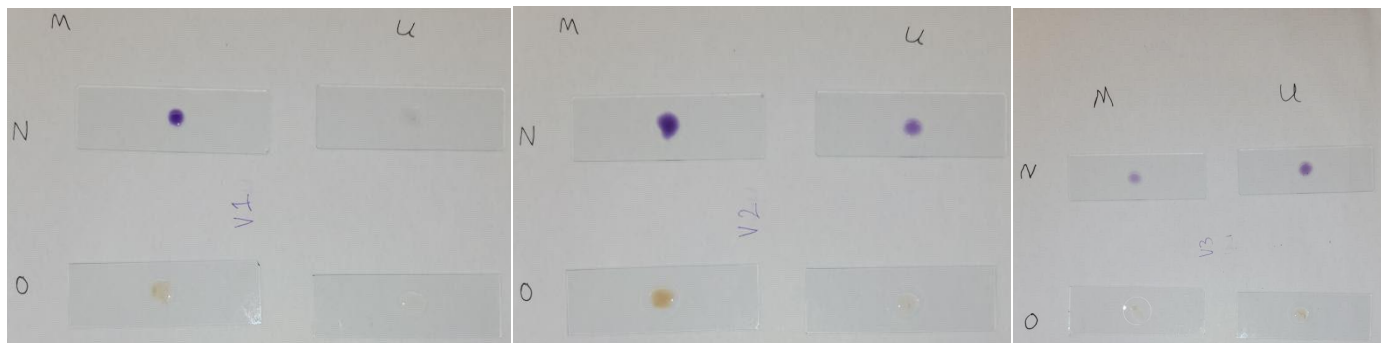
Whey Sample 3



Vegan Sample 1

Vegan Sample 2

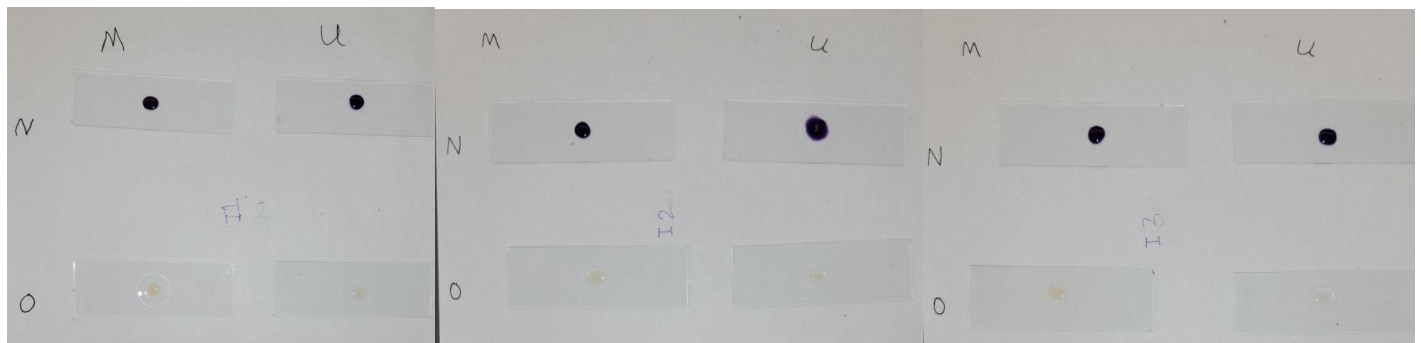
Vegan Sample 3



Isolate Sample 1

Isolate Sample 2

Isolate Sample 3



(Labeled incorrectly, but is isolate sample 3)

RGB (Red.Green.Blue) Values of the Ninhydrin Solutions

	Whey	Vegan	Isolate
Sample 1 Unmixed	2.0.5	64.64.63	1.0.1
Sample 1 Mixed	0.0.0	24.10.44	4.2.4
Sample 2 Unmixed	4.1.7	46.34.58	3.2.4
Sample 2 Mixed	0.0.0	24.13.41	2.1.3
Sample 3 Unmixed	3.2.7	45.37.55	2.1.4
Sample 3 Mixed	2.1.0	57.51.64	2.0.3

For reference, 0,0,0 is black and 255,255,255 is white

Protein concentration given RGB values of solution (g/L)

	Whey	Vegan	Isolate
Sample 1 Unmixed	200+	N/A	200+
Sample 1 Mixed	200+	85	200+
Sample 2 Unmixed	200+	60	200+
Sample 2 Mixed	200+	85	200+
Sample 3 Unmixed	200+	60	200+
Sample 3 Mixed	200+	N/A	200+

We can be confident that Whey and Isolate are 200+ because the colour approaches (0,0,0), which is consistent with the graph. All vegan samples are estimated, using the error bars to align color with protein concentration. Sample 1 unmixed and Sample 3 Mixed Vegan are N/A because their RGB value does not have a corresponding protein concentration without making irrational estimations.

Discussion

The protein table lists whey and isolate as containing protein content past 200g/L. This puts them out of the range of the graph we were mapping these RGB values to. From this we can deduce that the protein content of both Whey and isolate protein products far out whey that of Vegan, however, the inability to exactly calculate the content of the whey and isolate renders a massive limitation of our study and should be analyzed further. We believe this limitation is due to improper techniques in our measuring. To obtain protein content values that were measurable we should have tested our protein samples with a lower dilution. Due to this being a novel study, we did not have the prior information to understand the right dilution to move ahead with in our experiment. The dilution calculation was based on the manufacturers listed grams of protein per volume of scoop, which was different for each brand. This was then calculated to standardize a set protein ratio in each sample. In future studies, this ratio must be reduced.

Another reason for the inflated protein content data could be due to the dissolution rate of the whey and isolate proteins. From observations, it was noticed that these samples, before mixing, almost exhibited saturation from the protein powder after the initial 20 seconds of light mixing. This light mixing was not meant to dissolve the protein fully but rather spread out the powder evenly throughout the solvent so that proper mixing can occur during the 5 minute mixing period. The fact that protein saturation occurred with minimal mixing suggested that there was too much protein added to the whey and isolate samples, resulting in unmeasurable protein content. This differed with vegan protein, which exhibited a measurable range of values. Our

hypothesis suggested that only isolate would exhibit higher protein levels than the other types due to its higher purity and lower amount of non-protein compounds. However, because the data indicates that both isolate and whey protein vastly outnumbered vegan in protein content, there must be an undeniable factor that resulted in this anomaly. This is most likely due to the chemical make-up of the vegan protein. As the protein is extracted from plant-based sources, it does not contain as many different amino acids which are present in the other two proteins (whey and isolate both contain the nine essential amino acids - the building blocks of protein).

From the table of results, there are two anomalies present with the vegan samples labeled with the abbreviation "N/A". With sample 1 mixed and sample 3 mixed, the RGB data retrieved could not be calibrated with the graph. In simpler terms, the color emitted by the ninhydrin reagent was not strong enough to pinpoint to an exact protein concentration. Both of these samples are repetitions of the same protein type with the goal of being performed in control, thus, allowing us to draw conclusions that the main cause for these outliers were down to experimental and human error. Error from the human side would be most associated with the mixing portion of the experiment. If a sample was not mixed in the same starting conditions as its repeated trial, they would yield different results. The pre-mixing that occurred before the controlled mixing allowed human interaction with the protein samples to influence the results, which may have caused the difference in values.

Another error mentioned was experimental. The main source for experimental error came from the photos that were taken. A Samsung cell phone was used to capture the shots of the "Ruhemanns purple". The cellphones' sensor may not have been big

enough to capture all the light reflecting off the ninhydrin mixed solutions. Another challenge was the ability to maintain a controlled environment when taking the photos. Light pollution from the classroom, such as the ceiling lights emitting glare onto the glass cover slip, could have potentially affected the RGB values of the samples. Much was done to attempt to eliminate this factor including changing the angle of the camera and cover slips, however, a perfectly controlled experiment was almost impossible given the conditions.

After a thorough analysis of the chart data, it reveals that there were potential errors in our experimental process, particularly concerning the time and gram measurements. These values were critical yet imprecise, deviating from the ideal time and mass required for the experiment. A significant area of concern is the possibility that we miscalculated the grams of substances needed. This misjudgment could have led to skewed results.

However, it's important to note the errors we couldn't have made. The centrifuge, hot plate, and mixing rods in our setup were all automated, which effectively eliminates the possibility of human error in their operation. This assurance narrows down the potential sources of error in our experiment.

Moving forward, our focus should be on rectifying these identified issues. The primary error appears to have been the excessive use of protein in the mixture. In future experiments, it will be crucial to significantly reduce the quantity of protein used. Despite the lack of results in this initial trial, it's important not to discard the potential of this test entirely. The fact that human error was a contributing factor suggests that a second

experiment, with adjusted variables and more precise measurements, could yield substantially different and potentially successful results.

Conclusion

In conclusion, our study aimed to compare the dissolvability between different types of commercial protein powder, but we encountered significant limitations due to the protein content of whey and isolate exceeding the measurable range of our graph. This discrepancy indicates that whey and isolate proteins have substantially higher protein content than vegan protein, but our inability to accurately quantify these values poses a major limitation to our study. This issue likely stemmed from improper dilution techniques, a hypothesis supported by our observation of protein saturation in whey and isolate samples with minimal mixing. This contrasted with the vegan protein, which showed measurable values and differed in chemical composition. On the other hand, further complicating our results were anomalies in the vegan protein samples, where RGB data could not be calibrated accurately. These discrepancies suggest experimental and human errors, particularly in the pre-mixing phase, which could have influenced the results. Additionally, the use of a Samsung cell phone for capturing the Ruhemann's purple images might have led to inaccuracies due to limitations in the sensor and challenges in controlling the light environment.

Reflecting on these challenges, our study underscores the importance of precise measurement and controlled experimental conditions. The automated elements of our experiment, such as the centrifuge, hot plate, and mixing rods, ensured accuracy in those areas, but human errors in other aspects, like dilution and pre-mixing, significantly

impacted our findings. Even though the setbacks in this initial study, the potential for success in future experiments remains, provided that these identified issues are addressed and rectified.