

Does Protein Concentration of Different Types of Dietary Supplements for Athletes Vary According to Protein Particle Length?

Original Research

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Abstract

Introduction: Protein microparticles are crucial for their sensory properties and applicability in food production. This study has the objective of correlating protein particle length and content in different sources of protein supplements.

Methods: An analytical observational study with protein supplements (n=30) from different sources was carried out to estimate the protein content and the particle length in the samples. Protein content data was obtained via total nitrogen content. The samples were also analyzed for protein particle lengths using the scanning electron microscope.

Results: The comparison between the average particle length of each group of protein supplements analyzed has shown significant statistical difference ($F=139.6$; $p<0.001$). Whey Protein Isolate showed higher particle length, compared to other supplements ($57.7\pm42.1\ \mu\text{m}$; $p<0.001$). However, no evidence of a significant difference in protein concentrations among supplements was detected ($F=1.072$; $p=0.4004$). In addition, no evidence of a significant correlation between protein concentration and particle length was observed ($R^2=0.001$; $p=0.8476$).

Conclusions: Although a difference in the protein particle length of different supplements for athletes was found, there is no correlation between protein concentration and particle length in the studied protein supplements from different sources Whey Protein Concentrate, Whey Protein Isolate, Blend Whey Protein, Veggies, Beef and Albumin.

Key Words: Protein Supplements, Particle Length, Granulometry

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Introduction

Proteins are the ones responsible for building muscle mass, being essential to the human diet. This macronutrient has a central role in developing and maintaining the skeletal muscle mass. Athletes and recreationally active individuals require a larger protein intake than the general population. Muscle protein synthesis is elevated after exercise, promoting a strong anabolic response, so enough protein must be consumed to support that response¹.

Currently, protein supplements are trending products on the market, and they come in numerous varieties. Whey protein, from milk protein, is one of the most famous, but protein supplements from many other sources are available

for consumption. These products may be labelled with the claim “source of protein”, if the protein content represents at least 12%, or be labelled as “high protein” if protein content corresponds to at least 20% of the energy value, respectively². Usually, animal protein sources tend to have greater concentrations of protein, with albumin, meat and milk protein being excellent animal sources³.

Depending on the protein source, different amounts of macronutrients and micronutrients will make up the supplement. In the past, isolated vegetable protein was considered less nutritious and an incomplete source of essential amino acids, but this perception has evolved and currently, it is considered a healthy option that meets protein recommendations and needs for humans⁴. It is widely recognized that functional properties and physicochemical characteristics emerge from the interaction of proteins with water and other food elements and are of great use in determining the effectiveness of proteins as food ingredients. These properties impact the appearance, structure, texture, viscosity, mouthfeel, and flavor retention of the product⁵. The functional properties of whey protein products are not exclusively determined by their chemical composition (mainly protein content), but also by other factors, such as the methods used during the production process in industry⁶.

Microparticles from protein supplements can vary in size from a few tens of nm to a few tens of μm . The microparticles, considered soluble and insoluble aggregates of controlled size, usually have better functional properties than the native form of proteins⁷. The particle size of proteins, in the form of microparticles, is crucial for their sensory properties and applicability in food production. In the case of whey protein, using appropriate processing conditions, it is possible to obtain Whey Protein Microparticles (WPM) in a spherical format, with high creaminess and water retention capacity, which can be used as a fat substitute due to the "ball bearing" effect⁸, resulting in a pleasant mouthfeel similar to the creaminess of milk fat, or as a functional ingredient for targeted structure modification in various food products. WPM with an average particle length between 0.1-3.0 μm and dispersed in water are perceived as smooth and rich⁹. The limit between a soft and slightly mealy mouthfeel for WPM is reported to be around the particle length of 20.0 μm ¹⁰.

In literature, there are many investigations on protein supplements and their effects on muscle protein synthesis, however, there are few studies that evaluate the particle length of protein supplements. Given the issues raised, the present study has been designed to investigate the particle length of different types of protein supplements for athletes and relate the particle length with the content in protein supplements.

Scientific Methods

An analytical observational study was carried out with protein supplements from different sources, including Whey Protein Concentrate (WPC), Whey Protein Isolate (WPI), Whey Protein Blend (Blend WP), isolated proteins from vegetables (Veggies), beef protein isolate (Beef) and Albumin protein isolate (Albumin). This study is part of the research project “Bromatological and Adulterant Analysis in Protein Food Supplements” (project number 10776), approved by the Scientific Committee of the School of Health and Life Sciences of the Pontifical Catholic University of Rio Grande do Sul (PUCRS).

Samples

Protein supplements from different national and international brands available in physical and online stores were purchased for this study. The choice of protein supplement brands for this study considered the following criteria: 1) products labeled as protein supplements; and 2) products sold in Brazil. There was no restriction for national or imported brands, provided it was possible to purchase them in a physical store or digital commerce. A total of 30 different supplements were purchased.

The products arrived at the University’s premises, were kept in their original packaging, and handled as recommended by the manufacturers. Initially, the 30 supplements were categorized and labelled by protein source, into the six groups listed at the beginning of this section: WPC (n=6), WPI (n=4), Blend WP (n=9), Veggies (n=7), Beef (n=1), and

Albumin (n=3). The analysis occurred at the Laboratory of Food Analysis of PUCRS in Porto Alegre, Rio Grande do Sul, Brazil.

Analysis of Protein Content

Individual samples were prepared for protein content analysis in an electronic analytical scale, model AY220G, Shimadzu brand. To estimate the protein content of the samples, data from a previous stage of the project was used. The data was obtained via total nitrogen content by the Kjeldahl method, according to the Association of Official Analytical Chemists – AOAC (1998). The protein percentage was calculated by multiplying the mean value of the total nitrogen percentage by the factor 6.25 in Velp Scientifica equipment with a DK 20 digestion unit (Italy) according to equation 1 ¹¹:

The calculation for the determination of total proteins:

$$\frac{V \times 0.14 \times f}{P}$$

V = difference between the number of mL of 0.05 M sulfuric acid and the number of mL of 0.1 M sodium hydroxide spent in the titration

P = number of grams of the sample

f = conversion factor (6.25)

For this study, the previously obtained percentage estimates were uniformized to match the standardized mass of 30 grams.

Field Emission Scanning Electron Microscopy (FE-SEM) Granulometry

All 30 samples were analyzed for protein particle lengths using the scanning electron microscope (FE-SEM) at the Central Microscopy and Microanalysis Laboratory - PUCRS, with support from the laboratory technician. In this process, each sample was affixed onto a carbon tape for the insertion of solid samples atop a sample stub. These metal bases containing the samples were then placed into a metallizer machine, where the samples were coated with a 5 nm-thick layer of gold (Au) using a physical material deposition technique known as sputtering. The metallic coating is necessary to render the samples conductive (protein supplements are non-conductive as they are not metallic) to generate high-resolution images in the FE-SEM. The deposited thickness must be controlled accurately to avoid affecting the actual sample surface.

Following this process, the samples were placed in the microscope's sample chamber, and the analyzed area was defined by regions of interest. Three random points (spots) were selected on each of the samples, and their particles were analyzed at magnifications of 100x (1mm), 500x (300µm), 1,000x (100µm) and 2,000x (50µm) at each point, resulting in 12 images per sample, 360 images in total.

Image Analysis for Granulometry

All different protein particles were observed and measured, leading to a comparison of the lengths of particles in each type of supplement. The particle measurements were made by ImageJ software. To evaluate particle length, firstly the dimension was calibrated to 300 µm to collect data for particle diameter, area, and number of particles. Particle diameter was used as a proxy for the particle length. For this analysis, the 500x amplitude was chosen, as it is an amplitude that allows better visualization of the particles. 3 images per sample from FE-SEM were used, with at least 100 particle measurements per image. This totaled over 300 length measurements per protein supplement, 9,000 measurements overall.

Statistical Analysis

The analysis of the results occurred through descriptive and inferential statistics. To comprehend the behavior of the variables studied (continuous), the authors performed a Kolmogorov-Smirnov normality test to determine data normality. The constant parameter information mean $\pm$ standard deviation (SD) is presented. Toward the comparison between products, the authors opted for the hypothesis test analysis of variance (ANOVA) with Tukey post-hoc. For correlation tests, Pearson estimation is used. A significance level p value < 0.05 was considered. Charts were created using the GraphPad Prism software version 9.0 for Windows (San Diego, USA).

Results

Protein concentration – no difference between groups

The general characteristics of the 30 samples are presented in Table 1. No significant difference was observed in the protein content between the samples analyzed (ANOVA $F = 1.072$; $p = 0.4004$).

Table 1. Description of general characteristics of the protein supplements.

Dietary Supplements	Sample (n = 30)	Protein (g/ 30g of sample)
Whey protein concentrate (WPC)	6	19.18 $\pm$ 3.46
Whey protein isolate (WPI)	4	23.09 $\pm$ 0.94
Whey protein blend (Blend WP)	9	18.37 $\pm$ 4.49
Isolate proteins from vegetables (Veggies)	7	19.78 $\pm$ 2.81
Beef protein isolate (Beef)	1	19.91
Albumin protein isolate(Albumin)	3	17.91 $\pm$ 5.07

Data are Means $\pm$ SD

The evaluation between the protein concentrations of each group of supplements, observed in Panel B (Figure 1), was made using the ANOVA statistical test. Results did not identify significant differences among the samples ($p = 0.4004$). Using the Tukey test as confirmation, none of the multiple comparisons performed presented mean values below the threshold.

Particle length - WPI presented greater particle length than other groups

Data from the analysis of the sampled protein supplements, as well as illustrative images of the protein particles of each type of supplement, seen through the FE-SEM, are presented in Figure 1. The images magnified to 50 μm (Panels D, E, F, G, H, I), are enlarged images from the protein particle, to illustrate what they look like.

The comparison between the average particle length of each group of protein supplements analyzed is presented in panel A. WPI showed higher particle length than other protein supplements ($p < 0.001$; power=85.92%). Additionally, WPC showed higher particle length when compared to Blend WP, Beef and Albumin ($p < 0.001$; power=78.38%). Also, Blend WP presented higher particle size than Veggie, Beef and Albumin ($p < 0.001$; power=78.38%). Veggie protein supplements showed higher particle size when compared to Beef protein supplements ($p < 0.001$; power=76.37%).

Particle length vs. protein concentration – no correlation

To analyze the relationship between protein concentration and particle length for the 30 samples, Pearson estimation was used. No significant correlation ($R^2 = 0.001$; $p = 0.8476$) between protein concentration and particle length was observed (Panel C).

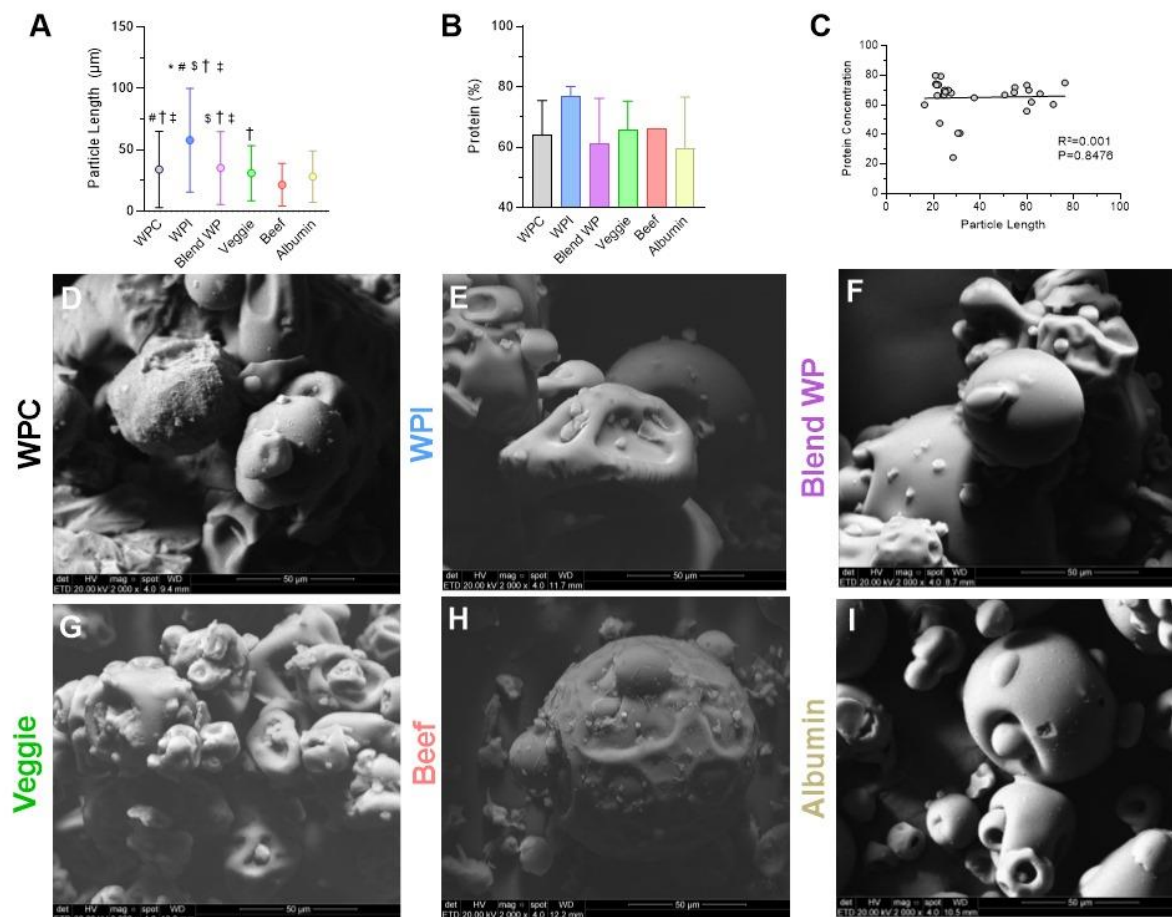


Figure 1. Data are Means $\pm$ SD. ANOVA test with Tukey post-hoc test. (A) Analysis of particle length in micrometers (μm) for each group of samples. * $p < 0.001$ versus WPC; # $p < 0.001$ versus Blend WP; \$ $p < 0.001$ versus Veggie; † $p < 0.001$ versus Beef; ‡ $p < 0.001$ versus Albumin. (B) Analysis of protein concentration (%) in each group of samples. (C) Pearson correlation between protein concentration and particle length of each sample. Panels D, E, F, G, H and I represent microscopic images of the supplement's protein particles (scale of 50 μm).

Discussion

Protein supplements are a highly debated and studied topic in human and, more specifically, athletes' nutrition. Despite the importance of particle length for sensory properties and applicability in food production⁸, not many investigations evaluate the protein particle length present in protein supplements; and studies evaluating the relationship between particle length and protein concentration of the supplements were not found in the main databases. The main finding of this study was that protein supplements from different sources differed in terms of particle size. According to the Use of Heat Treatment to Improve the Functional Properties of Proteins study¹², WPC aggregates with a small to medium particle size (1-3 μm), would be suitable to be applied as fat substitutes, when subjected to heat treatment. In the present study, WPC size values of $33.98 \pm 31.03 \mu\text{m}$ were obtained, with the minimum value, on average, being 2.90 μm . Comparing these values with the study previously mentioned, a small to medium particle size could be considered for WPC particle length. However, the particle length values observed in this study are higher, on average, than those found in the literature. Moreover, WPI presented the higher mean particle-length values when compared to all other supplement groups.

In this study, Beef and Albumin presented lower average values than any of the other groups. A study about the Nutritional Quality Evaluation of Commercial Protein Supplements¹³, concluded that Albumin Isolate Protein had a lower digestibility than expected from literature results. This suggests that a study comparing particle size and digestibility of protein supplements could bring relevant input to this discussion.

Another study, on physicochemical and morphological characteristics, and lubricating properties of specific size whey protein particles by acid or ion aggregation¹⁴, recognized that the size of the microparticles is vital for their oral lubrication characteristics. When the size of the particles is less than 10 μ m, a size below the threshold of particle perception by the human oral mucosa, the particles produce a fat-like texture. The average values for particle length in this study were not lower than 21.48 μ m (average particle length value for the Beef Protein group), so, it would be interesting to analyze the mouthfeel and lubricating properties of bigger protein particles (similar to those found in this study) with an average length of tens of μ m. However, a study considering the human oral mucosa would be necessary to reach more concrete conclusions. Although most research on whey protein particles has focused on exploring the impact of particle size on their lubricating behavior, a gap remains in investigating other particle properties, such as the surface characteristics of these particles¹⁴, and their impact on physicochemical characteristics and mouthfeel.

In the present study, regarding the protein concentration on protein supplements, no significant difference was found among the sampled groups. According to the Final Report on the Analysis of Protein Supplements for Athletes¹⁵, WPC can provide from 29% to 89% of protein, depending on the type of product. The lower the level of concentrated protein, the higher the levels of fat and lactose, which may contain large amounts of immunoglobulins and lactoferrins, not suitable for those who are lactose intolerant. WPC is mostly used as a food additive, due to its low cost and manufacturing process. The isolated form should be the purest form of whey, containing around 90% or more protein in its composition. Most WPI are fat-free and contain less than 1% lactose, making them the most suitable for people with intolerance. It has all the vitamins and minerals of milk, as well as all essential, non-essential and conditionally essential amino acids. Nevertheless, the 4 samples of WPI analyzed in this study presented a protein concentration lower than 80%, well below expectations.

Although, by the regulatory standard, WPC may contain a lower protein percentage compared to WPI, in this study there was no difference in protein concentration between the two types of whey protein, which may lead to questioning the adoption of WPI on athletes' nutrition. However, protein supplements have other important characteristics, in addition to protein concentration, which differ depending on the protein source and can be decisive when chosen by the consumer, such as lactose and fat content, digestibility and price.

Literature indicates that proteins isolated from albumin generally have a protein content close to 80% (w/w, dry basis)¹⁶. As for proteins from vegetable sources, pea protein isolate powder, depending on the processing treatments, generally has a protein content between 75% and 80% (w/w, dry basis)¹⁷, while soy protein powder isolate generally has a protein content greater than 90% (w/w, dry basis)¹⁸. Even so, in the present study, it was observed that neither Veggie proteins nor Albumin proteins differed in terms of protein concentration compared to other supplements from different sources.

Protein supplements contain information on their labels, which are essential so that the consumer makes the right choice according to their individual needs. Considering that most of its consumers are athletes or physically active individuals, it must be considered that protein supplements are taken for a nutritional purpose¹⁹. If protein supplements with larger particle sizes do not have a higher protein content, it is worth questioning why WPI, seen as an elite supplement by athletes, have such a higher market value and whether it would be worth its cost.

Conclusions

Although a difference in the protein particle length of different supplements for athletes was found, there is no correlation between protein concentration and particle length in the studied protein supplements from different sources (WPC, WPI, Blend WP, Veggies, Beef and Albumin). This finding can inspire the food industry to further research new methods to increase sensory properties of different protein supplements, especially veggie protein supplements due to their low acceptance by the physically active public.

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