

ORIGINAL ARTICLE

COMPARATIVE ANALYSIS OF THE PROTEIN CONTENT AND
NUTRITIONAL LABEL OF WHEY PROTEIN, PLANT-BASED, AND ANIMAL
PROTEINS SUPPLEMENTS

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Highlights: 1. Protein-based dietary supplements are within tolerance limit. 2. There is no difference in protein between animal and plant-based supplements. 3. The dietary supplement industry has improved its quality of products over the past.

PRE-PROOF

(as accepted)

This is a preliminary and unedited version of a manuscript that has been accepted for publication in Context and Health Journal. As a service to our readers, we are providing this initial version of the manuscript as accepted. The manuscript will still undergo revision, formatting, and approval by the authors before being published in its final form.

<http://dx.doi.org/10.21527/2176-7114.2024.48.14497>

How to cite:

Fabris LV, dos Santos LM, Stefani GP. Comparative analysis of the protein content and nutritional label of whey protein, plant-based, and animal proteins supplements. Rev. Contexto & Saúde, 2024;24(48): e14497

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ABSTRACT

The consumption of protein supplements is increasingly part of the everyday life of adults in general and is associated with a healthier lifestyle. However, a constant lack of commitment to the quality of these products by companies poses risks to consumers' health. Therefore, this study aims to analyze the protein content and compare it with the nutrition label of different protein supplements. This is an analytical original investigation. It was verified whether the food supplements comply with the label presented through food analysis (Kjeldahl method, ash content and moisture percentage) of the composition of various national and international brands ($n=51$). A total of 51 different protein-based supplements sold in Brazil were analyzed. The results showed that the protein values advertised on the nutritional label are similar to what the product has (P value $> 0,05$) and are within the 20% tolerance limit according to the current law. Also, there are similarities between different types of products (P value $> 0,05$) regarding their ash and water content. In conclusion, the nutrition facts of protein supplements reflect the reality of the brand of these products.

Keywords: Dietary supplements; Food analysis; Food labeling.

Abbreviations

ANVISA: National Health Surveillance Agency;

Blend WP: Mixture of different types of proteins of animal origin and plant-based proteins;

WPC: Whey protein concentrate.

WPI + WPH: Mixture of isolated and hydrolyzed whey proteins;

WPI: Whey protein isolate.

Análise comparativa do conteúdo de proteínas e rótulo nutricional de suplementos de whey protein, proteínas vegetais e animais

RESUMO:

O consumo de suplementos proteicos faz cada vez mais parte do cotidiano dos adultos em geral e está associado a um estilo de vida mais saudável. Porém, o constante descomprometimento com a qualidade desses produtos por parte das empresas traz riscos

à saúde dos consumidores. Portanto, este estudo tem como objetivo analisar o teor de proteína e compará-lo com a rotulagem nutricional de diferentes suplementos proteicos. Esta é uma investigação analítica original. Verificou-se se os suplementos alimentares cumprem o rótulo apresentado através da análise dos alimentos (método Kjeldahl, teor de cinzas e percentagem de umidade) da composição de diversas marcas nacionais e internacionais (n=51). Foram analisados 51 diferentes suplementos à base de proteínas comercializados no Brasil. Os resultados mostraram que os valores proteicos divulgados no rótulo nutricional são semelhantes aos do produto (P-valor > 0,05) e estão dentro do limite de tolerância de 20% conforme legislação vigente. Além disso, existem semelhanças entre os diferentes tipos de produtos (P-valor > 0,05) quanto ao teor de cinzas e água. Concluindo, as informações nutricionais dos suplementos proteicos refletem a realidade do farelo desses produtos.

Palavras-Chaves: Suplementos dietéticos; Análise de alimentos; Rotulagem de alimentos

INTRODUCTION

The consumption of dietary supplements is evident among those who practice physical exercise and athletes at all levels of sport ¹. The usage of supplements is often present in adults and is associated with healthy habits, such as maintaining a balanced diet, exercising, and avoiding obesity and smoking ². Nonetheless, there is evidence that some supplements pose health risks, as several athletes have failed drug tests due to undeclared ingredients in supplements.

When talking about sports nutrition, athletes wildly use animal-based proteins, especially whey protein, and plant-based proteins ³. Their consumption is related to post-exercise recovery, the increase in skeletal muscle mass in response to strength training and transportable nutrition on specific occasions ¹. Thus, the consumption of a protein-rich diet has significantly increased in recent years, according to a survey by Fortune Business Insights ⁴. Also, according to research carried out by the American market consulting firm Zion Market Research (2021), the whey protein market was estimated at US\$9.2 million in 2020 ⁵. Additionally, its value is expected to expand at an annual growth rate of 8.3% between 2021 and 2028.

Although some companies are careful to provide high-quality products, there are well-documented issues with the integrity of commercially available dietary supplements. In 2014, the National Health Surveillance Agency (ANVISA) from Brazil prohibited the distribution and commercialization of protein supplements due to non-compliance with the label and amounts of carbohydrates above 20% of the declared value tolerance limit for more or less stipulated in the Regulation Technician on Nutritional Labeling of Packaged Foods – RDC nº 360/2003⁶. A quantitative chemical composition analysis of 15 samples of Whey Protein showed that 73.3% of the supplements did not meet the resolution for carbohydrates and 13.3% for proteins, causing harm to consumers both from a nutritional and economic point of view⁷. In another investigation, a study that analyzed the protein concentration in different types of national protein supplements found that 100% of the samples contained less protein than stated on the nutrition facts but within the tolerated limit of 20% variation⁸.

In this study, the authors considered the lack of data on the quality of protein food supplements available on the market. The main objective of the analysis in question is to investigate the protein content of protein supplements, verifying whether they comply with their label arrangements through the bromatological analysis of the composition of different national and international brands. Such a study ought to promote greater transparency for the consumer and security for the professional nutritionist/dietitian who wants to prescribe products based on the information available on the label. Additionally, we believe that a lower protein present in dietary supplements could mitigate the intended recovery effects of protein supplements in exercise practitioners and athletes. The hypothesis being tested is that the protein values, mainly, are not in accordance with the information in the nutritional table presented.

MATERIALS AND METHODS

Study Design and Sample Obtaining

This is an analytical observational study of available dietary supplements for purchase in Brazil. There was the purchase of food supplements from different national and international brands for this study. They were available in physical and online stores. The products arrived at the University's premises, then kept in their original packaging, and packaged as recommended by the manufacturers. The only criteria considered to

guide the choice of food supplement brands were: 1) protein supplements and 2) sold in Brazil. There was no restriction for national or imported brands as long as it was possible to purchase supplements in the physical market or digital commerce. After purchasing all available dietary supplements within the University's budget, the protein supplements were then adequately storage in the laboratory. The analysis occurred in 2022 at the Laboratory of Food Analysis of the Pontifical Catholic University of Rio Grande do Sul (PUCRS) in Porto Alegre, Rio Grande do Sul, Brazil. And the analyzed samples were weighed using an electronic analytical scale, model AY220G, Shimadzu brand and stored in their original packaging.

Data Extraction from Nutritional Label Information

Since one of our objective were to compare the real protein content of the supplements with the nutritional label, we extracted data from the label. The authors used supplements based on proteins from different sources for the experimental research. They could have been animal-based or plant-based, although there was a possibility of a combination of them. The distribution of macronutrient data from the supplements was extracted from the packages and grouped in an Excel spreadsheet in a standardized way. The established standard form was a portion of 30 grams of bran, facilitating the normalized comparison of the food analysis concerning the label.

Methodology for Food Analysis

Determination of protein nitrogen by the modified Kjeldahl method

The total nitrogen content of the samples was determined by the Kjeldahl method, according to the Association of Official Analytical Chemists – AOAC (1998). All the analysis was performed in triplicate. 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 ⁹.

The calculation for the determination of total proteins:

$$\frac{V \times 0.14 \times f}{P} \quad (1)$$

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)

Determination of moisture loss by desiccation (direct drying in a kiln at 105°C)

Therefore, the authors weighed 2 to 10 g of the sample in a previously tared porcelain capsule. The next step corresponds to heating for 3 hours (at 105°C), then cooling the fragments in a desiccator to room temperature. Subsequently, they were again weighed, and the heating and cooling operations were repeated until the observed weight was constant. All the analysis was performed in triplicate. The percentage of moisture or volatile substances at 105°C was calculated according to equation 2⁹.

The calculation for determining moisture loss:

$$\frac{100 \times N}{P} \quad (2)$$

N = number of grams of moisture (loss of mass in g)

P = number of grams of the sample

Determination of waste by incineration (ash)

Acquiescing the rules of Instituto Adolfo Lutz (1985), the authors used the gravimetric method by muffle incineration. Based on the weight loss that occurs when the product is incinerated at 560°C and cooled to room temperature, the gravimetric method allows the weighing of the sample. Also, the heating and cooling operations occurred repeatedly until the observed weight was constant. All the analysis was performed in triplicate. The calculation of ash takes place according to equation 3⁹.

The calculation for determining the amount of ash:

$$\frac{100 \times N}{P} \quad (3)$$

N = number of grams of ash

P = number of grams of the sample

Statistical analysis

The analysis of the results occurred through descriptive statistics. In order 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) for parametric variables and median and interquartile range (IQ) for non-parametric variables were presented.

Toward the comparison between products, the authors opted for the hypothesis test analysis of variance (ANOVA) with Bonferroni post hoc for parametric variables and the Kruskal-Wallis test with Dunn post hoc for non-parametric variables. A significance level P value < 0.05 was considered¹⁰. Analyzes using SigmaPlot statistical software version 12.0 for Windows (San Jose, USA) and GPower software version 3.1 for Windows (Düsseldorf, Germany) furthermore occurred. Graphs were created using the GraphPad Prism software version 7.0 for Windows (San Diego, USA).

RESULTS

The 51 protein supplements analyzed were grouped into six different categories: a mixture of different types of proteins of animal origin and plant-based proteins (Blend WP), a mixture of isolated and hydrolyzed whey proteins (WPI + WPH), whey protein isolate (WPI), whey protein concentrate (WPC), plant-based protein (Plant-based) and other types of proteins, such as albumin, collagen and meat protein (Others).

The values of the means and standard deviations of the energy, carbohydrates, and sodium nutrients in each category are part of the research (Table 1). Likewise, there are the medians and interquartile ranges of fat and fiber nutrients. All values were taken from the nutrition fact on the label of each supplement. There was a standardization to 30 grams of bran.

Table 1: Distribution of macronutrients in the information available on the labels of supplements included in the study according to the class of each product.

Nutrient	Blend WP (n=14)	WPI + WPH (n=5)	WPI (n=4)	WPC (n=11)	Plant-based (n=11)	Others (n=6)	F (ANOVA)	P value
Energy (Kcal)	117.44 ± 3.88	111.40 ± 8.89	108.19 ± 5.43	115.49 ± 5.49	114.08 ± 8.70	105.62 ± 6.21	3.743	0.0064
Carbohydrate (g)	6.54 ± 5.07	2.90 ± 1.85	2.13 ± 1.13	8.58 ± 5.80	1.45 ± 1.51	7.26 ± 7.68	3.531	0.0089
Fat (g)	1.47 (0.71)	0.57 (0.84)	0.15 (0.30)	1.51 (0.45)	4.46 (6.55)	0.23 (0.37)	-	<0.0001
Fiber (g)	0.15 (0.30)	0.05 (0.12)	0.19 (0.39)	0.02 (0.07)	2.08 (1.24)	0.31 (0.72)	-	<0.0001
Sodium (mg)	86.34 ± 44.05	70.65 ± 22.35	80.03 ± 27.67	100.35 ± 65.62	250.03 ± 160.88	178.39 ± 122.84	5.218	0.0007

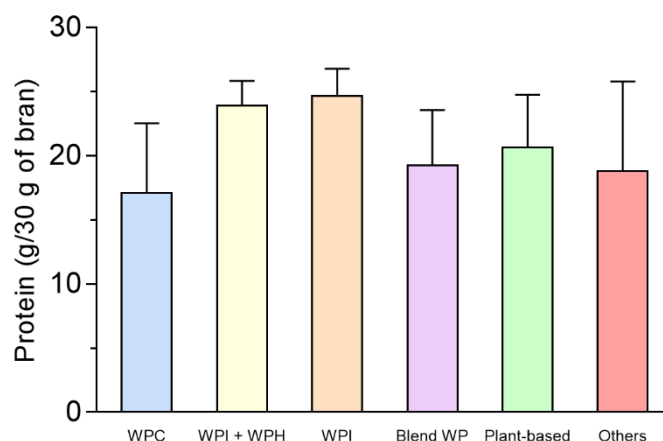
Data presented in mean ± SD or median (IQ). Blend WP: a mixture of two or more types of proteins of animal and/or plant-based; WPI + WPH: whey protein isolate and hydrolysate; WPI: whey protein isolate; WPC: whey protein concentrate; Others: proteins isolated from other sources (other animal proteins).

There are significant differences in energy, carbohydrate, fat and fiber content in protein supplements

Regarding the energy value, there was a significant difference between the Blend WP and the Others categories. Regarding the carbohydrate nutrient, there was a discrepancy between WPC and Vegetal and between Blend WP and Vegetal. As for the fat content, there was a disparity between the Plant-based category with WPI and the WPI + WPH. The Plant-based category is different from all others in terms of fiber content. And, regarding the sodium value, the Plant-based category differs from all the others, except for the Others category. All these cited differences have P value < 0.05.

Protein supplements have different protein content among them, but the nutritional label reflects the protein content from the analysis

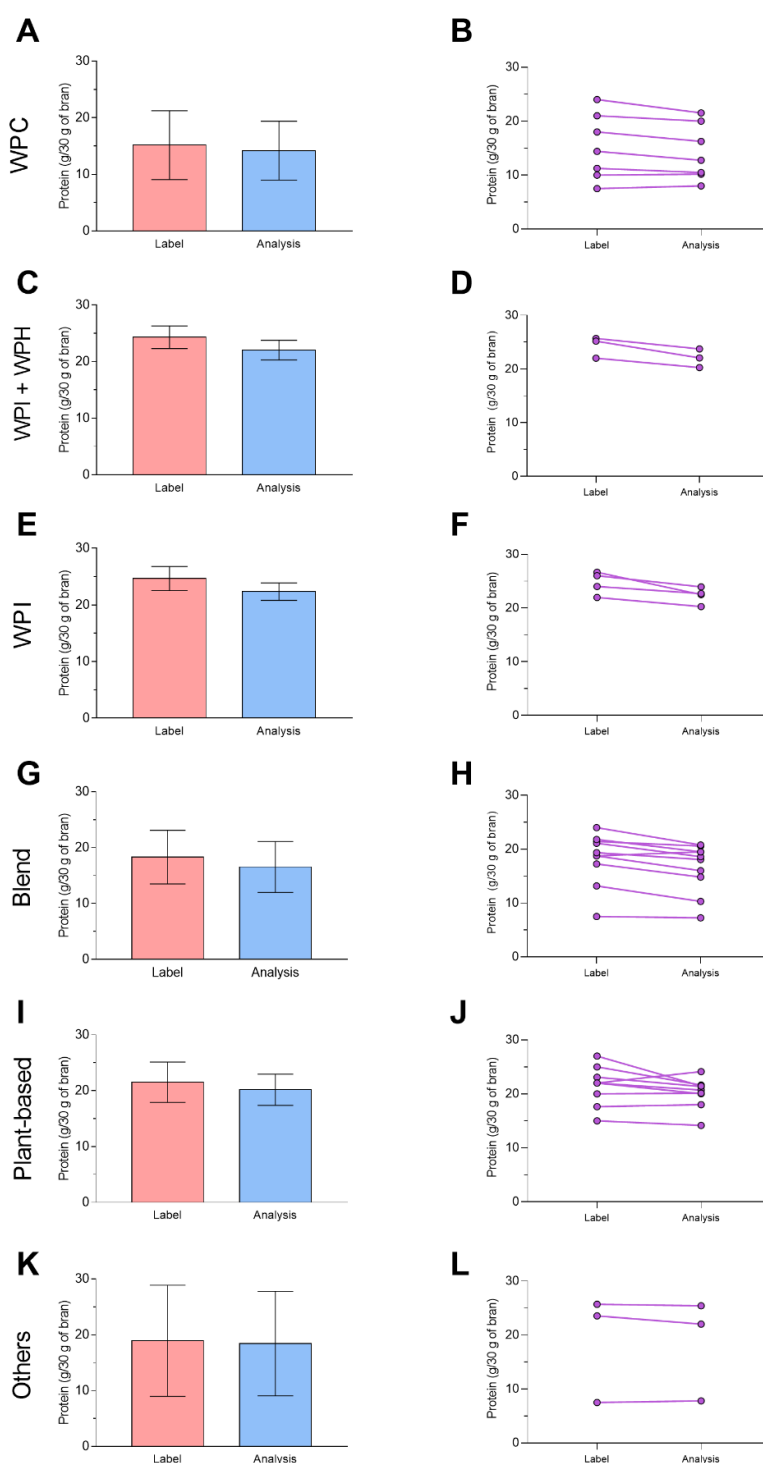
Figure 1 represents the comparison between the different categories of protein-based supplements to their declared protein contents in the nutritional table, standardized in 30 grams of bran. The analysis showed no difference in the sample: all products showed the same protein content for 30 grams of bran according to the between-label evaluation (P value > 0.05).



Data presented in mean \pm SD. Blend WP: a mixture of two or more types of proteins of animal and/or plant-based; WPI + WPH: whey protein isolate and hydrolysate; WPI: whey protein isolate; WPC: whey protein concentrate; Others: proteins isolated from other sources (other animal proteins).

Figure 1: Comparison of the protein content available on the label between different categories of protein supplements.

Figure 2 compares the protein values declared on the nutritional label with the analytical value obtained in duplicate, in 30 grams of bran, for each category. The analysis demonstrates that the values shown on the nutritional label are similar to what the bran contains in terms of protein (P value > 0.05) and that the vast majority are within the 20% tolerance limit, following the current law.



Data presented in mean \pm SD. Blend WP: a mixture of two or more types of proteins of animal and/or plant-based; WPI + WPH: whey protein isolate and hydrolysate; WPI: whey protein isolate; WPC: whey protein concentrate; Others: proteins isolated from other sources (other animal proteins).

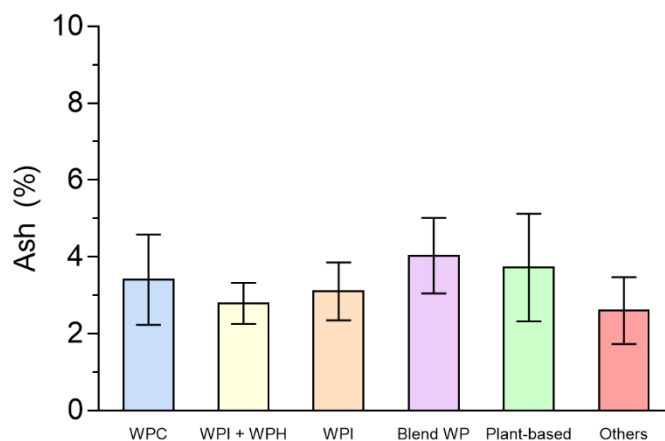
Figure 2: Comparison between the declared protein on the nutrition label with the bromatological analyzes in different protein supplements. The left panels represent label variations compared to the bromatological

analysis of all products within the dietary supplement class. The right panels represent label variations compared to the bromatological analyzes for each product within the same dietary supplement class.

Among the protein supplements analyzed, only two had protein content outside the limit tolerated by legislation, one of plant origin and the other of animal origin (a mixture of three types of whey protein). The results showed that ten supplements had their protein content varying between a rate of 10 to 19.99% difference from what was once analyzed and declared. There were 24 supplements between 0.01 and 9.99%. Also, seven supplements out of these 24 had the analytical protein content above the labeling.

No difference in the inorganic matter and total mineral levels among different protein supplements

The inorganic matter and total mineral levels of the analyzed supplements were represented through the ash content (Figure 3). In relation to dairy products, a value between 0.7 and 6.0% of ash is expected, characterizing adequate nutrition and possible adulterations when above the stipulated values. In the present study, all products were within this range. There was no significant difference among them (P value > 0.05).

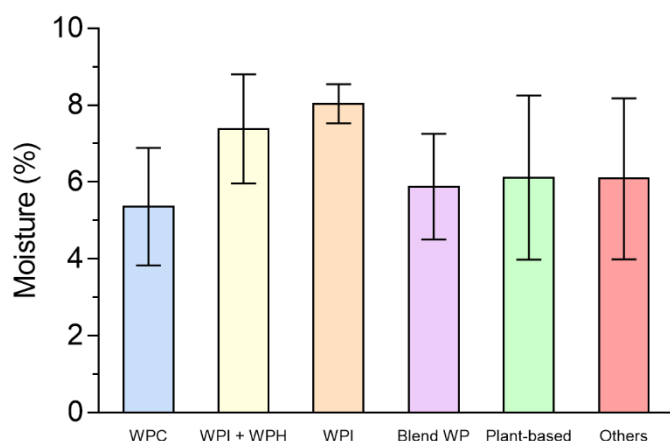


Data presented in mean \pm SD. Blend WP: a mixture of two or more types of proteins of animal and/or plant-based; WPI + WPH: whey protein isolate and hydrolysate; WPI: whey protein isolate; WPC: whey protein concentrate; Others: proteins isolated from other sources (other animal proteins).

Figure 3: Comparison of ash content between different categories of protein supplements.

No difference of moisture and water content among different protein supplements

The moisture and water content of the analyzed supplements (Figure 4) shows no difference between the supplement categories (P value > 0.05). Moisture values, within limits, bring quality to the products, taking into account stability, storage, packaging, and processing. Comparison-wise, dairy products must contain up to 4.0% moisture. According to the analyzes carried out, only three products reached this value. However, all supplements in question had moisture values below 10.0%.



Data presented in mean \pm SD. Blend WP: a mixture of two or more types of proteins of animal and/or plant-based; WPI + WPH: whey protein isolate and hydrolysate; WPI: whey protein isolate; WPC: whey protein concentrate; Others: proteins isolated from other sources (other animal proteins).

Figure 4: Comparison of moisture content between different categories of protein supplements.

DISCUSSION

The present study shares the behaviors of different protein-based supplements concerning the actual protein composition and its quality through ash and moisture contents. With the increasing demand for food supplements, mainly protein supplements, the need to have greater control over the quality of these products becomes evident. Adulterations and nutrient contents outside the values accepted by legislation have been part of this class of products for years. According to a study, there were significant differences in the caffeine content shown on the label in relation to that analyzed in the laboratory ¹¹.

Also, while working on microbiological contamination, such supplements showed poor hygienic quality when found the presence of secondary pathogens, molds, and yeasts in dietary supplements ¹². With this, the distortion of data declared on the nutritional label

does not respect the consumer's rights. Along with this issue, the indiscriminate consumption of supplements associated with unreliable information can damage the consumers' health ¹¹.

Differences between bran and the nutritional label have been noticed for a long time and still happen today, as shown by a study that quantified the protein content in supplements based on whey protein isolate. Half of the samples had protein values lower than the nutritional label declared ¹³. Another study concluded that all experimental values regarding protein percentages in a whey protein sample were lower than those indicated on the nutritional labels ¹⁴.

Meanwhile, fortunately, the current investigation denotes that surveillance of companies in the protein supplement industry is more efficient. The results shown are positive in terms of compliance with legislation for the vast majority of products analyzed. This analysis aimed to categorize the supplements among types of proteins instead of generalizing them, making it possible to understand diverse options available on the market. This diversity is perceived in relation to the nutritional information described in the tables and to the actual protein values and other product characteristics, such as moisture and ash.

The variability of the data of some nutrients present on the nutrition facts of whey protein supplements is limpid, as evidenced by a study in which 90.7% of the evaluated brands were not within the legislation standard ¹⁵. Yet, regarding the comparison between the data expressed in the nutritional table of the study in question, there was no statistical difference between categories. In conclusion, the amount of protein does not have to be a determining factor when choosing the type of protein used. The fact that they are similar is a good feature, considering a consumer with the power to choose. As clients highly consume whey protein supplements, and as the nutritional table is the dominant source of information about the product's characteristics, the regulation of labeling is urgent to reduce risks to consumers' health ⁸.

The protein content in whey protein supplements found in this study varies according to the type of processing. The whey protein concentrate (WPC) contains between 29 and 89% protein. The whey protein isolate (WPI), produced by filtering milk proteins, consists of at least 90% protein. Lastly, the whey protein hydrolysate (WPH) must have at least 95% protein in its composition ¹⁶. The process of WPH happens

through the enzymatic hydrolysis of whey protein, resulting in a high content of peptides and free amino acids. In sum, the expectation was to find percentages similar to these when analyzing the different categories of protein supplements.

The authors considered its protein content to analyze the different categories of whey protein (Blend WP, WPI + WPH, WPI, and WPC). The one that stood out negatively was the whey protein concentrate, for having the lowest percentage, but within the range between 29 and 89 %. The WPI + WPH and WPI categories, which should be purer, also showed unsatisfactory results. None reached the expected parameter of at least 90 % protein in their composition, even though they continue to be the samples with the highest amount of protein per portion (30.0 grams). The class containing different types of plant-based proteins had its protein content below the WPI + WPH and WPI classes but above the BLEND WP and WPC classes.

Protein supplements evidently must have protein in their composition. It is a simple assumption among people who purchase or prescribe it. All categories showed at least 50% protein in their composition, except for WPC. However, the only groups that showed at least 20.0 grams of protein in a 30.0-gram serving of bran were WPI + WPH, WPI, and plant-based. Vegetarian individuals have infrequent options, but vegetarian protein supplements seem to follow the legislation. On the other hand, omnivorous individuals have a wide variety of protein supplements. Supplements based on WPI or a mixture of whey protein isolate and hydrolyzate seem to be the best option.

Regarding the inorganic matter, the ash content found ¹⁴ showed considerable levels of total minerals, ranging from 2.29 to 3.75 %. Like the study in question, the values were within the appropriate range (Figure 3), showing more security to the population that consumes this class of supplement. All foods, whichever the industrialization method they have been subjected to, contain water. Such constataion represents the moisture in the food, to a greater or lesser extent ⁹. Consequently, determining the moisture content of protein supplements is vital since water can be a culture medium for microorganisms, causing undesirable changes in food, in this case, leading to loss of quality. In general, the greater the water activity, the greater the perishability of the food. As for moisture (Figure 4), dietary supplements with low content have lower water activity and are less prone to decomposition ¹⁴. Such an aspect is vital

to food preservation. In this study, the supplements proved suitable for their packaging and storage.

The present study has some limitations regarding the sample size. Several new brands are entering the dietary supplements market, and there has been an increase in the variety of the type of protein or protein blends used by companies. In addition, the number of products analyzed by category was not proportional. The results of this work are of paramount importance for the public that consumes and prescribes protein supplements. Other types of supplements are not in the corpus of research. For future investigations, an aminogram analysis to compare what is available on the supplement label with what is present in the bran could interest fellow researchers.

CONCLUSION

We conclude that the vast majority of national and international protein supplement brands sold in Brazil comply with the legislation regarding protein content, as well as inorganic matter and moisture. However, the tolerance limit for these values is significantly high, so the products do not have the quality they should be offered and do bring harm to the consumer who consumes these protein supplements and to the health professionals who prescribe them. Regarding this research, we believe that surveillance in health, especially in protein supplements, is a continuous effort to offer the truthful concentration of alleged macronutrients in the dietary supplement nutritional facts. That is, in the future we believe that another study could be conducted to evaluate the overall quality and protein content of dietary supplements in Brazil and in other countries if this scenario continues to be the observed.

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Received: 19/5/2023

Accepted: 15/12/2023

Published: 21/02/2024

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All authors have approved the final version of the text.

Conflict of interest: There are no conflicts of interest.

Financing: Does not have financing.

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Editor: Dr. Matias Nunes Frizzo

Editor-in-chief: Dra. Adriane Cristina Bernat Kolankiewicz

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