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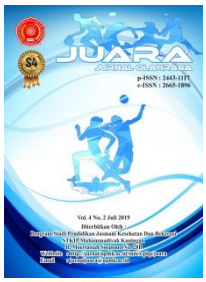

Analysis of Protein Content on Commercial Protein Supplement in Indonesia

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Abstract

Commercial Protein Supplements circulating freely nowadays are consumed not only by athletes but also by the wider community, especially sports activists who rely upon it to improve performance or merely for aesthetic interests. This study analyzes the nutritional content of the five supplements circulating freely in Indonesia to compare them with the claims on the packaging label. We examined five samples of popular supplements in Indonesia obtained online and then tested them in three copies for each sample. The actual total protein in the supplement sample is measured using the Kjeldahl method, where the appropriate nitrogen material is converted to a percentage of protein by multiplying a factor of 6.38 in duplicate. Then the research data processed and analyzed using Microsoft Excel 2016 computer programs, then by Statistical Program for Social Sciences (SPSS), consecutively. Relationships between different tests were evaluated by calculating the relevant correlation coefficients (Pearson linear correlation) ($p < 0.05$). Analysis of variance (ANOVA) was used to compare the differences between products with different manufacturing processes ($p < 0.05$). Based on the analysis results, only two of the five samples studied have a minimum of 80% compatibility with the claim on the packaging label. Several main factors including inaccurate analysis, preparation processing on products that triggers protein denaturation, inadequate storage, and regulations that have just been set so that producers are still free to write Nutrition Value Information with a relatively low level of accuracy. This research is expected to help the evaluation for producers and the government (BPOM) to provide correct information to consumers so that maximum physiological benefits can be obtained.

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INTRODUCTION

Nutritional supplements, especially protein supplements, play a role in improving sports performance and aesthetic reasons. The consumption of protein itself affects the nutritional status (Rachmayani *et al.* 2018). The consumption of dietary supplements is carried out by athletes at both the amateur and professional levels (Knapik *et al.* 2016) and has grown in popularity in recent years (Maughan 2013). Apart from athletes, nutritional supplements are also in demand by regularly exercising to get the ideal body composition or other aesthetic reasons. Nutritional supplements consumed by athletes must meet specific dietary requirements to optimize daily training performance or during the competition (Close *et al.* 2016) states that dietary supplements must be able to overcome nutritional deficiencies and can directly improve sports performance or maintain and restore health and immune function (Kuswari et al. 2019).

Whey Protein (WP) is a supplement with high nutritional quality, is quickly absorbed, and is a good source of essential amino acids, especially branched-chain amino acids (BCAAs) such as leucine isoleucine and valine (Almeida *et al.* 2015). In recent years, the consumption of whey protein (WP) supplements has been recommended to stimulate muscle protein synthesis in physically active individuals, especially individuals who do resistance training, such as those who exercise in the fitness center or athletes (Phillips 2012). Besides, scientific research has proven a relationship between physical activity and consumption of whey protein which can stimulate increased use of protein from supplements in the body (Børsheim *et al.*, 2002).

Another type of protein supplement is Protein Powder Supplement (PPS), namely whey protein in powder form, one of the most frequently consumed nutritional supplements, both by professional and amateur athletes and people who exercise for non-sports purposes (Sánchez-Oliver *et al.* 2011). Most PPS contain whey protein; however, some PPS contain different plant protein, milk, and/or meat hydrolysates. Considering the high demand from fitness enthusiasts (sportsmen) as consumers of WP, these products are expected to contain a minimum amount of nutrients of high biological value to optimize their physiological benefits.

Research related to evaluating total protein content (TP) in various WP supplements manufactured by companies in the United States and Brazil has been conducted before. The study compared the TP content, and protein value of each WP supplement informed on the product label. The results showed that supplements had nutritional content that did not match the claims on the packaging label. Protein supplements are generally evaluated in terms of their net protein content, regardless of reducing amino acids during heating.

Not only that, with the increasing number and diversity of consumers, protein

supplements are now starting to get more attention, especially in terms of the tangible benefits and results that consumers feel after consuming them. Law No.8 of 1999 on consumer protection in Article 4 states that consumers have the right to obtain correct, transparent, and honest information regarding the condition and guarantee of goods/services (Supreme Audit Agency 1999). The inaccuracy of nutritional label claims with what is contained in these products is directly a form of violation of the Law and is very detrimental to consumers. This condition will impact the level of consumer confidence in the development and the level of product purchasing. Based on this description, it is necessary to conduct research related to the analysis of nutritional content in supplements used by the public and athletes and compare them with claims on packaging labels, especially accessories circulating in Indonesia today.

METHODS

Design, Location, and Time

This study used a cross-sectional study design and was a quantitative analytical study that studied the differences between the protein content of protein supplement products circulating in Indonesia. The research was conducted in Jakarta from December 2019 until January 2020, and the total protein analysis was carried out at the Food Testing Laboratory accredited by PT. Saraswati Indo Genetech, located in Bogor, West Java.

Materials and Tools

Materials used were sulfuric acid, selenium mix as a catalyzer, distilled water, sodium thiosulfate, methylene blue, methyl red coloring, and standardized chloric acid. Tools used were 20-mesh screener, magnetic stirrer, Kjeldahl flask, distillation flask, burette, measuring flask, Erlenmeyer flask, and titration toolset.

Procedures

Content analysis is carried out to invest in the information on the product label. Based on the method used in several previous studies (García *et al.* 2013; Koo *et al.* 2018), data collection on selected protein supplement products was done on products sold freely in the online market. We identified five products in circulation based on the category of "protein supplement" or "whey protein," and three copies of each brand were acquired in December 2019. All nutritional value information listed on the product is then stored for further analysis. Samples vary from powder to liquid form.

Next, the five samples with three copies went through the total protein test using the Kjeldahl method accredited in duplicate. The test process is carried out in several stages, starting from (1) the sample preparation stage: all materials must pass through a 20-mesh screen to ensure the sample is homogeneous. (2) Decomposition whereby the sample, after being weighed, is put into a Kjeldahl flask with acid and catalyst then added to decompose until the non-volatile ammonium sulfate is formed. During this process, protein

nitrogen is liberated from ammonium ions, while carbon and hydrogen as trace elements are converted to carbon dioxide and water. (3) Neutralization and distillation in which the decomposed product is diluted with water and added with sodium thiosulfate to form ammonia which, when sterilized, will produce boric acid (containing indicators of methylene blue and methyl red, AOAC Method 991.20). (4) Titration in which the borate anion (several nitrogens) will be titrated with standardized HCl to then be calculated into the percentage of protein by multiplying a factor of 6.38 (Association of Official Analytical Chemists 1990; Nielsen 2017)

Data Analysis

The data obtained were processed and analyzed using Ms. Excel 2016 and the Statistical Program Social Sciences (SPSS) consecutively. The relationship between the different tests was evaluated by calculating the relevant correlation coefficient (Pearson linear correlation) ($p < 0.05$). Analysis of variance (ANOVA) was used to compare the differences between products with different manufacturing processes ($p < 0.05$). The results were expressed as mean ± standard deviation (SD).

FINDINGS AND DISCUSSION

Findings

Based on the five Protein Supplement samples we studied, they contained an average of ±26.2 g of protein in each serving and/or ±55g / 100g of product. To facilitate reading the data, the amount of protein/serving of the sample are presented in table 1.

Table 1. The amount of Total Protein in samples label.

Sample	Serving Size	Protein / Serving
Sample 1	38 g	20 g
Sample 2	33.50 g	25 g
Sample 3	35 g	25 g
Sample 4	38 g	25 g
Sample 5	395 ml	32 g

Based on the total protein test results of the five protein supplement samples we studied, with *Simplo and Duplo* measurements of all samples with three copies each, samples containing a mean of ±36 g of protein in 100g of the sample is presented in Table 2.

Table 2. Results of Total Protein from each sample

Sample	Code	Protein Test Results
Sample 1	A	32.18 g
	B	32.27 g

Sample	Code	Protein Test Results
	C	31.16 g
	A	73.08 g
Sample 2	B	73.98 g
	C	74.06 g
	A	66.34 g
Sample 3	B	65.09 g
	C	65.66 g
	A	18.06 g
Sample 4	B	17.32 g
	C	17.70 g
	A	4.46 g
Sample 5	B	4.58 g
	C	4.12 g

Based on these results, we can compare the average test results with the claims stated on the label. It is known that two of the five samples tested had conformity of £60% TP of the test results with the claims stated on the product label as presented in table 3.

Table 3. Comparison of Total Protein On Label with Laboratory Tests / serving size samples

Sample	Protein Content on Label	Test Results Mean Protein Ingredients / Serving Size	Percentage of Compliance to Total Protein as Stated
Sample 1	20 g	12.11 g	61 %
Sample 2	25 g	24.69 g	99 %
Sample 3	25 g	22.99 g	92 %
Sample 4	25 g	6.72 g	27 %
Sample 5	32 g	17.33 g	54 %

Discussion

Protein intake is essential for exercising (Kuswari et al., 2021), while protein supplementation helps athletes during their performance (Kuswari, 2017). Regarding Nutritional Value Information (ING) writing on products widely circulating in Indonesia, it is still not a primary public concern. Even the previous regulations held by the Food and Drug Supervisory Agency (BPOM) of the Republic of Indonesia stated that the inclusion of ING was still voluntary. Even so, the Government, in this case, BPOM, has issued the latest regulations regarding the writing and inclusion of ING on processed food labels in PBPOM No. 22 of 2019. This newest

regulation requires the inclusion of ING in processed food products for all producers who are not a part of micro-businesses (having an annual sales result of at most IDR 300,000,000) by attaching the nutritional analysis results obtained from government laboratories and/or laboratories that are otherwise accredited with the provisions of at least 80% of the value listed in the ING Table (Indonesian Food and Drug Supervisory Agency 2019). This regulation was enforced as of August 22, 2019, and still tolerates all products with a distribution permit from BPOM a maximum of 30 months after this regulation comes into effect. This could be the main reason there are still 3 out of 5 Protein Supplement products widely circulating that do not have an accuracy of >80% of what is written on the product label (Table 3).

When viewed from the consumer's point of view, the inconsistency of TP on label claims and test results on three of the five samples tested would undoubtedly be very detrimental to consumers who have chosen to take protein supplements to get the maximum physiological benefits. The percentage of suitability in some samples is relatively low, ranging from 26% to 60% only. Of course, the supplementation function is not optimal. It will even harm consumers who already have specific target fulfillment targets because, basically, nutritional supplements must overcome dietary deficiencies and can directly improve sports performance or maintain and restore health and immune function (Close 2016; Mountjoy *et al.* 2018). In this case, not only was there a violation of Law No.8 of 1999 concerning Consumer Protection, but a more significant domino effect could occur and would be detrimental to both parties (consumers and producers) (Supreme Audit Agency 1999). Research (Chung *et al.*, 2012) states that consumer distrust of producers plays an essential role in shaping consumer attitudes and purchase intentions, defeating consumer knowledge and the health value of these products. The doubt of consumers that arises either because they do not feel improvement in themselves or just hearing from others will significantly affect their attitudes and purchase intentions of products being marketed even though they already know the health benefits of these products. ING needs to be corrected by producers to uphold consumer rights and pay more attention to writing claims on labels because maintaining consumer confidence is the key to successful sales of a product.

On the other hand, the difference in the TP content in the laboratory test results with the claims on the protein supplement label is more than the threshold can be caused by various factors, one of which is the method of calculating the total protein by only counting the presence of nitrogen and subtracting the amount of all amino acids (Schönfeldt *et al.* 2019). Nitrogen in this regard does not come solely from amino acids in the protein; purines, pyrimidines, vitamins, creatine sugar, creatinine, and free amino acids can contribute to the total amount of nitrogen present in a product (Pellett & Young 1980). Not only that,

the compounds (2S, 4R) -4- hydroxyproline and L-hydroxyproline (C5H9O3N), which are hydrolyzed from gelatin (the main component of collagen), also function as other nitrogen fillers obtained from the skin of pigs, cows, or their bones (Gomez-Guillen *et al.* 2011). Even other non-food sources of nitrogen, such as melamine, can also contribute to total nitrogen in processed foods and were found in 78% of protein supplement products in South Africa, although they are still within safe limits. Melamine, a multi-amine molecule, can be used as a non-protein nitrogen material that incorrectly increases the amount of protein content in a product by simply expanding the presence of nitrogen without contributing to the amino acids in the product (Gabriels *et al.* 2015).

The next factor that could cause the difference in TP is that most PPS contain whey protein, but some PPS contain different vegetable proteins, milk, and/or meat hydrolyzates. PPS processing can go through chemical or enzymatic processes to achieve a stage of isolation and concentration, either by heat treatment (evaporation) or ultrafiltration. The final stage of the process is drying by heat application through rollers or atomization. These steps can result in partial denaturation of the protein. Bernard *et al.* (2011) stated that the manufacture of whey protein powder using the Spray Drying method at temperatures of 170 ° C and 260 ° C resulted in protein denaturation values of 30% and 47%. This denaturation can cause unwanted reactions and affect the nature of the nutrients in the product (Rufián-Henares *et al.*, 2004).

The manufacturing process but the storage of protein supplements also affects the levels of nutrients in them. If a product is stored for an extended period, it can damage the nutritional properties of the supplement. Le *et al.* (2011) analyzed milk and whey protein concentrates stored for one year at various temperatures (25 - 40° C) and relative humidity (44%, 66%, 85%). One of the most significant changes caused by heating, drying, and storage is the Maillard reaction (MR). The Maillard reaction is a non-enzymatic browning reaction in food between reducing sugars and amino acids, peptides, or proteins. MR produces various complex components called Maillard reaction products (MRP) (Van Boekel 1998). However, when protein supplements are made in milk, lactose will block the lysine amino group to form lactulosyllysine, which can change the bioavailability of protein, and chelating can affect the value and quality of protein (Tamanna & Mahmood 2015).

CONCLUSION

Nutritional supplements, especially protein supplements, play a role in improving sports performance and aesthetic reasons. The consumption of dietary supplements is carried out not only by athletes at both amateur and professional levels but also for ordinary sports activists and has continued to grow in popularity in recent years. Several previous studies in the United States and Brazil showed

inconsistent levels of accuracy between the total protein count compared to the nutritional value information on the packaging. This level of accuracy is essential to ensure consumers get the maximum physiological benefits and manufacturers who can have the trust of consumers in these products. However, this problem occurs in Indonesia because several previous studies have had more or less the same results, where three out of five protein supplements in Indonesia have a total protein value <80% of what is stated in the Nutritional Value Information. This can be caused by various things, ranging from newly enforced regulations, manufacturing processes that trigger protein denaturation, inaccurate protein measurement methods, and poor product storage. The current study will continue with new rules the government has implemented to help evaluate freely available products on the market. Research with a more varied and actual sample of supplement products currently popular in the community with a series of nutritional claims is needed to evaluate and educate the public on the quality and accuracy of shares on the product label.

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