

Quality of creatine supplements: a study based on content and labeling analysis

Qualidade dos suplementos de creatina: um estudo baseado na análise do teor e da rotulagem

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ABSTRACT

Introduction: Increased consumption of supplements aiming at improving performance in training and gaining muscle mass contributed to the rise of sports supplements, with an emphasis on e-commerce, where the practicality of the purchase and sale of these products is preponderant. **Objective:** Aiming to contribute to the health of consumers, the objective of this work was to evaluate the quality of creatine supplements sold online through content and labeling analysis. **Method:** Six creatine samples from brands sold on e-commerce were compared with a reference sample purchased at a pharmacy manipulation. The Kjeldahl method was used to measure creatine, and a checklist based on RDC No. 243/2018 for the analysis of the labels. **Results:** After analyzing the samples, two failed in relation to creatine content, which was below the 20% limit established by the RDC No. 429/2020. In the labeling research, it was observed that the majority of samples (83%) were in disagreement with legislation current, and only sample C's label met all the criteria evaluated. **Conclusions:** Considering the results obtained, it is evident that there is a need for oversight specifically directed at supplements given that the discrepancy between the amounts found raises concerns regarding the quality and safety of the products being marketed. It is important to highlight that scientific literature on this topic remains insufficient, even in the face of the significant growth of this market, necessitating research in the area of quality control of sports supplements to verify compliance with quality and safety requirements.

KEYWORDS: Quality Control; Nutritional Supplement; Creatine

RESUMO

Introdução: O aumento do consumo de suplementos visando melhor performance nos treinos e ganho de massa muscular contribuiu para a ascensão do mercado de suplementos esportivos, com destaque para o *e-commerce*, em que a praticidade de compra e venda desses produtos são preponderantes. **Objetivo:** Avaliar a qualidade dos suplementos de creatina, comercializadas *online*, por meio da análise de teor e de rotulagem, almejando contribuir com a saúde dos consumidores. **Método:** Foram analisadas seis amostras de creatina de marcas vendidas no *e-commerce* e comparadas com uma amostra de referência adquirida em farmácia magistral. Para o doseamento de creatina foi utilizado o método Kjeldahl e, para a análise dos rótulos, um *checklist* baseado na RDC nº 243/2018. **Resultados:** Após a análise das amostras, duas foram reprovadas em relação ao teor de creatina, que se encontrava abaixo do limite de 20%, estabelecido pela RDC nº 429/2020. Na pesquisa de rotulagem, foi observado que a maioria das amostras (83%) estava em desacordo com a legislação vigente e apenas o rótulo da amostra C atendia a todos os critérios avaliados. **Conclusões:** Considerando os resultados obtidos, é evidente a necessidade de fiscalização específica direcionada aos suplementos, haja vista que a discrepância entre os teores encontrados levanta preocupações quanto à qualidade e autenticidade dos produtos comercializados. É importante salientar que a literatura científica ainda é insuficiente sobre o tema, mesmo diante do crescimento expressivo desse mercado, necessitando de pesquisas na área de controle de qualidade de suplementos esportivos para constatar se cumprem os requisitos de qualidade e segurança.

PALAVRAS-CHAVE: Controle de Qualidade; Suplementos Nutricionais; Creatina



INTRODUCTION

Food supplements are non-drug bioactive substances, enzymes, and probiotics intended for healthy people. The insertion of food supplements is carried out with the aim of supplementing the diet nutritionally and is not indicated for the treatment or prevention of diseases¹.

There has been a notable increase in the consumption of supplements over the years, especially sports supplements aimed at gaining lean mass. This market has become increasingly lucrative and is also popular in e-commerce due to its practicality². In this context, one of the most popular supplements among athletes and physical activity practitioners is creatine. Its growing demand is largely because it is a substance that has been widely studied and proven to be effective in improving physical performance³.

α -Methyl guanidino acetic acid, known as creatine, was discovered by the French physiologist Michael Chevreul around 200 years ago; subsequently, other researchers of the time began and improved studies on this substance over time. Thus, in 1992, during the Barcelona Olympics, the use of creatine began to become popular as an alternative for improving training performance⁴.

Creatine as a nutritional supplement improves training performance and helps to achieve better results in gaining muscle mass⁵. However, the safe and effective use of supplements such as creatine depends on regulations that ensure both the quality of the products, and the clarity of the information provided to consumers.

In this regard, Normative Instruction (IN) No. 28 of July 26, 2018, published by the Brazilian National Health Surveillance Agency (Anvisa), establishes specific criteria for food supplements. This standard defines the list of constituents, limits of use, permitted claims, and labeling requirements for these products. In the case of creatine, the claim approved for labeling is that "creatine helps to increase physical performance during repeated short-term, high-intensity exercise", provided that the minimum dose of 3,000 mg is respected, as described in Annex III of the aforementioned IN⁶.

In addition, in July 2018, a new regulatory framework for food supplements was implemented through RDC No. 243, of July 26, 2018, which establishes the health requirements for this category of products. However, the lack of adequate supervision compromises compliance with the rules. This regulatory framework covers all categories of food supplements, from those intended for infants to vitamin and mineral supplements, so it does not ensure that products aimed at athletes are fully compliant with the resolutions imposed^{7,8}.

With this in mind, the aim of this study was to assess the quality of creatine supplements sold through e-commerce, checking whether the creatine content in the formulation corresponds to that declared by the manufacturers on the packaging label. In addition, the product labels were analyzed for

compliance with current legislation. From this assessment, possible fraud and irregularities in the marketing of these supplements can be identified, thus contributing to the protection of consumer health.

METHOD

Six samples of creatine from different brands sold on e-commerce were analyzed and compared with a reference sample (R) of Chinese origin, batch 220524-C11, with a content of 98% to 102%, characterized as a white crystalline powder, not very soluble in water, as specified in the technical report, purchased from a magistral pharmacy.

The samples were initially subjected to visual inspection to assess macroscopic aspects, using a stereoscopic magnifying glass (Tecnival) at 400x magnification. Moisture was also analyzed using the gravimetric method at 105 °C, as described in the Brazilian Pharmacopoeia.

The creatine content was determined using the Kjeldahl method, the official method of the Association of Official Analytical Chemists for determining the total nitrogen content of foods⁹. The Kjeldahl method is a widely accepted analytical test in the food industry. Its principle consists of the indirect quantification of the food's total protein, based on the direct measurement of the nitrogen present in the sample and subsequent multiplication by a conversion factor¹⁰. The method basically involves three stages: digestion, distillation, and titration. Initially, the sample is catalytically digested in sulfuric acid, with the quantitative conversion of the nitrogen present in the organic matrix to ammonium sulphate; the digest obtained is then alkalized with sodium hydroxide and the ammonia formed is distilled off by vapor drag; the ammonia is then collected in boric acid, forming ammonium borate, which in turn is titrated with hydrochloric acid of known concentration¹¹.

The balanced chemical reactions of the three steps of the method can be seen below:

1. Digestion: $\text{Sample} + \frac{\text{H}_2\text{SO}_4}{\Delta} \rightarrow (\text{NH}_4)_2\text{SO}_4$
2. Distillation: $(\text{NH}_4)_2\text{SO}_4 + 2\text{NaOH} \rightarrow \text{Na}_2\text{SO}_4 + 2\text{NH}_3 + 2\text{H}_2\text{O}$
 $\text{NH}_3 + \text{H}_3\text{BO}_3 \rightarrow \text{NH}_4\text{H}_2\text{BO}_3$
3. Titration: $\text{NH}_4\text{H}_2\text{BO}_3 + \text{HCl} \rightarrow \text{H}_3\text{BO}_3 + \text{NH}_4\text{Cl}$

Creatine was measured as follows: initially, about 0.2 g of each sample was weighed on a semi-analytical balance (Radwag, model PS 360/C/1), placed in a digester tube containing 1.0 g of a catalytic mixture composed of potassium sulphate and copper sulphate in a ratio of 95:5 ratio, plus 5 mL of AP sulfuric acid; these were digested under heating in a digester block (Marconi, model MA 4025) until 350 °C was reached and the organic matter was completely destroyed (the solution was clear and



bluish-green). After cooling the samples, the Kjeldahl tubes containing the sample were coupled to a nitrogen distiller (Solab, model SL - 47), with around 20 mL of 40% sodium hydroxide added. The condensed solution was poured into an Erlenmeyer flask containing 10 mL of 2% boric acid solution and a mixed indicator (methyl red and bromocresol green). The solution obtained (75 mL) was titrated with standardized 0.1N hydrochloric acid (HCl) until the color changed from green to pink.

To express the protein value of various food matrices, it is necessary to convert the nitrogen (N) content using a factor based on the percentage of nitrogen present in most proteins, which is generally 16%; in this case, the factor obtained by dividing 100 by the percentage present in the protein (100/16) is 6.25. The variation in the percentage of nitrogen in food allows other factors to be adapted, as is the case with creatine. The creatine content was calculated according to the variables of volume of HCl used in the titration, sample weight, HCl factor and conversion factor (3.12) based on the percentage of nitrogen in creatine (32.02%). The factor was obtained by dividing 100 by the percentage of nitrogen present in creatine (100/32.02)¹². Using the equations below, it was possible to obtain the percentage of total nitrogen¹³ and creatine, respectively, of the samples being analyzed.

$$\% \text{ Total nitrogen} = \frac{(V_a - V_b) \cdot N \cdot F_c \cdot 0,014 \cdot 100}{M}$$

$$\% \text{ Creatine} = \% \text{ Total nitrogen} \cdot F (3,12)$$

In which:

% Total nitrogen = Percentage of total nitrogen in the sample;

V_a = Volume of HCl used to titrate the sample;

V_b = Volume of HCl used to titrate the blank;

N = HCl normality;

F_c = HCl correction factor;

M = Sample mass;

F = Conversion factor.

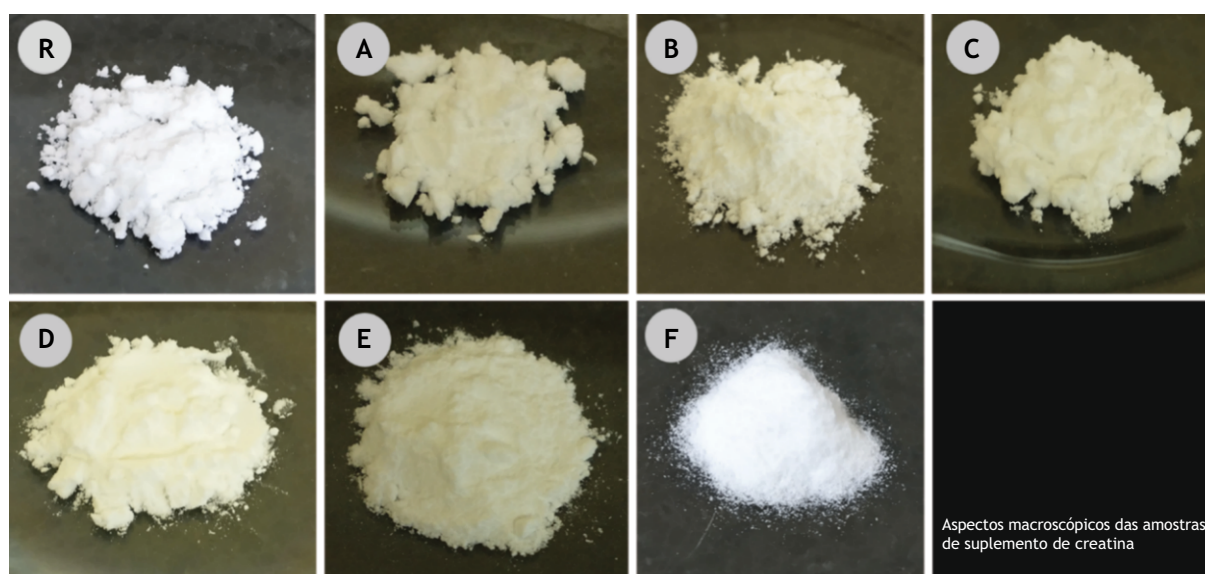
All samples were analyzed in triplicate. The analyses were carried out in the Bromatology Laboratory at the Center for Education and Health at the Federal University of Campina Grande.

To verify the presence of carbohydrates in the formula, a qualitative analysis was carried out using a suspension containing water and creatine, to which a drop of lugol, equivalent to 50 µL, was added.

To analyze the creatine labels, a structured form proposed by Arevalo and Sanches¹⁴ was used, following RDC No. 243/2018, which sets out the health requirements for supplements. The labels were also analyzed in terms of the requirements established by IN No. 28/2018⁶.

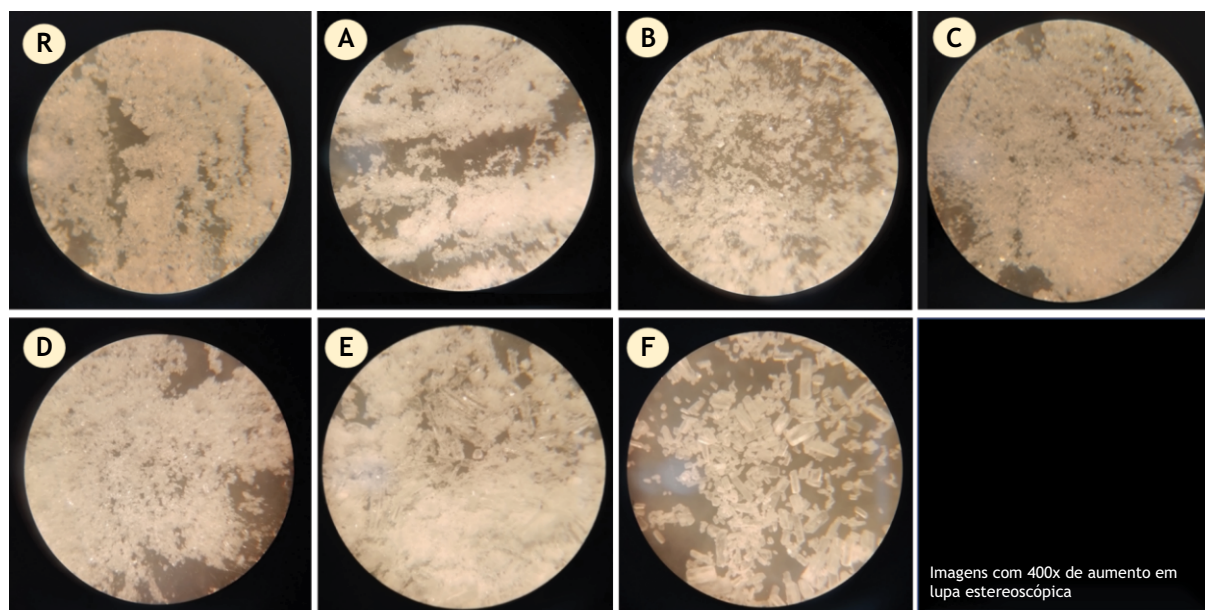
RESULTS AND DISCUSSION

When evaluating the macroscopic aspects of the creatine samples investigated, it was observed that samples A, B, C, E, and F had the same characteristics as the reference sample (R) - white, crystalline powder. However, as seen in Figure 1, sample D did not have the same crystalline appearance as the others. Regarding the common hygroscopic characteristic of creatine, sample F was the only one that apparently did not form lumps, which may be associated with the crystalline morphology observed in Figure 2F.



Source: Prepared by the authors, 2023.

Figure 1. Visual aspect of the creatine samples used in the research.



Source: Prepared by the authors, 2023.

Figure 2. 400x magnification stereoscopic images of creatine samples.

When evaluating the samples using a stereoscopic magnifying glass (Tecnival) at 400x magnification, small crystals could be seen in samples (A, B, C, D) and the reference; sample E appeared as a mixture of larger rectangular crystals and small crystals similar to the other samples. However, the crystals in sample F had shapes that differed from the others, showing rectangular shapes of various sizes. In turn, these shapes may be related to their greater stability in terms of absorbing moisture from the environment when compared to the other creatine samples (Figure 2).

Table 1 shows the percentage moisture values of the samples analyzed, which ranged from 6.24 ± 0.18 (E) to 12.70 (P).

The occurrence of different crystalline forms in a solid can modify various physicochemical properties such as: melting point, solubility, physical and chemical stability, and thermal behavior. These characteristics can affect the bioavailability, hygroscopicity, stability, and consequently the efficacy of the product¹⁵.

Table 1. Moisture content of creatine samples (n = 3).

Sample	Humidity (% $\pm$ SD)
R	12.70 ± 0.01
A	12.18 ± 0.05
B	10.14 ± 0.14
C	12.30 ± 0.09
D	10.40 ± 0.10
E	6.24 ± 0.18
F	12.16 ± 0.02

Source: Research data, 2023.

Although it was not possible to establish a correlation between the moisture content and the morphology of the crystals in the samples, it should be noted that products with high water contents act as a substrate for the multiplication of microorganisms and favor degradation reactions, generating significant losses in quality and compromising safety¹⁶.

In this sense, in the absence of a moisture limit for creatine-based supplements, further studies are suggested with the aim of establishing a moisture content limit as a quality parameter for products of this type.

The results obtained for the content of the samples, according to the Kjeldahl method, ranged from 0.48% (sample D) to 96.26% (sample C), with the reference sample showing a content of 97.55%, slightly below the value stated in the analysis report (98% to 102%). However, samples A (96.3%), C (96.26%), and F (96.98%) showed percentages close to the content of the reference sample and within the limits set by the legislation. On the other hand, samples D (0.48%) and E (70.46%) showed variations in creatine content outside the minimum limit recommended (20%) in RDC No. 429 of October 8, 2020, as shown in Table 2.

Of the samples used, only sample B (44.55%) stated on its label that it contained the added carbohydrate maltodextrin in a 1:1 ratio. In this case, the content found was consistent with what was declared. In addition, to apply the recommended dose of 3 g/day, sample B indicated on its label that 6 g/day should be used to reach the recommended daily dose of creatine. The other samples only mentioned creatine in the list of ingredients, without mentioning the addition of carbohydrates.

According to Cardoso, Seabra, and Souza¹⁷, maltodextrin is a type of carbohydrate derived from starch that has undergone



a hydrolysis process, generating an oligosaccharide that is easily absorbed and is often used in sports supplements due to its ability to increase the glycemic response. This action allows glucose levels to be maintained during training, being a strategy to avoid excessive insulin release and preserve glycogen reserves for longer, which can help improve performance during exercise. In this sense, Mazzarella¹⁸ observed that combining creatine with maltodextrin enhances creatine absorption in muscle tissue.

Although the incorporation of maltodextrin in the formula shows significant results in performance during physical exercise, this addition is not clearly highlighted on product labels. Information about its presence is usually presented in small spaces, making it difficult to see. This increases the chances of consumers getting confused and consequently buying a product that isn't pure creatine.

Regarding the percentage of variation allowed by the guidelines, Resolution No. 429/2020¹⁹ determines a tolerance of $\pm 20\%$ in

relation to the nutrient values declared on the label. Therefore, samples D and E showed differences outside the limit tolerated by Anvisa, corresponding to 99.52% and 29.54% variation, respectively, and were considered to have failed quality control. Samples A, B, C, and F showed no irregularities in terms of the difference between the content described on the label and the content found.

The Brazilian Association of Nutritional Product Companies (Abenutri)²⁰ also carried out a survey on the dosage of creatine sold in Brazil. In this study, the contents of 30 brands were evaluated, seven of which were rejected for exceeding the variation limit of $\pm 20\%$ in accordance with RDC No. 429/2020; among these rejected samples, three had no creatine content in the formula at all. In addition, variations close to the limit determined by Anvisa were observed in some brands.

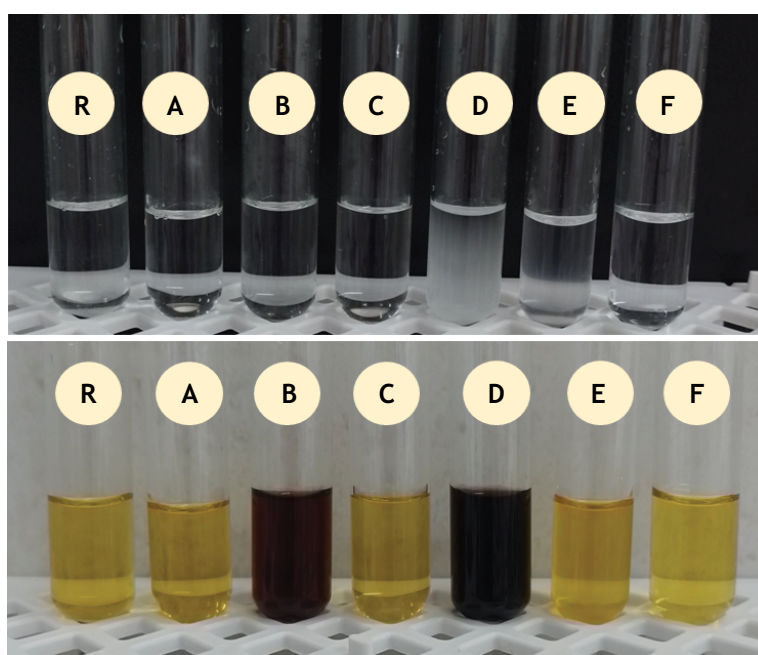
In the study carried out by Cruz¹², some supplements for athletes (protein hydrolysate, glutamine, BCAA, and creatine) were evaluated for their content using different methods, including the Kjeldahl method. Of the five creatine samples analyzed, only one showed a variation of more than $\pm 20\%$. In addition, the Kjeldahl method was considered efficient in the analysis, being equivalent to the instrumental methods applied in the study.

When assessing the presence of carbohydrates in the creatine samples, turbidity was observed in sample D, while the other suspensions were clear. This indicates the possible presence of carbohydrates in sample D, since the reaction with lugol resulted in dark coloration in the suspension (Figure 3). Lugol is known to react with starch molecules, producing a dark color when the carbohydrate is present.

Table 2. Comparison between the labeled creatine content and that found using the Kjeldahl method.

Sample	Labeled content (%)	Content found (%)	Difference (%)
R	100 $\pm$ 2	97.55	- 0.45
A	100	96.30	- 3.70
B	50	44.55	- 5.45
C	100	96.26	- 3.74
D	100	0.48	- 99.52
E	100	70.46	- 29.54
F	100	96.98	- 3.020

Source: Research data, 2023.



Source: Prepared by the authors, 2023.

Figure 3. Creatine suspensions with and without lugol.



When lugol was added, samples B and D tested positive for the presence of carbohydrates. On the label, sample B indicated the addition of maltodextrin; however, sample D's label only reported the presence of creatine in the formula and even used the expression "100% pure", which differs from the result obtained, as the sample had an insignificant creatine content, only 0.48%. Thus, incorrect labeling can lead consumers astray, since it presents false information, leading potential customers to have a misconception about the product on offer.

According to Dal Molin et al.²¹, the growing number of food supplements available on the market, together with the large number of stores selling them, can contribute to the ineffectiveness of the inspection of these products. In addition, the ease of purchasing through e-commerce can lead consumers to have access to low-quality products, leaving them susceptible to purchasing a fraudulent product.

To evaluate the product labels, a form based on RDC No. 243/2018 was used, through which 16 labeling parameters were analyzed according to the standards imposed by Anvisa. Examining questions 1 to 5, all the labels contained the designation "food supplement" plus the pharmaceutical form (Q1) in capital letters (Q3) and, next to the product brand (Q2), contrasted with the label background (Q5); however, the labels of samples A and F did not have the designation in bold (Q4), as shown in the Chart.

In terms of frequency and consumption (Q7), all the products were in agreement, but in terms of warnings (Q8 to Q10), some brands differed. In this respect, the labels of samples A, B, D, and E did not display the warnings "This product is not a medicine" and "Keep out of reach of children"; in addition, the labels of samples A, B, and D did not display the phrase "Do not exceed the daily consumption recommendation indicated on the packaging".

Chart. Checklist according to Resolution 243/2018.

Items to note	Sample					
	A	B	C	D	E	F
1. Does the product contain the designation "food supplement" plus its pharmaceutical form?	yes	yes	yes	yes	yes	yes
2. Is the designation close to the product brand and in legible characters?	yes	yes	yes	yes	yes	yes
3. Is the statement in capital letters?	yes	yes	yes	yes	yes	yes
4. Is the statement in bold?	no	yes	yes	yes	yes	no
5. Does the declaration contrast in color with the background of the label?	yes	yes	yes	yes	yes	yes
6. Does the product have an indication for use for the population group for which it is indicated, including the age group in the case of children?	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable
7. Does the product contain the quantity and frequency of consumption for each of the population groups indicated on the label?	yes	yes	yes	yes	yes	yes
8. Does the product bear the prominent, bold warning "This is not a medicine"?	no	no	yes	no	no	yes
9. Does the product have a prominent, bold warning "Do not exceed the daily consumption recommendation indicated on the package"?	no	no	yes	no	yes	yes
10. Does the product have a prominent, bold warning "Keep out of reach of children"?	no	no	yes	no	no	yes
11. Does the product contain storage instructions, even after opening the package?	no	no	yes	yes	yes	no
12. Does the product contain identification of the species of each strain in the list of ingredients of food supplements containing probiotics?	not applicable	not applicable	not applicable	not applicable	not applicable	not applicable
13. Does the product have any words, trademarks, images, or any other graphic representation, including in other languages, claiming to have a medicinal or therapeutic purpose?	no	no	no	no	no	no
14. Does the product contain words, marks, images, or any other graphic representation, including in other languages, claiming to contain unauthorized or prohibited substances?	no	no	no	no	no	no
15. Does the product feature words, marks, images, or any other graphic representation, including in other languages, stating that food is unable to provide the necessary components for health?	no	no	no	no	no	no
16. Does the product feature words, brands, images, or any other graphic representation, including in other languages, claiming to be comparable or superior to conventional foods?	no	no	no	no	no	no

Source: Research data, 2023.



Regarding preservation, even after opening the package (Q11), the labels of products A, B, and F did not express the recommendation. On the questions about words, brands, images, or any other graphic representation (Q13 to Q16), the products were following the rules. Questions 6 and 12 were classified as “not applicable” as they did not fit into the label analysis of creatine supplements.

According to the work of Arevalo and Sanches¹⁴, who applied the same questions regarding Resolution No. 243/2018 to 130 supplements for athletes, including 14 creatine supplements, it was found that 100% of the labels complied with questions Q7, Q11, Q13, Q14, Q15, and Q16 applied in the study. However, questions Q1 (61.5%), Q2 (49.2%), Q8 (76.2%), Q9 (76.2%), and Q10 (74.6%) did not comply with the items listed on the form.

When carrying out labeling analyses on 69 samples of creatine, both domestic (60.8%) and imported (39.2%), Mendes²² found that most of the non-conformities came from the labels of imported creatines, totaling 77.7%. In addition, the absence of bold highlighting in mandatory expressions and the presence of prohibited images and expressions were the most frequent among the non-conformities found.

The labels of the samples were also assessed regarding the specifications defined by IN No. 28/2018. It was observed that samples A and B did not include the mandatory warning: “This product should not be consumed by pregnant women, nursing mothers, and children.” In addition, samples A, B, and D did not present the specific authorized claim: “Creatine helps increase physical performance during repeated short-duration, high-intensity exercise.”

Based on research by Dal Molin et al.²³, after analyzing 44 sports supplements of various types purchased through e-commerce, 11.36% of the samples did not have a nutritional table or similar information on their label, while 29.54% had incomplete information. Furthermore, a dosage test was carried out, which found that 70% of the samples exceeded the limits recommended by Anvisa.

The results of this study revealed that two (33%) of the six samples of creatine powder supplements failed the dosage test due to their low creatine content, which was in disagreement with the limit established by the regulatory standards. In addition, sample D, one of the failed samples, showed an intense positive reaction for the presence of carbohydrates, in contrast to the

creatine content declared on the label, characterizing it as a fraudulent product. In addition, this same sample showed several inconsistencies on its label.

Although there are regulatory standards for dietary supplements, oversight in this area is precarious. In addition, the growth of e-commerce has made it easier to buy and sell low-quality products, increasing the risk of acquiring fraudulent supplements. It is essential to be wary of prices that are well below market value, as many frauds are carried out to reduce the cost of the final product. It is crucial that consumers are aware of the origin of these products and check that they have quality certifications and seals.

CONCLUSIONS

The analyses carried out revealed irregularities in the content and labeling of the brands of these samples. The dosage test revealed variations in creatine content from 0.48% to 96.26%. In the label analysis, according to the requirements described in RDC No. 243/2018, only the label of sample C was fully compliant. In terms of compliance with IN 28/2018, labels A, B, and D were not in line with the legislation. As a result, it was found that only sample C met all the parameters assessed in this research, fully complying with the resolutions in force.

This wide discrepancy between creatine levels raises concerns about the quality and authenticity of the products on the market, suggesting the possibility of quality deviation or even fraud. It also highlights the need for stricter inspection by regulatory bodies. In addition, it is important for consumers to be vigilant when purchasing supplements, especially when they find products with prices significantly below the market average, which could be an indication of possible fraud.

When comparing the results of this research with existing scientific literature, it was possible to observe that other studies also corroborate the findings, both in terms of analyzing the content of supplements and aspects related to labeling.

In addition, it is important to highlight the lack of studies and research into the quality control of food supplements. Through new studies, it is possible to expand knowledge and establish quality parameters with the respective acceptance limits for the production and marketing of supplements, providing a solid basis for future regulatory updates.

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Authors' Contributions

Cardoso FBS, Azevedo MGB, Souza JBP - Conception, planning (study design), acquisition, analysis, data interpretation, and writing of the paper. Dantas CEA - Planning (study design), analysis, data interpretation, and writing of the paper. All the authors approved the final version of the paper.

Conflict of Interest

The authors inform that there is no potential conflict of interest with peers and institutions, political or financial, in this study.



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