Proteins and protein components for sportspeople:quality control of dietary supplements

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ABSTRACT

Quality control of protein supplements intended for a large audi- ence of consumers such as sportspeople is particularly important.A case study on quality control of dietary supplements containingprotein and protein components is presented. The objective of thestudy was to evaluate the conformity of the quantities of aminoacids, essential and branched-chain amino acids, declared on the label through measurements with chromatographic analytical tools.16 sportspeople supplements from different European countries were tested. Analysis of concentrated whey protein highlighted some differences between the label and what was experimentallydetermined; in these samples some amino acids (6 amino acidsout of 19) exceeded the maximum tolerance (>20%) regulated by the European Commission. To a lesser extent, analysis of the otherclasses revealed amino acid concentrations that exceeded the max-imum analytical tolerance percentage. As regards the essential andbranched amino acid supplements, it was seen that the declared quantity conforms with that determined experimentally.

1. Introduction

Dietary supplements (FDA definition) or food supplements (European Food Safety Authority definition) (Durazzo et al. 2022a) are reported to be concentrated sourcesof nutrients (i.e. vitamins and minerals) presenting physiological or nutrition-al effect sold in 'dose' form. In Europe, since 2002, dietary supplements have been regulatedas foods by Directive 2002/46/EC (2002) which has defined these formulations as protecting consumers from potential health risks by ensuring that no misleading information is provided. Their use is addressed 'to correct nutritional deficiencies, maintaining an adequate intake of certain nutrients, or to support specific physio-logical functions' (EFSA webpage).

Dietary supplements should not replace regular food intake. Although health guide-lines do not encourage the use of dietary supplements, the supplement market is subject to growing interest, which causes increased consumer exposure to potential risks (Lentjes 2019). However, it should be emphasised that with respect to concerningfoods, dietary supplements provide micronutrients and other types of substances with physiological or nutritional effects in quantity predetermined and in particular dosage forms; therefore, the development and production of the supplements require spe-cialised skills for the qualitative/quantitative control of the formulation used.

Over the past few decades, there has been an increase in the exploitation and processing of compounds from various sectors of agriculture and agro-food industries as an innovative direction to identify sustainable alternative sources of bioactive molecules (Giammarioli et al. 2013; Durazzo et al. 2018).

With the increase in demand and with the attempt to reduce production costs, it could be lost the fundamental component of a particular supplement, the quality. Consumer satisfaction, and above all safety, should be the main objective of those who produce food and even more so of whom produces food supplements.

It is worth mentioning the current international perspective by Durazzo et al. (2022b) on the importance and effectiveness of utilising and applying rigorous ana-lytical techniques and adopting harmonising metrological principles in the study of dietary supplement products and ingredients, particularly medicinal plants and other botanicals.

Products sold on the market often contain ingredients that significantly vary in their chemical composition from the information on the labels (Dwyer et al. 2018, Mannino et al. 2020).

The global sports nutrition market size was valued at 40.0 billion in 2021 and is expected to expand at a compound annual growth rate (CAGR) of 8.5% from 2022 to 2030 (Catalani et al. 2021). The consumption of sports nutrition products is rapidly increasing among fitness enthusiasts and active lifestyle consumers and the most sought-after supplements were 'Whey Protein', followed by 'Branched Chain Amino-Acid', 'Creatine', 'Multivitamin supplements' and 'Nitric Oxide boosters'.

There is no universal analytical method that can ensure the quality control of all parameters of dietary supplements. The authors marked that the most analytical technique selected for the analysis of biologically active substances in dietary supplements is HPLC coupled with a photodiode array or ultraviolet and visible detectors.

Table 1. Samples tested and divided according to their formulation and on what is declared on the label and classified according to FoodEx2, Revision 2.

	Samples	FoodEx2 (Revision 2 (Code)
Whey protein concentrate dietary supplements	WPC_1, WPC_2, WPC_3, WPC_4 and WPC_5	(A02PN) Whey powder or similar food,
Whey protein isolate dietary supplements	WPI_1, WPI_2 and WPI_3	(A02PN) Whey powder or similar food,
Whey protein hydrolysed dietary supplements	WPH_1, WPH_2, WPH_3 and WPH_4	(A02PN) Whey powder or similar food,
Essential amino acids-based dietary supplements	EAA_1, EAA_2 and EAA_3	(A03SY) Protein and amino acids supplements
Branched-chain amino acids-based dietary supplements	BCAA	(A03SY) Protein and amino acids supplements

On the other hand, the management of data on dietary supplements is a priority: integrating food supplement database and infrastructures following a standardized and harmonized approach in the perspective of interoperability (Durazzo et al. 2020;

Dwyer et al. 2021; Saldanha et al. 2021). This paper aims at evaluating, on dietary supplements containing proteins and protein components, if the values declared onthe label correspond to the measurements obtained through a qualitative and quan- titative analysis performed with chromatographic analytical instruments on the essen- tial and branched chain amino acids.

2. Results and discussion

The benefits of muscle protein synthesis linked to consuming protein-rich foods after exercise are known; an increase in the presence of protein-based supplements on the market has produced an industry multi-billionaire. Whey protein (WP), which is the soluble protein fraction in milk serum that is obtained during cheese and casein production, hasbeen studied since the 1970s as a source of high biological-value proteins (Bulut & Akin2012). In this work, some commercial supplements containing whey protein were sub-jected to qualitative and quantitative analyses through HPLC-FLD for the analysis ofessential and branched-chain amino acids (Di Stefano et al. 2020; Cardullo et al. 2022). The supplements samples tested were 16 from different European countries (Italy, Spain, Sweden, France and Portugal) and from the United Kingdom and have beendivided according to their formulation and what is declared on the label as reportedin Table S1. Moreover, the description and classification system, FoodEx2 (Revision 2), developed by the European Food Safety Authority (EFSA), was utilised for codingproducts in line with data harmonisation and standardisation procedures (Table 1) (EFSA, 2015, 2016, 2019a,b, 2020, 2021, 2022, 2023).

Tables S2–S8 reported the amino acids values declared in label and those exper-imentally determined in HPLC-FLD and coefficient of variation CV% (which represents the percentage difference between the values declared on the label and those deter-mined experimentally). The results were expressed in grams of amino acids on 100 grams of product.

For dietary supplements, the European Commission has established a tolerance value maximum ±20% (i.e. must contain at least 80%) of the declared content for vitamins and minerals and other bioactive substances (European Commission, 2012).

2.1. Whey protein concentrate dietary supplements (WPC)

In Table S2, sample WPC_1 has 18 amino acids of which 3 amino acids (glycine, methionine and proline) had a CV% ranging between −23% and −32%, 14 amino acids have a

valuethat falls within ± 20% of tolerance; tyrosine had a determined value greater than thevalue indicated on the label, in fact it had a CV% equal to 3%. Sample WPC_2 had 18amino acids of which 4 amino acids (leucine, valine, cysteine and proline) with a CV%between −21% and −32%, while the others 14 amino acids were within the allowed limit.Sample WPC_3 has 18 amino acids of which 6 (serine, glycine, methionine, alanine, tryp-tophan and glycine) showed a CV% ranging between −23% and −35%. In Table S3, WPC_4contains 19 amino acids of which threonine, phenylalanine and isoleucine which have aCV% between −27% and −34%; the others 16 amino acids fall within the range indicatedby the European Commission guidelines. Sample WPC_5 (Table S3) contains 19 aminoacids of which tyrosine and phenylalanine with a CV% −31% and −33% respectively, valinehave a CV% of +22%. In all samples (WPC_1, WPC_2, WPC_3, WPC_4, WPC_5) (Table S2 and Table S3), the amount of some single amino acids experimentally determined was lower than that reported on the label. Referring to total amino acids content, for WPC_1CV% is 7.58%, for WPC_2 CV% is 10.41%, for WPC_3 CV% is 9.16%, for WPC_4 CV% is 8.71%, and for WPC_5 CV% is 9.10%. Despite the lower values determined, the differencedoes not exceed the value imposed by EC law (tolerance of ± 20%); therefore, they complywith the guidelines of the European Commission.

2.2. *Whey protein isolate dietary supplements (WPI)*

In Table S4, sample WPI_1 contains 19 amino acids of which serine, isoleucine, lysine and proline have a CV% between −23% and −27%; sample WPI_2 contains 19 amino acids of which serine, aspartic acid and proline had a CV% between −21% and −24%;sample WPI_3 contains serine, valine and tryptophan with a CV% between −21% and
−31%. In all whey protein isolate dietary supplements (WPI_1, WPI_2, WPI_3; TableS4) amino acids values determined experimentally were sometimes lower than that stated on the label. Referring to the total amino acids content, for WPI_1 CV% is 7.82%, for WPI_2 CV% is 8.08%, for WPI_3 is 6.35%, therefore they respected the guidelines of the European Commission.

2.3. *Whey protein hydrolysed dietary supplements (WPH)*

Sample WPH_1 (Table S5) contains 19 amino acids and all fall within the range indicated by the EC guidelines; sample WPH_2 contains 19 amino acids of which tryp-

tophan and arginine had a CV% between −27% and −28%, valine had reported aCV% of +28%. Sample WPH_3 contains 18 amino acids of which proline had a CV%of +31%. In Table S6, sample WPH_4 contains 19 amino acids of which arginine andtyrosine, with CV% in a range −25% −38%.

2.4. Essential and branched-chain amino acids-based dietary supplements

Samples EAA_1, EAA_2, EAA_3 (Table S7), and BCAA (Table S8) contained amino acids within a tolerance value maximum ± 20% therefore they comply with the guidelines of the European Commission. In Table S9, retention time (min.), coefficient of deter- mination (R^2) and linear regression model of external standards used for amino acid determination were reported.

3. Conclusions

The presented investigation aims at evaluating if the values declared on the label of whey protein supplements correspond to the measurements obtained through a qualitative and quantitative analysis with analytical instruments.

The analysis was satisfactory, in fact, the amino acids declared on the label were detected through the chromatographic study in HPLC-FLD instrument. The quantitative analysis highlighted differences between what was noted on the label and what was determined experimentally, especially for concentrated whey protein supplements (WPC). The latter presented some amino acids (6 amino acids out of19) that exceeded the maximum tolerance (± 20%) required by the European Commission.

Measurements from other classes of isolated and hydrolysed whey protein supplements (WPI-WPH) revealed the presence of some amino acid values lower than that stated on the label.

As regards the essential and branched amino acid supplements (EAA and BCAA), no determined amino acids exceeded the CV% ± 20%. Referring to total amino acidscontent, the difference did not exceed the value imposed by EC law (tolerance of ±20%), therefore all samples complied with the guidelines.

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