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# Investigation of sport supplements quality by Raman spectroscopy and principal component analysis

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ABSTRACT

In this work, sport supplements were investigated by Raman spectroscopy. Samples were obtained from health foods shops, gyms and sports centers covering a wide range of available supplement powders. A systematic comparison of Raman spectra of the analyzed supplements allowed identifying the supplement type through the characteristic vibrational modes of carbohydrates and proteins. The protein supplements were identified by Raman bands at 1650, 1250 and 1004 $cm^{-1}$, while the spectral range between 1200 and 800 $cm^{-1}$ was useful to identify the carbohydrate supplements. Due to the diversity in composition of sport supplements, a chemometric tool such as principal component analysis (PCA) was employed to assist in the interpretation of Raman spectra, allowing also the identification of compounds present in sport supplements. Especially, the Raman scattering of aromatic and aliphatic amino acids residues contributes to the existence of bands characteristic for the different types of proteins. This kind of information is very important for the quality control of these products, for detecting the presence of fraud or a sample composition in disagreement with the label, thus ensuring the provenance of the supplements.



## 1. Introduction

Among many commercially available dietary supplements, there are products composed by specific macro- and micro-nutrients formulated specifically to athletes. In general, such products are suitable as complementary food and may contribute to enhance the athlete's performance, where nutritional needs can vary with the sport modality and also with intended achievements in health [1].

Such commercial supplements may be found in various forms and the most common types are: (i) those composed mainly by whey protein (known simply as "whey protein supplement"), (ii) supplements consisting mostly of carbohydrates, such as malto-dextrin and (iii) supplements formulated with proteins and carbohydrates (known in Brazil as "hyper caloric supplements"). Moreover, these types listed above are the most widespread in gymnastic clubs [2].

These supplements are in constant innovation; new products appear continuously in the market with a great diversity in their composition. There is a wide variety of commercially available brands, but in Brazil some of them are not approved by our regulatory agency (ANVISA) [3], and this fact represents danger for the consumers' health.

Despite the important collective health concerns, little scientific information regarding the quality of dietary supplements has been reported. Some issues have not been adequately dealt with, such as possible contaminations inherent to the production chain [4] or even falsifications. The sport supplements may suffer different kinds of fraud, like presence of undeclared or prohibited ingredients, protein content lower than the label declared, use of proteins of different origin and low nutritional value and unreliable dosages of specific nutrients [5]. In the last years, several analytical methods have been developed and improved with the aim to evaluate overwhelmingly the presence of steroids and synthetic drugs [5–7]. Most of these investigations employ separation techniques and mass spectrometry based methods.

On the other hand, several studies show that the use of these products has been disseminated increasingly among the practitioners of physical activities, including those who are not athletes [8,9]. In addition, the use of supplements usually occurs without the supervision of a physician or other qualified professional, constituting a worrisome scenario that is consolidated not only in

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Brazil but also in other countries [8–10]. Therefore, we consider that studies aimed at assessing the nutritional adequacy of these products in relation to the declared contents of its components, as well as the existence of undesirable impurities are important issues for food science.

Since the protein amount is the component of supplements most related to the product final cost, the aim of this study was to explore Raman spectroscopy as a screening technique to determine qualitatively the protein content of different commercial supplements. The advantage of this technique, compared to the conventional titration procedure (Kjeldahl titration) is that the former is employing little or no sample preparation. Also, Raman spectroscopy has some advantages over FTIR spectroscopy since water does not interfere strongly in the Raman spectra, and functional groups of interest, such as C—C, C—H, C=C bonds produce strong Raman signals, but weak bands in the FTIR spectra. Furthermore, based on the literature [11–13], the analytical use of Raman spectroscopy together with chemometric tools has gradually been shown to be applicable for quality control of manufactured products. Food components, like proteins, carbohydrates, lipids and vitamins have been determined by Raman spectroscopy [12].

The development of our analytical method took into account that proteins characteristic Raman scattering bands exist depending on the instrumental conditions [14]; additionally, to facilitate the interpretation of the spectra, chemometric methods were employed, specifically methods for exploratory analysis. Principal Component Analysis (PCA) was used, and it allowed building a statistical model to identify the different types of supplements studied. Besides, based only on the spectral measurements, it was possible to differentiate the protein type that is present in the supplements analyzed.

## 2. Materials and methods

### 2.1. Samples

Samples were obtained from Brazilian market places, such as health foods shops, gyms or sports centers covering a wide range of available types of sports supplements powders (18 brands). These 18 samples of sport supplements were composed of various ingredients, as proteins (whey, egg, beef, soy), carbohydrates (maltodextrin), amino acids and other elements. The samples were from domestic and imported manufacturing and comprised different price ranges. In addition, spectra of pure maltodextrin, creatine, collagen and a whey protein concentrate (80%) were recorded for comparison purposes.

### 2.2. FT-Raman measurements

Raman spectra were obtained for sport supplements as received, without any chemical or physical pretreatment. The FT-Raman spectra were recorded using a RFS 100 FT-Raman Bruker spectrometer equipped with a Ge detector and using excitation by the wavelength of 1064 nm from a Nd:YAG laser. A few milligrams of the samples were pressed by hand into an aluminum cup. The laser light was focused onto the sample, and the scattered radiation was collected at 180°. Three replicate Raman spectra were recorded for each sample, each in different days, using a laser power ranging from 25 to 50 mW. FT-Raman spectra were collected at 4 $cm^{-1}$ resolution over the range of 3500–400 $cm^{-1}$. Two spectral sets were obtained, the first set comprised 256 accumulated scans (~7 min) and was used for spectral characterization of the sport supplements; for the second one the spectra were obtained with 32 scans only (~1 min) and this set was employed for chemometric analysis. These two experiments were performed in order to evaluate the influence of the acquisition time on the chemometric results. Similar results were obtained using these two experimental conditions indicating that satisfactory results can be obtained within *ca.* 1 min. The software Opus 6.0 (Bruker Optik, Ettlingen, Germany) was employed for Raman data acquisition and data treatment.

### 2.3. Chemometric analysis

Raman spectra were baseline corrected using a concave rubber band method with 18 iterations available in OPUS 6.0 (Bruker Optik, Ettlingen, Germany). Baseline corrected spectra were imported into the Matlab software. The data set was composed by all replicated Raman spectra. Before our chemometric analysis, all spectra were normalized using vector normalization to reduce the influence of physical variables. After data pre-processing, PCA was conducted with spectral data in the region of 1800–400 $cm^{-1}$. The number of principal components was selected based on the highest fractions of variance explained together with interpretations of the model. The Raman spectra before and after pre-processing are found in the Supplementary material (Figs. 1S and 2S).

## 3. Results and discussion

Examples of FT-Raman spectra (1800–800 $cm^{-1}$) recorded with 256 scans are presented in Fig. 1. Different compositions of sport supplements are displayed in Fig. 1a with the significant spectral regions for each food component highlighted. The spectra present characteristic bands of the main ingredients (whey protein and maltodextrin), as presented specifically in Fig. 1b. The Raman spectrum of the supplements with a high protein content show intense bands at 1660 $cm^{-1}$, due to the C=O stretching vibration mode (Amide I), associated with the CONH group of proteins [14,15]. This band is also observed for the high-calorie supplements, however, with lower intensity. In addition, the Raman spectra show bands at 1608 and 1580 $cm^{-1}$, which have contributions from the C=C stretching mode of phenylalanine and tryptophan residues, respectively [14].

A weak Raman band at 1553 $cm^{-1}$ is observed in protein supplements, which was assigned to the Amide II mode ($\delta_{N—H}$ and $\nu_{C—N}$) [16,17]. The strong Raman band at 1450 $cm^{-1}$ is observed in all three types of supplements and is attributed to the CH deformation mode. For Raman spectra of protein samples, aliphatic amino acids residues show bending modes (δ CH) near 1450 $cm^{-1}$ [18]; carbohydrates compound also present Raman band in this region. This band cannot be assigned to a vibrational mode specific for a single component. The Raman spectra for the high-calorie supplements (Fig. 1a, (B and C)) show a group of bands in the region between 1400 and 1300 $cm^{-1}$, that is due to C—C—H and C—O—H deformation modes [13]. However, the spectral profile is different due to the presence of other carbohydrates. These high-calorie supplements also show a Raman band at 1265 $cm^{-1}$, described as $\tau(CH_2)$ deformation mode.

Other important bands of proteins correspond to the amide III mode, which appear in the region between 1350 and 1220 $cm^{-1}$ for the Raman spectra of the high-protein supplements. This band is associated to coupled C—N stretching and N—H deformation of the peptide group [14,15]. The well-defined band that appear at 1004 $cm^{-1}$ for the proteins and high-calorie formulations can be attributed to the ring (benzene) vibration of phenylalanine [19]. The location and intensity of this band are insensitive to the conformation or environment; therefore, it can be used as an internal reference for normalization of the Raman spectra of proteins [19].