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Short communication

PCA based screening for melamine adulteration in supplements for sport nutrition using vibrational spectroscopy tools

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Introduction

Supplements for sport nutrition have increased demand and consumption nowadays. However, a deficient regulation for these products permits the manufacturer, importer, supplier, or distributor to be responsible for their quality and has made them the target of adulteration for economic gain (Wójcicki, 2020). For example, the supplements can be diluted and/or mixed with cheap, readily available, relatively odorless, colorless, and tasteless substances to mask inferior quality, increase volume, or substitute natural constituents. Unfortunately, economically motivated adulteration sometimes may have negative health implications (Karunathilaka et al., 2018). One of the most common adulterants in protein supplements for sport nutrition is melamine due to its' high nitrogen content (66% by mass). The drawbacks of traditional assays for nitrogen quantification allow a possibility of melamine adulteration (Wójcicki, 2020). Melamine consumption may cause nephrolithiasis, chronic kidney inflammation and bladder carcinoma (Hau et al., 2009). This substance was identified in the 2007 pet and human food recalls as well as the 2008 global milk product food safety scares. Melamine contamination was also documented in other reports for a wide range of food products, including milk, infant formula, frozen yoghurt, pet food, biscuits, candy, and coffee drinks (Balabin and Smirnov, 2011). Current analytical methods for melamine determination are mainly chromatography-based (e.g., HPLC, GC-MS), which are time consuming, expensive, labor-intensive and require complex sample pretreatment as well as well-trained personnel. Vibrational spectroscopy-based methods are highly desirable for the analysis of adulterants because they are easy to use, provide rapid analysis, require no sample preparation, and multiple analyses are possible on a single test portion due to the methods' non-destructive nature. Combined with chemometric approaches, they can be used as targeted analytical tools for comprehensive screening as well as reliable quantification of specific adulterants (Karunathilaka et al., 2018). The aim of this study was to evaluate the feasibility of ATR-FTIR and Raman spectroscopy as analytical tools for detection and quantification of melamine in different supplements for sport nutrition.

Materials and methods

Eleven commercially available supplements for sport nutrition produced by various manufacturers and purchased from local stores were included in our investigation. All studied samples represent three different types of supplements: protein powders, amino acid powders, and creatine. Melamine (2,4,6-triamino- 1,3,5-

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triazine) with 99% purity was purchased from Sigma Aldrich, USA. For ATR-FTIR measurements representative powder samples were placed on the ATR diamond stage of Carry 600 instrument (Agilent, Germany) and scanned in the wave number range 4000-650 cm^{-1}, 32 scans per sample and resolution 4 cm^{-1}. Raman spectroscopy was performed with a fiber optic probe from ATR 3000 DH portable Raman spectrometer (Optosky, China), 1064 nm laser. The laser power was varied in the range of 100-400 mW, while the integration and excitation times were in the range of 30-60 s.

Statistical analysis was performed using PCA (SIMCA 14.1 software) to evaluate the spectral data with or without spectral preprocessing. Further Hierarchical clustering analysis (HCA) was employed to classify the samples according to their spectral characteristics.

Results and discussion

The FTIR PCA model was built using the 1800-650 cm-1 spectral range to eliminate artifacts due to the presence of water in the samples (R2X=0.852, 2 components). The score scatter plot revealed distinctive clustering of the samples. The first component could resolve the samples into the following groups: Group 1 (Creatine), Group 2 (Glutamine, Milk protein powder + Soy protein isolate), Group 3 (BCAA, Beef protein, Pea protein), Group 4 (Vegan protein and Whey protein), Group 5 (Melamine). The second component could additionally resolve the Group 3 into BCAA samples and protein samples (Fig. 1). The HCA confirmed the above-mentioned sample clustering in the score scatter plot.

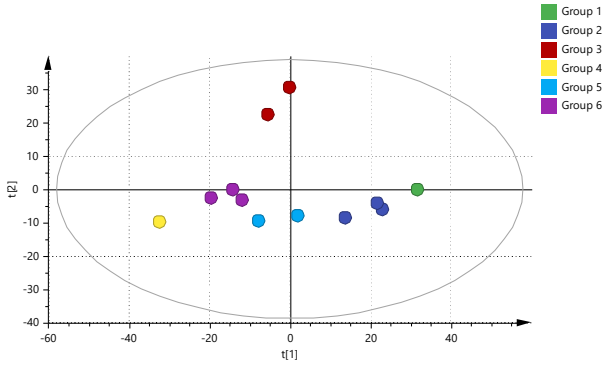

Fig. 1. PCA score plots of SNV-treated FTIR spectra of selected supplements.

The PCA score scatter plot from the first (R2X=0.29) and the fourth component (R2X=0.13) of the model from the SNV-transformed Raman spectra in the range of 1700-195 cm^{-1}, revealed the most appropriate sample clustering (R2X=0.9) (Fig. 2). However, the HCA could not efficiently resolve the melamine and creatine samples due to similar spectral attributes. The presence of volatile compounds in some of the samples caused physicochemical changes during the spectral acquisition. thus producing significant spectral artifacts in the 'melamine specific' regions, thus affecting the resolution capability of the model.

The results obtained from the PCA modeling indicated the advantageous capability of FTIR-ATR compared to Raman spectroscopy for comprehensive screening and/or quantification of melamine adulteration.

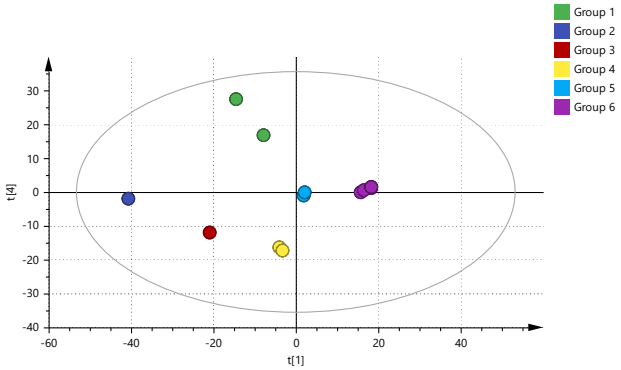

Fig. 2. PCA score plots of SNV-treated Raman spectra of selected supplements.

Conclusion

PCA based modeling of FTIR and Raman spectra of selected supplements were successfully applied for initial screening of melamine adulteration. The models revealed that FTIR was a better technique in resolving the samples according to their chemical content in contrast to the Raman spectroscopy which presented occurrence of spectral artifacts due to physicochemical changes during spectral acquisition. Further experiments will be performed to build PLS based quantification models.

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