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Arrowroot and Cassava Mixed Starch Products Identification by Raman Analysis with Chemometrics

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Abstract: Food frauds present a major problem in the foodstuff industry. Arrowroot and cassava may be targeted in adulteration and falsification processes. Raman analysis combined with chemometric techniques was proposed to identify the mixing and adulteration of these foodstuffs in commercial products. 67 cassava and 5 arrowroot samples were prepared in laboratory. 21 cassava and 5 arrowroot commercial samples were purchased in local stores. Raman assays were performed in the range of 400 to 2300 cm^{-1}. Principal component analysis with K-means clustering was used to identify the adulteration of these products. It was possible to observe the separation of three different groups in the data, these groups labelled group 1, 2 and 3 were correspondent to cassava-like samples, mixed samples, and arrowroot-like samples, respectively. Despite the visual analysis related to sensory characteristics and the visual analysis of each Raman spectrum of cassava and arrowroot not being able to differentiate these foodstuffs, the chemometric approaches with the Raman specters data were able to identify which samples were pure arrowroot, pure cassava and which were mixed products. The proposed approach showed to be an effective tool in the investigation of fraud for arrowroot and cassava.

Keywords: food; fraud; adulteration

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1. Introduction

Maranta arundinacea (L.) (arrowroot) is a South American tropical crop with starch valued in the international market for its quality for application in fine confectionary. The literature shows that this starch produces a paste of greater lightness and digestibility, than more common starch sources such as cassava, and can compete with commercial modified starches [1,2].

Arrowroot has been widely cultivated in Brazil, but has lost ground in the last 50 years, reaching near extinction. Thus, other starches produced from different raw materials at the industrial level, such as cassava and corn, have gradually replaced arrowroot, although they do not present the same characteristics when compared to those produced from arrowroot, such as easy digestibility and gelatinization capacity [2].

However, the scarcity of arrowroot starch on the market implies a price increase and favors the occurrence of fraud through substitution with cheaper sources, such as commercial cassava or potato starches. The major challenges in identifying this fraud are the impossibility of visual discernment and the usual inconclusive analytical approaches [3].

Food fraud is a growing problem in the food industry, compromising the credibility of the business and, in the worst cases, the health of the consumer. These fraudulent products, when used as ingredients, produce different results from those expected for the original counterpart. This can be exemplified with tuberous starches from different vegetal sources. Due to the physical and sensory similarities, fraud in the form of substitution or counterfeiting in these products is hardly noticed by consumers.

The major composition of cassava and arrowroot products are starches, which intrinsic macro structural arrangement, leads to granules of different sizes and shapes. Other typical compounds, can be also explored for differentiation, such as amylose and amylopectin [4]. Sanitary inspection and quality control are mainly responsible for identifying these frauds, however, new analytical approaches to evaluate the differences between similar products, such as arrowroot and cassava, are constantly in demand [5,6].

Techniques such as spectroscopic approaches combined with chemometric techniques and microscopic analysis are commonly used to identify pure starches or quantify intentionally blended starches. However, operational costs and the need for trained professionals are drawbacks to overcome [7,8]. Thus, the continued development of new techniques associated with chemometrics would broaden knowledge by expanding the possibilities of identifying fraud in starches. Among the promising techniques, Raman Spectroscopy allows the chemical identification of minor compounds and also the macro structural characterization of different polymorphs. Chemometric approaches, such as principal component analysis and K-means clustering, can explore and assist in the observation correlated patterns with specific samples within a group [9].

In this sense, the objective of this study was to evaluate the differences between arrowroot and cassava starches using Raman associated with principal component analysis and K-means clustering as an alternative for the identification of fraud in starches.

2. Materials and Methods

Starch was extracted in the laboratory from 67 cassava and 5 arrowroot samples, compared to 21 cassava and 5 arrowroot samples purchased from local businesses. The samples were coded as C for cassava and A for arrowroot, as presented in Table 1.

Table 1. Scheme for the labelling of cassava and arrowroot samples.

Laboratory Extraction		Commercial	
Cassava C1 to 67	Arrowroot A1 to A5	Cassava C68 to C88	Arrowroot A6 to A10

Raman assays were performed in sextuplicate ($n = 6$) with a resolution of 16–18 cm^{-1} on the Mira M-1 spectrometer (Metrohm, São Paulo, SP, Brazil). The spectral range was 400–2300 cm^{-1}, laser power of 100 mW and laser wavelength of 785.0 nm resulting in 1901 variables. Raman assays were performed for 88 cassava samples and 10 arrowroot samples ($n = 98$).

Raman specters were treated with a first-derivative Savitzky-Golay filter with a 2nd-degree polynomial and 21-point window. The dataset was further treated with the standard normal variate filter [9].

Rstudio IDE [10] for Rstudio 3.6.1 and R packages prospectr [11], mdatools [12], chemometrics [13] and ggplot2 [14] were used.

PCA was calculated through singular value decomposition. K-means clustering method was performed with the Hartigan and Wong algorithm [15]. The K-means was calculated with the first three principal components of PCA.

3. Results

To evaluate the visual similarities between the cassava and arrowroot spectra, Raman assays were performed. The average of the spectra is shown in Figure 1.

Arrowroot showed great similarities with the cassava spectra. Thus, in order to explore the possibility of differentiation between cassava and arrowroot, a principal component analysis with K-means was performed (Figure 2).

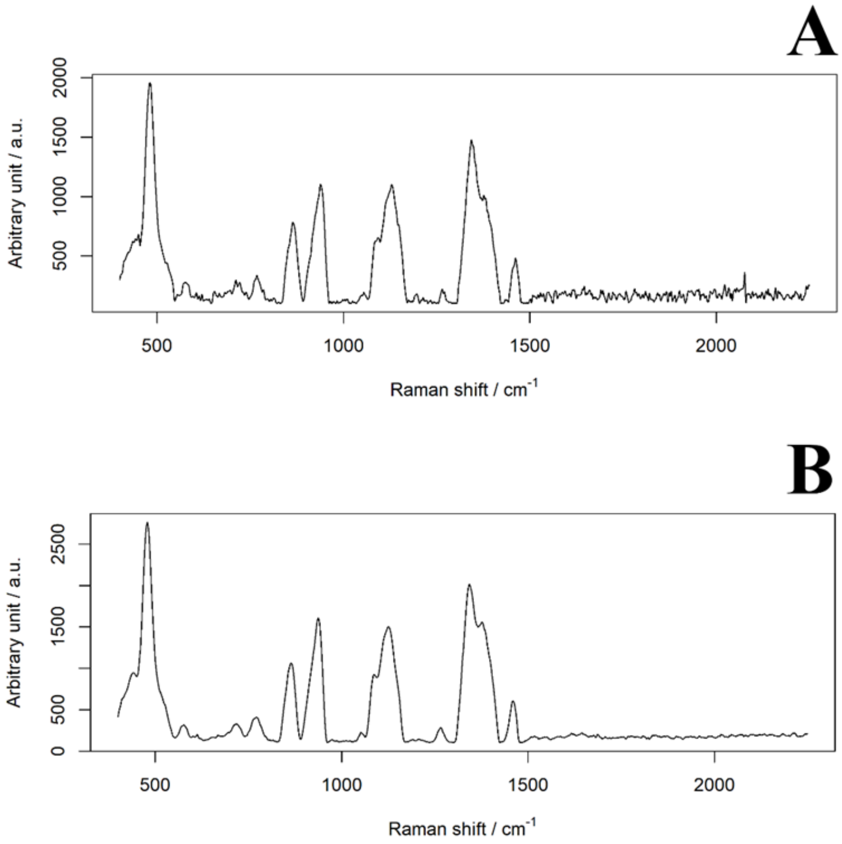

Figure 1. Mean untreated Raman spectra of arrowroot (n = 10) (**A**) and cassava (n = 88) (**B**).

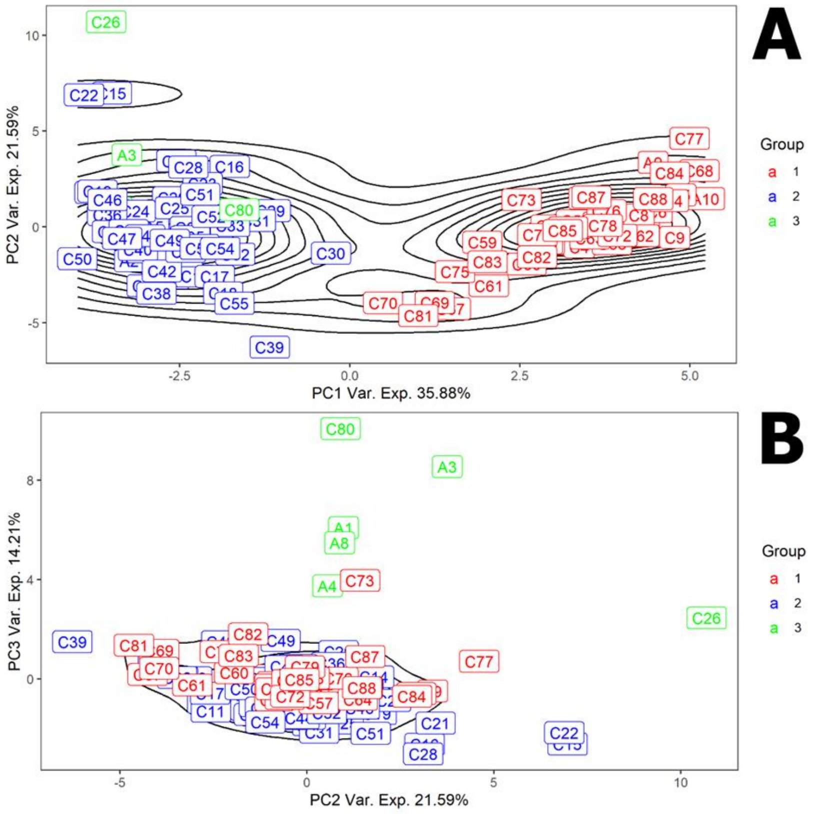

Figure 2. PCA plot of first and second components (**A**) and plot of second and third components (**B**) with K-means cluster assignments for cassava (C1–C88) and arrowroot (A1–A10).

4. Discussion

In Raman analysis (Figure 1), both spectra showed peaks in the regions between 600–1500 cm^{-1} which are linked to the C-C bond vibrations [16], which, probably derives from the high great starch content within both samples. Arrowroot showed great similarities to the spectra of cassava. From visual inspection of the product or differentiation of the specters, both products could not be distinguished from each other.

The standard deviation of mean arrowroot specter and cassava mean specter was very similar, being 316.38 and 467.23 (Arbitrary intensity units, as displayed in the specters of Figure 1), respectively. Likewise, the difference and division mean of cassava/arrowroot was 88.07 and 1.18, respectively.

Raman spectroscopy can be used, along with infrared analysis, to differentiate saccharide types such as monosaccharide (i.e., glucose), oligosaccharide and polysaccharides reference substances. However, complex samples such as powdered tubers and herbs, pose a greater challenge regarding differentiation, due to the presence of many substances, along with fiber and matrices that may hinder analytical signals [17].

Therefore, in order to determine these differences, a principal component analysis with K-means was performed (Figure 2).

The data density in the first three PC in PCA, showed that the data set was clustered into two large groups and a smaller scattered data group. Therefore, in order to separate the data into the likely groups of arrowroot, cassava and mixed products, a K-means for 3 clusters was performed. From the cassava dataset, 41 samples were assigned in group 1, 45 samples were assigned in group 2 and only 2 samples were assigned in group 3. Moreover, from the arrowroot samples, 2 were assigned to group 1 (A9 and A10), 4 were assigned to group 2 (A2, A5, A6 and A7) and 4 were assigned to group 3 (A1, A3, A4 and A8).

Group 3 seems to be related to arrowroot starch, since it grouped more samples from this botanical source, although two cassava starch samples were assigned to this group as well. Group 1 had a higher proportion of cassava starch samples. From this, group 2 may be related to mixed products, in which there is presence of both cassava and arrowroot starches.

Commercial sample A8 can be considered as pure arrowroot product, while commercial samples A6 to A10 (except A8) are most likely adulterated products.

Moreover, the laboratory extracted cassava products, i.e., C1–C67, were assigned 68.66% in group 2 and the commercial cassava products, i.e., C68–C88, 95.24% of the samples were assigned to group 1 (Table S1). Therefore, it can be inferred that the two large clusters observed in Figure 2A are relative to the separation between commercial cassava and laboratory extracted products.

5. Conclusions

The similarities between the spectroscopic profiles of cassava and arrowroot, makes their differentiation difficult by visual analysis. Due to the high similarity between the Raman spectra of arrowroot and cassava, the observed separation was likely derived from the content of components, namely starch. However, exploratory data analysis with k-means was able to elucidate the pattern differences in the Raman spectrum and differentiate pure arrowroot and likely adulterated arrow products. The model was able to separate between laboratory extracted and commercial products.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/polysaccharides2030043/s1, Table S1: K-means classification results for arrowroot and cassava in groups 1, 2 and 3.

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