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Detection of counterfeit stevia products using a handheld Raman spectrometer

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ABSTRACT

Stevia is a highly appreciated natural sweetener because it can be consumed by diabetic patients. Due to the increasing popularity of stevia during the last years, counterfeit products have been making their way into the market. Raman spectroscopy is a versatile analytical technique that can be used for control tasks and handheld modern devices expand its possible applications to instant *in situ* measurements. The Raman spectra of six commercial stevia products (five purchased in Bolivia and one in Germany) were recorded and compared to the spectra of standards of rebaudioside A and stevioside as well as the spectra of the artificial sweeteners sodium cyclamate and sodium saccharin. Based on the Raman spectroscopic data, it was verified that three of the Bolivian products were counterfeit products and another one was rich in maltodextrin. The Raman spectra of one Bolivian product and the German one revealed rebaudioside A and stevioside as major components. Raman spectroscopy was capable of detecting contents as low as 5% (w/w) of sodium cyclamate during measurements of stevia-sodium cyclamate mixtures. The results show that Raman spectroscopy can successfully be used to detect counterfeit stevia and underline its high potential for the detection of food adulteration.

1. Introduction

In recent years, several studies have warned of a dramatic increase in obesity. Since health issues such as diabetes, cardiovascular disease, hypertension and certain cancer types, among others, were associated to obesity [1–3], dietary alternatives and associated solutions are currently being explored. One of the substances that has been blamed for the worldwide increasing obesity is sugar; some researchers have suggested that sugar should be controlled and/or regulated like alcohol and tobacco, due to its negative effects on human health [2]. As a reaction, both people and companies started to search for sugar substitutes. Therefore the so-called “artificial sweeteners”, which are virtually calorie-free substances, have gained popularity in recent years. A review on the most important artificial sweeteners, their synthesis, metabolism and some health aspects was published by Chattopadhyay et al. [4]. The effects of artificial sweeteners on human health are, at least, controversial. Some researchers reported important negative effects on human health, so Suez et al. [5] reported that certain artificial sweeteners induce glucose intolerance. This type of bad publicity for artificial sweeteners give rise to a growing interest in products of natural origin. It is in this context that stevia, a sweetener extracted from the leaves of *Stevia rebaudiana*, has drawn the attention not only of consumers, but also of big companies. Currently, it is possible to find in the market soft drinks sweetened with stevia.

Stevia is a highly demanded sweetener that has gained relevance because it is suitable for diabetics. The main components of stevia are stevioside and rebaudioside A, which are white powders, 200–300 and 400 times sweeter than sugar, respectively, heat-stable, non-fermentable as well as anti-plaque and anti-caries agents [6]. In November 2011, the European Commission approved the use of stevia as a non-caloric sweetener in the European market. Due to the increasing popularity of stevia, stevia-based counterfeit products have been appearing in the market during the last years.

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Currently, there is a specific concern about the presence of counterfeit stevia in many South American countries. During the last months, many press articles have warned the population of the commercialization of counterfeit stevia, since the consumption of such products can put in risk the health of the consumers. It is clear that the adulteration or falsification of stevia in other continents cannot be ruled out either.

The adulteration or falsification of stevia is performed using common artificial sweeteners, which are cheaper than stevia. The predominant methods for the analysis of artificial sweeteners are based on chromatographic techniques (liquid chromatography, gas chromatography, ion chromatography and thin-layer chromatography), however, other analytical techniques were also proposed for specific cases [7]. For the analysis of the stevia components, chromatographic techniques are also applied [8,9]. While chromatographic techniques provide good and reliable results, they require special facilities as well as highly qualified operators. Spectroscopic techniques are interesting options, since quick measurements are possible. There are some reports on the application of spectroscopic techniques (*e.g.*, FTIR) for the analysis of both artificial sweeteners and stevia components [10–12]. In order to protect the consumers, control institutions in each country require analytical techniques and methods that allow them to respond quick and efficiently, ideally, by performing *in situ* verifications of the product quality. Currently available portable and handheld Raman spectrometers add important mobile applications to this spectroscopic technique in the areas where *in situ* quality control and detection of counterfeit products are required.

Raman spectroscopy is a remarkable analytical technique applicable to almost all disciplines of natural sciences [13]. Due to its non-destructive spectroscopic nature as well as minimum or no sample preparation requirements, Raman spectroscopy was used in the characterization of materials in several disciplines including microbiology, arts, archaeology, geology, atmospheric sciences and planetary exploration, among many others [14–21]. Raman spectroscopy was suggested for the quality control of certain products and forensic research [22–27]. There are also reports on applications for the detection of different sugars and artificial sweeteners [28–32], but to our best knowledge, no earlier report was published on Raman spectroscopic detection of falsification/adulteration of stevia products.

In this work, we present the average Raman spectra of rebaudioside A, stevioside and six commercial stevia products. The Raman spectra of the commercial stevia products were compared with the spectra of rebaudioside A and stevioside in order to evaluate the capability of Raman spectroscopy to verify their identity. Further comparisons with the Raman spectra of sodium cyclamate and sodium saccharin were done to determine whether a rapid inspection of Raman spectra of this kind of substances can be used to detect counterfeit stevia products.

2. Material and methods

2.1. Standards and commercial stevia products

The artificial sweeteners sodium cyclamate (analytical grade) and sodium saccharin (analytical grade) as well as the standards of stevioside hydrate (≥98% (HPLC)) and rebaudioside A (≥96% (HPLC)) were purchased from Sigma–Aldrich. Five commercial stevia products, labeled in this work as Samples 1–5, were purchased in local stores in Bolivia. One commercial stevia product, labeled in this work as Sample 6, was purchased in Germany. The declared composition and the appearance of the six commercial stevia products is provided in Table 1.

Six stevia-sodium cyclamate mixtures, containing 1, 5, 10, 30, 50 and 70% of sodium cyclamate were prepared by mixing appropriate amounts of the stevia product purchased in Germany (Sample 6) and sodium cyclamate. The Raman spectra of Sample 6 shows the expected Raman signals for rebaudioside A and stevioside, confirming the composition declared by the distributor.

2.2. Raman spectroscopy and data processing

Raman measurements were performed using a handheld Raman spectrometer, Progeny™ (Rigaku Raman Technologies, Wilmington, MA, USA), equipped with a 1064-nm Nd:YAG laser. The detection of the Raman scattered light was done by a Peltier cooled InGaAs detector. The total exposure time for a Raman spectrum was 5 s, and the laser power was 490 mW. The accuracy and precision of the Raman bands of the acquired spectra were below 3 cm^{-1} and 1.5 cm^{-1}, respectively. The measurements were performed through transparent plastic bags. Ten spectra were recorded for each sample, and each sample was measured at least three times on three different days. The same procedure was applied to the standards of rebaudioside A and stevioside. These spectroscopic data were used to calculate the average Raman spectra for each sample and the mentioned standards. Individual Raman spectra of sodium cyclamate and sodium saccharin were also recorded and used as reference for the interpretation of the bands in the average Raman spectra of the samples. For the evaluation of the stevia-sodium cyclamate mixtures, ten spectra were recorded for each mixture on the same day; the average Raman spectra of the mixtures were used for comparisons.

In order to evaluate the reproducibility of the measurements, another Raman spectrometer of the same model (see above) was used. Twenty spectra were recorded for each sample on two different days (ten samples each day) and these data were used to calculate the average spectra as well as their associated standard deviation (parameter used to evaluate the variations in the spectra between measurements).

In all cases, “R” software [33] was used for data processing (*i.e.*, calculation of the average spectra and standard deviation).

Table 1
Composition of commercial stevia products (Samples 1–6), according to the declared composition.

Sample[a]	Declared composition (label of the product)	Appearance
1	Dulcosides, steviosides, rebaudiosides without additives.	White powder
2	70% stevioside and 30% fructose-free aspartame	White powder
3	Without reported composition	White powder
4	Steviol glycosides and maltodextrins	White powder
5	95% stevioside and 5% maltodextrins	White powder
6	steviosides	White powder

[a] Samples 1–5 were purchased in local stores in Bolivia, while Sample 6 was purchased in Germany.