Characterization of Artificial Sweeteners (Steviana) and Investigation of (Aspartame, Saccharin and Sorbitol) using Raman Spectroscopy

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Most, Photons colliding with molecules do not change their energy after the collision (elastic collision) and the ensuing radiation is called Rayleigh scattering. Rayleigh scattering consists of photons that have the same frequency as the incident light. A very small number of the photons that collide with the, molecules exchange energy with them upon the collision (an example of in elastic collision). If an incident photon delivers an hv_0 quantum of energy to the molecule, the energy of the scattered photon reduces to h (v_0 -v) and the frequency of the scattering photon becomes (v_0 -v). On the contrary,

When an incident photon receives the hv energy from the molecule, the energy of the scattering photon rises to h (v_0+v) , and the frequency of the scattering photon becomes (v_0+v) .Scattering in which an incident photon exchanges energy with a molecule is known as Raman scattering [1].

Scattered light having the frequency of v_0 -v and that having the frequency of vibration v_0 +v are called "Stokes Raman scattering" and "anti-Stokes Raman scattering", respectively.

ABSTRACT

A selection of one common and commercially available table-top artificial Sweetener in Sudanese Markets (Steviana) was considered. The study showed that, the compositions of the sample (steviana) contained sorbitol as a natural low-potency sweetener, and it doesn't contain (Aspartame, Saccharin) as an artificial high-potency sweeteners which they are chemical additives and have hazardous on health. Raman peaks of these compositions were compared with previous studies and analysis, which they were identical with the obtained results. The samples were examined in powder and solvent forms. These samples were irradiated using 6mW Nd-YAG laser with 532 nm ModelHoribaLabRAM HR 3D at room temperature. These spectra provided free Raman fluorescence signatures in identifying the most significant peaks of these sweeteners. The results confirms and indicating excellent potentials of Raman spectroscopy for the assessment of Artificial sweeteners quality.

KEYWORDS: Artificial sweeteners, Natural sweeteners, Laser Raman spectroscopy Introduction:

Raman spectroscopy is a spectroscopic technique used in condensed matter physics and chemistry to study vibrational, rotational and other low frequency modes in a system [1].

It depends on the scattering phenomenon. In this context, scattering occurs due to collisions between photons and molecules. Generally, a photon collides with a substance, not necessarily only with a molecule. Irradiation of light with the frequency v0 upon a certain molecule brings a number of photons with the energy $E = hv_0$ to this molecule.

Stokes Raman scattering arises from interaction between a photon and a molecule that is in the ground state, while anti-Stokes Raman scattering is due to interaction between a photon and a molecule that is in the excited state. At ambient temperatures, most molecular vibrations are in the ground state and thus the anti-Stokes transitions are less likely to occur than the Stokes transitions, resulting in the Stokes Raman scattering being more intense. For this reason, it is usually the Stokes Raman spectrum that is routinely studied [1].

Actually it is the finger print of the molecule so it can be used to identify different materials. This work aimed to use Raman Spectroscopy to identify the compositions of the artificial sweeteners.

Motivation:

Artificial sweeteners have been in use since 1879, that is, since the discovery of Saccharin. Many others have been created since then and are currently being used by increasing numbers of people as an alternative to sugar in

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food and drink. These Sugar substitutes have a sweetening capacity that is hundreds of times greater than that of sugar while providing a pleasant sweet taste. Their very low energy content, such as few kcal per gram, can be regarded as negligible and makes them particularly suitable for weight maintenance or reduction as well as for other dietary needs. They also help in preventing dental decay, since they do not promote the growth of bacteria that cause cavities. The confectionery section of the food industry frequently needs to identify the type of artificial sweetener in a mixture prior to the introduction of the latter into the production chain. Several analytical methods based on different techniques are available for determining artificial sweeteners both qualitatively and quantitatively [2].

Classification of Sweeteners:

Artificial sweeteners have been classified as nutritive and non-nutritive depending on whether they are a source of calories. The nutritive sweeteners include the monosaccharide polyols (e.g., sorbitol, Mannitol, and xylitol) and the disaccharide Polyols (e.g., Maltitol and lactitol). They are approximately equivalent to sucrose in sweetness . The non-nutritive sweeteners, better known as artificial sweeteners, include substances from several different chemical classes that interact with taste receptors and typically exceed the sweetness of sucrose by a factor of 30-13,000 times. Nutritive sweeteners (e.g., sucrose, fructoseCIC and sorbitol) are generally recognized as safe (GRAS) by the Food and Drug Administration (FDA), yet concern exist about increasing sweetener intakes relative to optimal nutrition and health [2].

Health Aspects of Sweeteners:

Both nutritive and non-nutritive sweeteners have generated health concerns among health care providers and the public for many years. Concerns related to safety of non-nutritive sweeteners are addressed primarily in animal studies. Artificial sweeteners are present in many food consumed by whole world. Their use is beneficial in that they provide sweetness, increasing the palatability of food without the added sugar and resulting calories, an important adjunct to weight loss and diet regimens. Most artificial sweeteners are not metabolized by the body and are therefore considered safe. However, scientists disagree about safety because the

Results & Discussion:

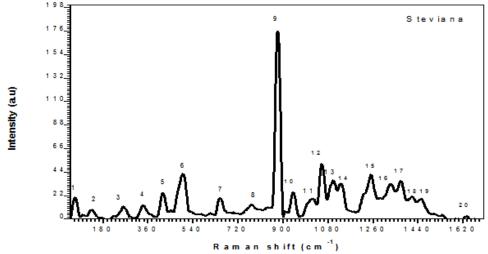


Fig (2) Raman spectrum of steviana powder in the range from 50 to 1700 cm⁻¹

Figure (2) illustrates Raman spectrum of the powder sample (steviana) in the range from 50 to 1700 cm⁻¹. Table (1) lists the main frequencies of the Raman spectra and the vibrational assignments.

metabolites of the "no metabolized" compounds have been shown to produce deleterious effects in mice, rats, and dogs. [3][4].

Material & Method:

Two samples of commercial sweeteners (steviana) ,were Investigated in this work by laser Raman spectrometer in the Range from 50 $\rm cm^{-1}$ to 4000 $\rm cm^{-1} for$ both powders and solutions.

Equipment:

In this work laser Confocal Raman Microscope spectrometer shown in the Figure (1) was used. The light source of this spectrometer is Nd-YAG laser with wavelength of 532 and output power of 6mW. The Raman shift in wave

Number and the change in intensities in of the scattered light were compared with data in the references and previous studies.

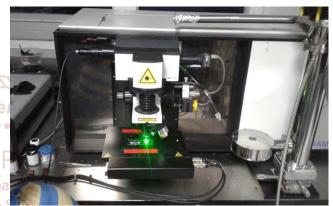


Fig (1) schematic diagram of laser Raman microscope spectrometer.

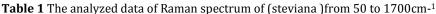
Samples preparation:

Samples were prepared as follows:

Sample: 🔪

- A. (steviana) powder.
- B. 5g of (steviana) powder dissolved in a boiling mineral water (25mL) 100C⁰ with HCl acid 5mL (PH 3.1).

| Table 1 The analyzed data of Raman spectrum of (steviana)from 50 to 1700cm-1 | | | | | | | |
|--|--------------------------------|----------------|--|------------|--|--|--|
| Peak No | Raman Shift (cm ¹) | Intensity (au) | Assignment | Reference | | | |
| 1 | 67 | 20.5 | Skeldef | [6] | | | |
| 2 | 140.0 | 9.4 | υ(0-Ag-0) | [6] | | | |
| 3 | 263.0 | 12.1 | sorbitol | [5][7] | | | |
| 4 | 342.0 | 13.6 | C–O–C sorbitol | [5][7] | | | |
| 5 | 416.3 | 25.6 | 0–H bending | [5][7] | | | |
| 6 | 500.0 | 42.7 | disulphide bridges | [8][7] | | | |
| 7 | 640.0 | 20.7 | Skeletal 5-ring deformation | [8] [7] | | | |
| 8 | 773.0 | 14.3 | twist CH ₃ Ring Stretching | [10][11] | | | |
| 9 | 876.0 | 176 | Skeletal vibration and C–H twisting | [5][7] | | | |
| 10 | 940.0 | 25.7 | C–C stretch of peptide backbone | [5][7] | | | |
| 11 | 1019 | 20.3 | C–NH ₂ stretching | [13] | | | |
| 12 | 1056.0 | 52 | C–O stretching and O–H bending | [5][7] | | | |
| 13 | 1093.0 | 36.7 | C–C stretching and O–H bending | [5][7] | | | |
| 14 | 1134.4 | 33.8 | C–O and C–C stretching, and O–H bending NH ₂ twisting | [5][13][7] | | | |
| 15 | 1250.0 | 60.5 | Amide III | [14][7] | | | |
| 16 | 1325.0 | 42.2 | CH ₂ wagging | [13][7] | | | |
| 17 | 1361.0 | 36.9 | tryptophan amino acid | [8][7] | | | |
| 18 | 1411.5 | 21.4 | CH ₂ , CH ₃ | [15][16] | | | |
| 19 | 1446 | 20 | CH2 bending C−H –CH ₃ | [8][12][7] | | | |
| 20 | 1632 | 3.4 | Asymmetric NH ₂ deformation | [13] | | | |



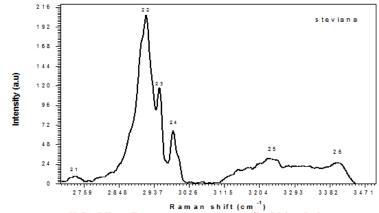


Fig (3) Raman spectrum of steviana powder in the range from 2700 to 3500 cm⁻¹.

Figure (3) illustrates Raman spectrum of the sample (steviana) in the range from 50 to 1700cm⁻¹. Table (2) lists the main frequencies of the Raman spectra and the vibrational assignments.

| Peak No | Raman Shift (cm ⁻¹) | Intensity (au) | Assignment | Reference | | | |
|---|---------------------------------|----------------|---|-----------|--|--|--|
| 21 | 2740.5 | 10.9 | | | | | |
| 22 | 2918.2 | 207.0 | νCH | [8] | | | |
| 23 | 2955.7 | 117.9 | Stretching (C6H ₂ ,C ₅ H) | [8][6] | | | |
| 24 | 2975.1 | 65.5 | νCH | [8] | | | |
| 25 | 3202 | 32 | Stretching (CH ₃) | [6] | | | |
| 26 | 3406 | 26.5 | N-H or O-H | [17] | | | |
| $\begin{array}{c} 224 \\ 196 \\ 168 \\ 168 \\ 140 \\ 112 \\ 84 \\ 56 \\ 28 \\ 28 \\ 28 \\ 7 \\ \end{array}$ | | | | | | | |

Table 2 The analyzed data of Raman spectrum of (steviana) from 2700 to 3500cm⁻¹

Fig (4) Raman shift (cm -1) Fig (4) Raman spectrum of steviana powder dissolved in boiling mineral water and HCL acid (PH3.1) in the range from 500 to 2500 cm⁻¹.

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Figure (4) illustrates Raman spectrum of the dissolved sample (steviana) in the range from 500 to 2500 cm⁻¹. Table (3) lists the main frequencies of the Raman spectra and the vibrational assignments.

| Table 3 The analyzed data of Raman spectrum of the dissolved (steviana) from 500 to 2500cm ⁻¹ | | | | | | |
|---|---------------------------------|----------------|--|-----------|--|--|
| Peak No | Raman Shift (cm ⁻¹) | Intensity (au) | Assignment | Reference | | |
| 27 | 540.0 | 1.7 | disulphide bridges | [9] | | |
| 28 | 620 | 3.7 | - | - | | |
| 29 | 773.0 | 1.8 | twist CH ₃ | [10][7] | | |
| 30 | 876 | 6.4 | Skeletal vibration and C-H twisting | [7][5] | | |
| 31 | 1040 | 11 | C–C and C–O stretching and O–H bending | [5][7] | | |
| 32 | 1117 | 13.2 | Magnesium carbonate | [7] | | |
| 33 | 1298.4 | 11.6 | C–N stretching and C–H bending | [5] | | |
| 34 | 1382 | 14.2 | V (COO) | [13] | | |
| 35 | 1506.0 | 13.6 | Amide II | [13] | | |
| 36 | 1632 | 19 | Asymmetric NH ₂ deformation | [18] | | |
| 37 | 1749 | 13 | C = O stretching N–H bending | [9][12] | | |
| 38 | 1885 | 12 | - | - | | |
| 39 | 1994 | 12 | - | - | | |
| 40 | 2121.0 | 11.8 | sothiocyanat | [18] | | |
| 41 | 2254.0 | 11 | Diazoniu | [16] | | |
| 42 | 2433 | 10 | - | - | | |

Table 3 The analyzed data of Raman spectrum of the dissolved (steviana) from 500 to 2500cm⁻¹

Through the analysis of the two samples it was found that some of the vibration modes of the Steviana sweeteners appeared as Follows:

- 1. vibration modes of (steviana powder) at (263.0, 342.0, 416.0, 599.0, 640.0, 773.0, 642.0, 1446, 876.0 very strong, 940.0, 1056.0, 1093.0, 1134.4, 1250.0, 1325.0) cm⁻¹, which were attributed to the sorbitol ($C_6H_{14}O_6$).
- Vibrational modes at (622.4, 818.0) cm⁻¹ which are attributed to Aspartame (C₁₄H₁₈N₂O₃) are not exist. Vibrational modes at (711.0, 1032, 1154.0, 1300.0) cm⁻¹, which are attributed to Saccharin (C₇H₄NNaO₃S) are not exist.
- 3. Vibrational mode at (1117 cm⁻¹) which is attributed Magnesium carbonate.

Conclusion:

The results presented in this work show that Raman [7] Marleen de Veij,a Peter Vandenabeele, etal ,(2008), spectroscopy technique is an efficient method to identify the compositions of the artificial sweeteners. It provides precise information about components found in the tow samples of Opener Spectroscopy.

the commercial sweeteners.

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