

RAPID IDENTIFICATION OF SOME SWEETENERS AND SUGARS BY ATTENUATED TOTAL REFLECTANCE-FOURIER TRANSFORM INFRARED (ATR-FTIR), NEAR-INFRARED (NIR) AND RAMAN SPECTROSCOPY

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ABSTRACT

The aim of the present study is to compare the vibrational characteristics of various sweeteners with different sugars by using attenuated total reflectance-Fourier transform infrared (ATR-FTIR), near-infrared (NIR) and Raman spectroscopy. For this purpose, ATR-FTIR, NIR and Raman spectra of several sugars and sweeteners were recorded. The spectra of all samples were examined in detail for each technique. Then certain values of sugars (sucrose, galactose, fructose, glucose) and sweeteners (saccharin, sucralose, neotame, acesulfame K and rebaudioside A) were compared. Also, the spectra of binary, triple and quaternary mixtures of some sweeteners and sugars were analyzed. The specific bands of each of the sugars and sweeteners were identified. The obtained spectral information can be used as a reference to develop useful spectral methods for routine controls of sweeteners and sugars. Moreover, the methods will be helpful for the rapid identification of sweeteners and sugars.

Keywords: Sweeteners, sugars, ATR-FTIR spectroscopy, NIR spectroscopy, Raman spectroscopy

BAZI TATLANDIRICI VE ŞEKERLERİN ZAYIFLATILMIŞ TOPLAM YANSIMA-FOURIER DÖNÜŞÜMLÜ KIZILÖTESİ (ATR-FTIR), YAKIN KIZILÖTESİ (NIR) VE RAMAN SPEKTROSKOPİSİ İLE HIZLI TANIMLANMASI

ÖZ

Bu çalışmanın amacı, çeşitli tatlandırıcıların farklı şekerler ile titreşimsel özelliklerinin, zayıflatılmış toplam yansım-Fourier dönüşümlü kızılötesi (ATR-FTIR), yakın kızılötesi (NIR) ve Raman spektroskopisi yöntemleri kullanarak karşılaştırılmasıdır. Bu amaçla; çeşitli şeker ve tatlandırıcıların ATR-FTIR, NIR ve Raman spektrumları kaydedilmiştir. Tüm örneklerin spektrumları her bir teknik için detaylı olarak incelenmiştir. Daha sonra şekerler (sakkaroz, galaktoz, fruktoz, glukoz) ve tatlandırıcılar (sakarın, sükraloz, neotam, asesülfam K ve rebaudioz A) için belirli değerlerin karşılaştırılması yapılmıştır. Ayrıca, bazı tatlandırıcılar ve şekerlerin ikili, üçlü ve dördü karışımlarının spektrumları analiz edilmiştir. Her bir şekerin ve tatlandırıcının spesifik bantları belirlenmiştir. Elde edilen spektral bilgi, tatlandırıcıların ve şekerlerin rutin kontrollerinde faydalı spektral yöntemlerin üretilmesi için referans olarak kullanılabilir. Ayrıca bu maddelerin hızlı bir şekilde tanımlanmasına da katkıda bulunacaktır.

Anahtar kelimeler: Tatlandırıcılar, şekerler, ATR-FTIR spektroskopisi, NIR spektroskopisi, Raman spektroskopisi

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INTRODUCTION

Sweetness in food is usually associated with sucrose, which is the most widely consumed sugar in the world. Because of many health problems associated with 'sugar' consumption, sweeteners are heavily used as sucrose substitutes in various food and beverages. Sweeteners and sugar alternatives in food technology include reduced calorie bulk sweeteners (maltitol, sorbitol etc.) and high intensity sweeteners (acesulfame K, aspartame and neotame, saccharin, sucralose etc.) (O'Donnell and Kearsley 2012). The permitted limit of sweeteners in food and/or beverages causes no safety problems. Nevertheless, it should be noted that synthetic sweeteners like all other purely synthetic food additives are xenobiotic substances which are foreign substances to humans. Therefore, the synthetic sweeteners are recommended to be used only when there is no alternative (Pussa 2008). There are some contradictory results in the literature regarding the safety of the synthetic sweeteners (Feehley and Nagler 2014; Mallikarjun and Sieburth 2015; Soffritti et al. 2014), and also negative consumer attitude towards them (Bearth et al. 2014). Hence, there is a growing interest in natural sweeteners, especially the natural high potency sweeteners.

Analysis and monitoring the content of sweeteners in food and beverage products using various methods (chromatographic, electrophoretic, and mass spectrometric methods, *etc.*) are also the subject of many studies in the literature (Dacome et al. 2005; Pane et al. 2015; Sakai et al. 2015). However, these current methods require the usage of expensive equipment, toxic chemicals and also long preparation steps. Hence, the analytical techniques enabling fast, sensitive and reliable discrimination, detection and analysis of sweeteners and/or sugars for routine control is of great importance. Spectroscopic techniques such as Fourier transform infrared (FTIR) spectroscopy, near-infrared (NIR) and Raman have been widely and successfully used for the rapid and sensitive analysis and authentication of food in recent years (Buyukgoz et al. 2015; Lohumi et al. 2015). However, there are few studies reporting the usage of spectroscopic

techniques for detection of sweeteners (Buyukgoz et al. 2015; Jentzsch et al. 2016b; Peica 2009).

In literature, there are various techniques proposed for the analysis of sweeteners and sugars. Analysis of acesulfame K has been performed using capillary electrophoresis (CE), capillary zone electrophoresis (CZE) and high-performance liquid chromatography (HPLC) (Bergamo et al. 2011; Fernandes et al. 2013; Hannisdal 1992). Neotame has been analyzed using CZE, HPLC and nuclear magnetic resonance spectroscopy (Hu et al. 2013; Yang and Chen 2010; Zell et al. 2000). Sucralose has been detected using liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS), high performance anion-exchange chromatography and CE (Ferrer and Thurman 2010; Hanco and Rohrer 2004; Stroka et al. 2003). LC/TOF-MS, CE, CZE and HPLC are also reported to be used in determination of saccharin (Bergamo et al. 2011; Fernandes et al. 2013; Ferrer and Thurman 2010; Hannisdal 1992). Analysis of rebaudioside A has been performed by using gas chromatography coupled to mass spectrometry (GC-MS) and hydrogen/carbon, nuclear magnetic resonance techniques (Dacome et al. 2005; Jentzsch et al. 2016b). Sugars (glucose, fructose, galactose and sucrose) have been analyzed by HPLC, GC, GC-MS, LC/MS amperometric, CE, CZE techniques (Antiochia et al. 2004; Banos et al. 2000; Bernardes et al. 2004; Carvalho et al. 2003; Cheng et al. 2008; Hui et al. 2009; Kuhnle et al. 2008; Liu et al. 2006; Luo et al. 2012; Periasamy et al. 2011; Rovio et al. 2008). Nowadays, there is a growing interest in the usage of spectroscopic methods for detection and/or analysis of various sweeteners and sugars. In the study of Peica (Peica 2009), surface-enhanced Raman spectroscopy (SERS) was used for the identification and characterization of aspartame. Stability and solubility of it at various pH values were also determined in their study. Buyukgoz et al., (Buyukgoz et al. 2015) also used SERS with silver nanoparticles for the quantification of aspartame in soft drinks. Using characteristic aspartame band of the SERS spectra, the amount of aspartame in water was detected and quantified. In a more recent study by Jentzsch et al., (Jentzsch et al. 2016a), Raman spectroscopy

was also used for the detection of adulteration of stevia products with other sweeteners. In the study of Shim et al., (Shim et al. 2008) ATR-FTIR was used in the analysis of acesulfame K in diet food. There are also some examples of use of Raman or NIR spectroscopy in the analysis of sugars such as sucrose and glucose (İlaslan et al. 2014; Mabood et al. 2015; Silveira et al. 2009).

The aim of this study was to develop the spectra of various sugars (sucrose, galactose, fructose, glucose) and sweeteners (saccharin, sucralose, neotame, acesulfame K and rebaudioside A) to show the differences among them to be used in generation of spectral methods and identify the specific band assignments of sugars and sweeteners in mixtures by using spectroscopic methods which could make a significant contribution to the rapid discrimination of sweeteners and sugars, and their quantitative analyses for routine control.

MATERIAL AND METHOD

Materials

The materials in this study were the widely used sugars and sweeteners. Acesulfame K analytical standard was provided from Supelco (Bellefonte, PA, USA). Neotame and sucralose standards were supplied from Sigma-Aldrich (St. Louis, MO, USA). High purity (99.5%) D(+)-glucose, D(+)-galactose and D(-)-fructose were provided from Sigma-Aldrich (St. Louis, MO, USA). Sucrose was purchased from Merck (Darmstadt, Germany). All of the samples were kept in the dark at room temperature until analyzed.

The binary, triple and quaternary mixtures of various sweeteners and sugars were also prepared gravimetrically in equal amounts. Accordingly, rebaudioside A and neotame constituted the binary mixture. Two different triple mixtures were prepared, containing Acesulfame K, neotame and sucralose as the first, and acesulfame K, rebaudioside A and glucose as the second one. Mixture of saccharin, glucose, galactose and neotame were used as the quaternary mixture. The mixture samples were also analyzed and specific bands assignments were shown in ATR-FTIR, NIR and Raman spectra.

ATR-FTIR, NIR and Raman spectroscopy measurements

The ATR-FTIR spectra were measured using a Thermo Nicolet iS50 FTIR (Thermo Fisher Scientific Co., Waltham, MA) spectrometer equipped with a single bounce diamond crystal and a deuterated triglycine sulfate detector. The sweetener powders were placed on the crystal and thus the evanescent wave could be absorbed by the powder. The ATR-FTIR spectra of sweetener samples were determined to be in the range of 400-4000 cm^{-1} with a resolution of 4 cm^{-1} . Each spectrum was collected from 32 scans in the absorbance mode.

NIR analysis was carried out using a Thermo Nicolet iS50 FTIR (Thermo Fisher Scientific Co., Waltham, MA) spectrometer with the smart diffuse reflectance accessory which maximizes the collection of diffusely scattered radiation, while minimizing the specular effect, resulting in the highest quality spectral result. The NIR spectra of sweetener samples were determined in the range of 4000-10000 cm^{-1} with a resolution of 8 cm^{-1} . Each spectrum was collected from 32 scans in the absorbance mode.

A DeltaNu Examiner Raman microscope (DeltaNu Inc., Laramie, WY) was used with a 785 nm laser source, motorized microscope with a stage sample holder and a CCD detector. The instrument parameters were as follows: 60 mW laser power and 60 s acquisition time. The spectra were obtained in the range of 200-2000 cm^{-1} . NuSpec™ spectra software was used for baseline correction of all Raman spectra. All measurements were performed at room temperature. Three measurements were averaged to produce one spectrum for each ATR, NIR and Raman measurements.

RESULTS AND DISCUSSION

ATR-FTIR characterization

ATR-FTIR spectra of samples were given in Fig. 1. The wavenumbers of the strongest bands were also given in Table 1 (Silverstein et al. 1981). As it can be seen from the Fig. 1, different characteristic bands were observed for all samples.

Table 1. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) bands of sweeteners and sugars.

Compound	ATR-FTIR bands (cm ⁻¹)
Acesulfame K	724(δ =C-H), 940(δ =C-H), 1173(stC-O, stC-N), 1290(stC-O, stC-N), 1589(stC=C)
Neotame	699(δ =C-H), 757(δ =C-H), 1540(stC=C), 1690(stC=O), 1728(stC=O), 2955(stO-H)
Sucralose	618(stC-Cl), 642(stC-Cl), 857(δ =C-H), 890(δ =C-H), 1001(stC-O), 1030(stC-O), 1092(stC-O), 3457(stO-H)
Saccharin	701(δ =C-H), 757(δ =C-H), 897(δ =C-H), 1175(stC-O, stC-N), 1332(stC-N), 1715(stC=O)
Rebaudioside A	888(δ =C-H), 988(δ =C-H), 1035(stC-O), 1066(stC-O), 1728(stC=O), 3356(stO-H)
Glucose	994(δ =C-H), 1019(stC-O), 1107(stC-O), 1146(stC-O), 3255(stO-H)
Fructose	781(δ =C-H), 976(δ =C-H), 1049(stC-O), 1147(stC-O), 3402(stO-H), 3521(stO-H)
Galactose	763(δ =C-H), 835(δ =C-H), 954(δ =C-H), 1043(stC-O), 3125(stO-H), 3200(stO-H), 3382(stO-H)
Sucrose	907(δ =C-H), 987(δ =C-H), 1049(stC-O), 1115(stC-O), 3327(stO-H)

δ =deformation, st=stretching

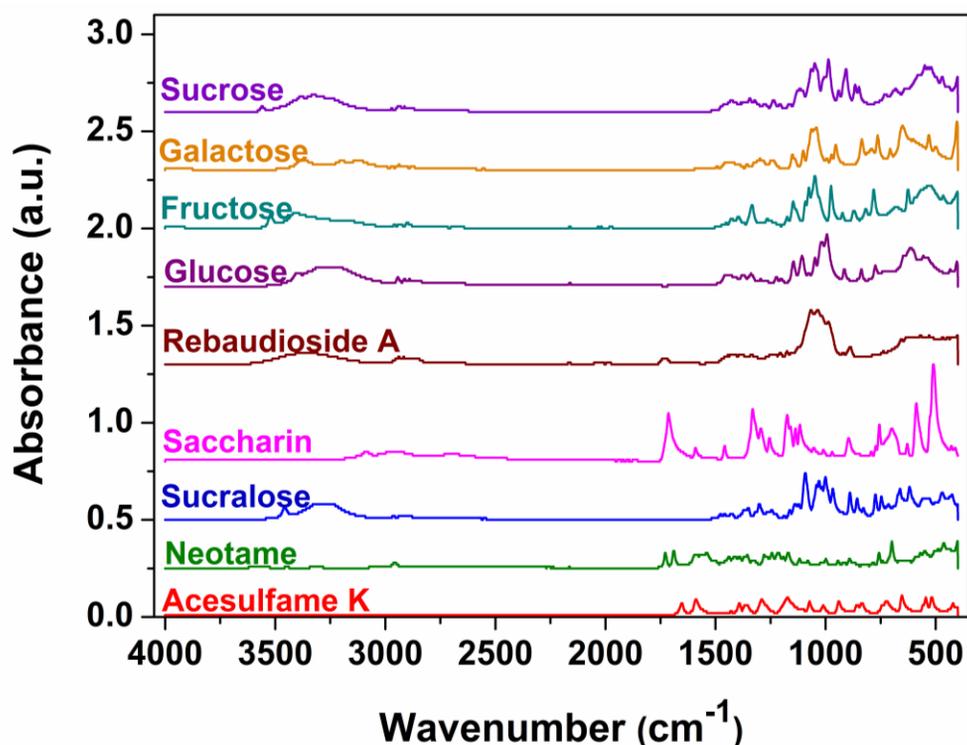


Fig. 1. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of sweeteners and sugars.

The strong intensity band of acesulfame K observed at 1173 cm^{-1} was attributed to stretching of C-O and C-N. Other significant bands with medium located around 1290 and 1589 cm^{-1} were assigned to stretching of C-O and C-N and stretching of C=C, respectively. The weak bands located around 724 and 940 cm^{-1} were assigned as deformation of C-H units.

The strong band of neotame located at 699 cm^{-1} was attributed to deformation of C-H. Other significant bands with medium intensity located around 757 , 1540 , 1690 and 1728 cm^{-1} were assigned to deformation of C-H, stretching of C=C, C=O and C=O, respectively. The weak band located at 2955 cm^{-1} was assigned as stretching of O-H.

The most intense band of sucralose located at 1092 cm^{-1} was assigned as stretching of C-O. Other significant bands with medium intensity located around 618 , 642 , 857 , 890 , 1001 and 1030 cm^{-1} were obtained. The bands observed at 618 and 642 cm^{-1} were assigned as stretching of C-Cl. The two bands observed at 857 and 890 cm^{-1} were assigned as deformation of C-H. The bands located at 1001 and 1030 cm^{-1} were both attributed to C-O stretching. The weak band located at 3457 cm^{-1} was assigned as stretching of O-H.

The sharp band of saccharin observed at 701 cm^{-1} was associated to a C-H deformation. Other bands with medium intensity located around 1175 , 1332 and 1715 cm^{-1} were found. The band observed at 1175 cm^{-1} was assigned as stretching of C-O and C-N. Another medium intensity band located at 1332 cm^{-1} was associated to a C-N stretching. The band located at 1715 cm^{-1} was attributed to stretching C=O. The weak bands located at 757 and 897 cm^{-1} were assigned as deformation of C-H units.

The strong intensity bands of rebaudioside A located at 1035 and 1066 cm^{-1} were attributed to stretching of C-O. The medium band of located around 988 cm^{-1} was assigned to deformation of C-H. Other bands of with weak located around 888 , 1728 and 3356 cm^{-1} were assigned to

deformation of C-H, stretching of C=O and stretching of O-H, respectively.

The strong bands of glucose observed around 994 and 1019 cm^{-1} were assigned to deformation of C-H and stretching of C-O, respectively. The medium intensity band located at 1107 cm^{-1} was attributed to stretching of C-O. The weak bands located at 1146 and 3255 cm^{-1} were associated to stretching of C-O and O-H, respectively.

The most intense band of fructose observed at 1049 cm^{-1} was attributed to stretching of C-O. The medium bands located around 781 and 976 cm^{-1} were both associated to C-H deformation units. The weak bands located around 1147 , 3402 and 3521 cm^{-1} were obtained. The band observed at 1147 cm^{-1} was assigned to stretching of C-O. The two bands observed at 3402 and 3521 cm^{-1} were attributed to stretching of O-H.

The sharp band of galactose located at 1043 cm^{-1} was assigned to stretching of C-O. Other significant bands with medium intensity located around 763 , 835 and 954 cm^{-1} were all associated to deformation of C-H. The weak bands located around 3125 , 3200 and 3382 cm^{-1} were all assigned to stretching of O-H.

The strong intensity of sucrose band located at 987 cm^{-1} was attributed to C-H deformation. The medium bands located around 907 and 1049 cm^{-1} were both assigned to C-H deformation. The weak bands located around 1115 and 3327 cm^{-1} were associated to stretching of C-O and O-H, respectively.

NIR characterization

NIR spectroscopy gives information about the overtones and combinations of fundamental vibrations. High vibrational frequencies of chemical bonds between light atoms, such as C-H, O-H, and N-H, result in overtone and combination bands which are detectable in region of $780\text{-}2500\text{ nm}$ (Osborne et al. 1993).

NIR spectra of samples are given in Fig. 2. The wavenumbers of the strongest bands are also given in Table 2 (Goddu and Delker 1960; Kaye

1954; Workman 2000). As it can be seen from Fig. 2, different characteristic bands were defined for all samples.

Table 2. Near-infrared (NIR) bands of sweeteners and sugars.

Compound	NIR bands (cm ⁻¹)
Acesulfame K	4028(CH combinations), 4321(CH, CH ₃ combinations), 4380(CH ₃ combinations), 4438(CH ₃ combinations), 5748(CH 1 st overtone), 5883(CH, CH ₃ 1 st overtone combinations), 6071(CH ₃ 1 st overtone).
Neotame	4039(CH combinations), 4438(CH ₃ combinations), 5197(CONH ₂ , RCO ₂ R ¹ 1 st overtone combinations), 6958(CH, ROH 2 nd overtone combinations).
Sucralose	4046(CH combinations), 4413(CH ₂ combinations), 5766(CH, CH ₂ 1 st overtone), 5848(CH, CH ₂ 1 st overtone combinations).
Saccharin	4130(CH combinations), 4427(CH ₃ combinations), 4680(RNH ₂ combinations), 6040(CH ₃ 1 st overtone), 8889(CH ₃ 2 nd overtone).
Rebaudioside A	4290(CH, CH ₂ , CH ₃ combinations), 4830(ROH combinations), 5211(RCO ₂ R ¹ 1 st overtone), 6983(CH, ROH 2 nd overtone).
Glucose	4219(CH, CH ₂ combinations), 4280(CH, CH ₂ combinations), 4388(CH ₂ combinations), 8345(CH, CH ₂ 2 nd overtone).
Fructose	4038(CH combinations), 4799(ROH combinations), 5833(CH, CH ₂ 1 st overtone combinations), 5944(CH, CH ₂ 1 st overtone combinations), 6831(ROH 2 nd overtone), 7287(CH ₂ 2 nd overtone), 8233(CH 2 nd overtone).
Galactose	4035(CH combinations), 4143(CH combinations), 4332 (CH, CH ₂ combinations), 4783(ROH combinations), 5774(CH ₂ 1 st overtone), 8360 (CH, CH ₂ 2 nd overtone combinations)
Sucrose	4313(CH, CH ₂ combinations), 4821(ROH combinations), 5914(CH, CH ₂ 1 st overtone combinations), 6960(CH, ROH 2 nd overtone combinations).

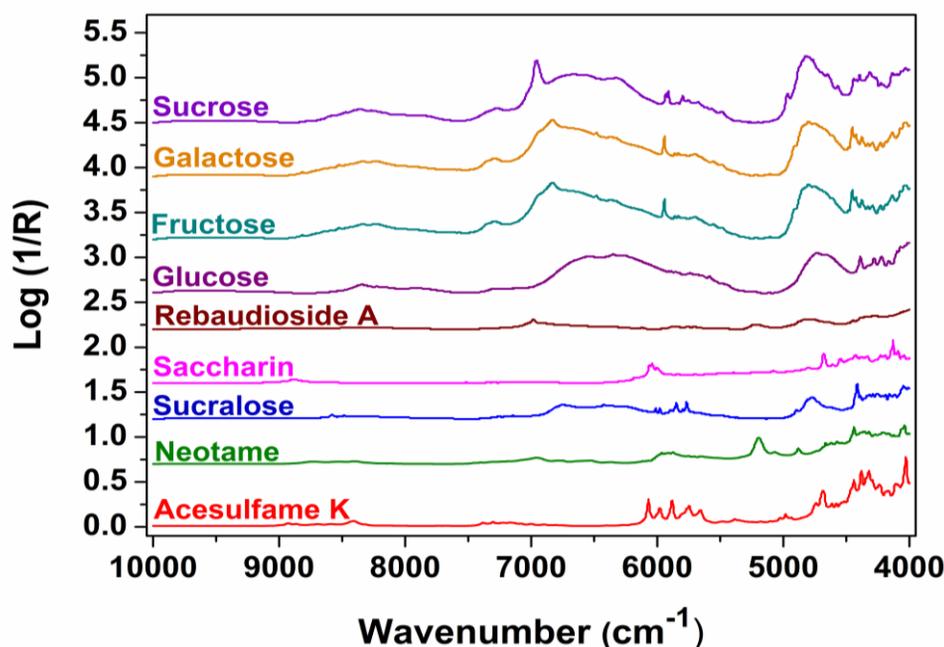


Fig. 2. Near-infrared (NIR) spectra of sweeteners and sugars.

The strong band of acesulfame K located at 4028 cm^{-1} was attributed to CH combinations. Other significant bands with medium intensity located around 4321 and 4380 cm^{-1} were obtained. The band observed at 4321 cm^{-1} was assigned to CH and CH_3 combinations. The band observed at 4380 cm^{-1} was associated to CH_3 combinations. The weak intensity bands around 4438 , 5748 , 5883 and 6071 cm^{-1} were obtained. The bands observed around 4438 and 5748 cm^{-1} were associated to CH_3 and CH 1st overtone combinations, respectively. The band observed at 5883 cm^{-1} was assigned to CH and CH_3 1st overtone combinations. The band located at 6071 cm^{-1} was assigned to CH_3 1st overtone.

The strong intensity bands of neotame observed around 4039 and 4438 cm^{-1} were attributed to CH and CH_3 combinations, respectively. The medium band located at 5197 cm^{-1} was associated to CONH_2 and RCO_2R^1 1st overtone combinations. The weak band located at 6958 cm^{-1} was assigned to CH and ROH 2nd overtone combinations.

The strong intensity band of sucralose observed at 4413 cm^{-1} was assigned to CH_2 combinations. The medium intensity band located at 4046 cm^{-1} was attributed to CH combinations. The weak bands located around 5766 and 5848 cm^{-1} were both assigned to CH and CH_2 1st overtone combinations.

The sharp intensity band of saccharin located at 4130 cm^{-1} was associated to CH combinations. Other significant bands with medium intensity located around 4427 , 4680 and 6040 cm^{-1} were attributed to CH_3 , RNH_2 and CH_3 1st overtone combinations. The weak band located at 8889 cm^{-1} was assigned to CH_3 2nd overtone.

The strong intensity band of rebaudioside A observed at 4290 cm^{-1} was attributed to CH, CH_2 and CH_3 combinations. The medium bands located around 4830 and 6983 cm^{-1} were obtained. The band observed at 4830 cm^{-1} was associated to ROH combinations. Another medium intensity band observed at 6983 cm^{-1} was associated to CH and ROH 2nd overtone

combinations. The weak intensity band located at 5211 cm^{-1} was assigned to RCO_2R^1 1st overtone.

The strong intensity bands of glucose located around 4219 , 4280 and 4388 cm^{-1} were attributed to CH and CH_2 combinations. The weak band located at 8345 cm^{-1} was associated to CH and CH_2 2nd overtone combinations.

The strong bands of fructose located around 4799 and 6831 cm^{-1} were assigned to ROH and ROH 2nd overtone combinations, respectively. The medium intensity bands located around 4038 and 5944 cm^{-1} were obtained. The band observed at 4038 cm^{-1} was associated to CH combinations. Another medium intensity band observed at 5944 cm^{-1} was associated to CH, CH_2 1st overtone combinations. The weak intensity bands located around 5833 , 7287 and 8233 cm^{-1} were attributed to CH, CH_2 1st, CH_2 2nd and CH 2nd overtone combinations.

The strong intensity bands of galactose observed around 4035 and 4783 cm^{-1} were associated to CH and ROH combinations. The medium bands located around 4143 , 4332 and 5774 cm^{-1} were obtained. The band observed at 4143 cm^{-1} was assigned to CH combinations. The band located at 4332 cm^{-1} was assigned to CH, CH_2 combinations. Another medium intensity band observed at 5774 cm^{-1} was assigned to CH_2 1st overtone. The weak band located at 8360 cm^{-1} was attributed to CH, CH_2 2nd overtone combinations.

The strong intensity bands of sucrose located around 4821 and 6960 cm^{-1} were obtained. The band observed at 4821 was associated to ROH combinations. Another strong band located at 6960 was associated to CH, ROH 2nd overtone combinations. The medium intensity located at 4313 cm^{-1} was assigned to CH, CH_2 combinations. The weak intensity located at 5914 cm^{-1} was attributed to CH, CH_2 1st overtone combinations.

Raman characterization

Raman spectroscopy presents detailed information about molecular vibrations which

depend on strength and types of chemical bonds (Zhu et al. 2011). Raman spectra of samples were given in Fig. 3. The wavenumbers of the strongest

bands were also given in Table 3 Anonymous, 2018; Torres 2007). Fig. 3 shows the different characteristic bands observed for the samples.

Table 3. Raman bands of sweeteners and sugars.

Compound	Raman bands (cm ⁻¹)
Acesulfame K	290(δ C-C _{aliphatic}), 350(δ C-C _{aliphatic} , δ C-CH ₃), 396(δ C-C _{aliphatic}), 547(δ C-C-O, δ C-CH ₃), 659(ν C-C _{alicyclic,aliphatic}), 747(ν C-C _{alicyclic,aliphatic}).
Neotame	263(δ C-C _{aliphatic}), 509(δ C-C-O, δ C-CH ₃), 620(ν C-C _{alicyclic,aliphatic}), 759(ν C-C _{alicyclic,aliphatic}), 946(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 1003(ν C-C _{alicyclic,aliphatic}), 1018(ν C-C _{alicyclic,aliphatic}), 1207(δ C-H _{ring} , ν C-C _{alicyclic,aliphatic}).
Sucralose	353(δ C-C _{aliphatic} , δ C-O-C, δ C-C-H ₃), 536(δ C-C-O, δ C-CH ₃), 624(ν C-Cl, ν C-C _{alicyclic,aliphatic}), 664(ν C-Cl, ν C-C _{alicyclic,aliphatic}), 710(ν C-Cl, ν C-C _{alicyclic,aliphatic}), 745(ν C-Cl, ν C-C _{alicyclic,aliphatic}), 775(ν C-Cl, ν C-C _{alicyclic,aliphatic}).
Saccharin	344(δ C-C _{aliphatic}), 384(δ C-C _{aliphatic}), 523(δ C-C-O), 704(ν C-S _{aliphatic} , ν C-C _{alicyclic,aliphatic}), 777(δ C-H, ν C-S _{aliphatic} , ν C-C _{alicyclic,aliphatic}), 1018(ν C-C _{alicyclic,aliphatic}), 1179(δ C-H _{ring} , ν C-C _{alicyclic,aliphatic}), 1595(ν C-C _{alicyclic,aliphatic} , ν C=C), 1698(ν C=C, ν C=O).
Rebaudioside A	355(δ C-C _{aliphatic} , δ C-O-C, δ C-CH ₃), 432(δ C-C-O), 739(ν C-C _{alicyclic,aliphatic}), 897(ν C-C _{alicyclic,aliphatic} , ν C-O-C), 1123(ν C-C _{alicyclic,aliphatic}), 1203(δ C-H _{ring} , ν C-C _{alicyclic,aliphatic}).
Glucose	346(δ C-C _{aliphatic}), 406(δ C-C _{aliphatic}), 542(δ C-C-O, δ C-CH ₃), 841(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 915(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 1074(ν C-O-C _{asym} , ν C-C _{alicyclic,aliphatic}), 1121(ν C-O-C _{asym} , ν C-C _{alicyclic,aliphatic}), 1345(δ C-H _{ring}).
Fructose	421(δ C-C-O), 525(δ C-C-O), 594(δ C-C-O), 627(ν C-C _{alicyclic,aliphatic}), 818(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 872(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 926 (ν C-O-C, ν C-C _{alicyclic,aliphatic}), 978(ν C-C _{alicyclic,aliphatic}) 1081(ν C-O-C _{asym} , ν C-C _{alicyclic,aliphatic}).
Galactose	358(δ C-C _{aliphatic} , δ C-O-C), 417(δ C-C _{aliphatic}), 532(δ C-C-O), 660(ν C-C _{alicyclic,aliphatic}), 704(ν C-C _{alicyclic,aliphatic}), 830(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 889(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 1068(ν C-O-C _{asym} , ν C-C _{alicyclic,aliphatic}), 1248(ν C-C _{alicyclic,aliphatic}).
Sucrose	402(δ C-C _{aliphatic}), 525(δ C-C-O, δ C-CH ₃), 640(ν C-C _{alicyclic,aliphatic}), 850(ν C-O-C, ν C-C _{alicyclic,aliphatic}), 922 (ν C-O-C, ν C-C _{alicyclic,aliphatic}), 1039(δ C-H _{ring} , ν C-C _{alicyclic,aliphatic}), 1124(ν C-O-C _{asym} , ν C-C _{alicyclic,aliphatic}).

δ =deformation, ν =vibration, t =torsion, st =stretching

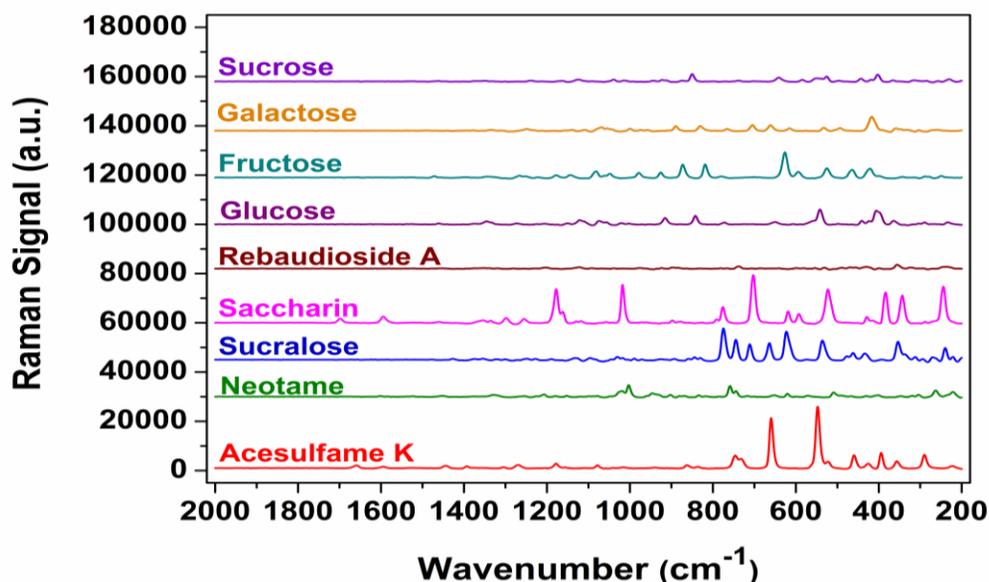


Fig. 3. Raman spectra of sweeteners and sugars.

The most intense bands of acesulfame K observed around 547 and 659 cm^{-1} were obtained. The band located at 547 cm^{-1} was assigned to deformation of C-C-O and C-CH₃. The band observed at 659 cm^{-1} was assigned to vibration of C-C_{alicyclic,aliphatic}. Other significant bands with medium intensity located around 290, 396 and 747 cm^{-1} were attributed to deformation of C-C_{aliphatic} and vibration of C-C_{alicyclic,aliphatic}. The weak band located at 350 cm^{-1} was associated to deformation of C-C_{aliphatic}.

The strong bands of neotame located around 759 and 1003 cm^{-1} were assigned to vibration of C-C_{alicyclic,aliphatic}. Medium intensity bands located around 263, 509, 946 and 1018 cm^{-1} were obtained. The bands observed around 263 and 1018 cm^{-1} were associated to deformation of C-C_{aliphatic} and vibration of C-C_{alicyclic,aliphatic}. The band located at 509 cm^{-1} was attributed to deformation of C-C-O and C-CH₃. The band observed at 946 cm^{-1} was attributed to vibration of C-O-C and C-C_{alicyclic,aliphatic}. The weak intensity bands located around 620 and 1207 cm^{-1} were assigned to vibration of C-C_{alicyclic,aliphatic} and deformation of C-H_{ring}.

The strong intensity bands of sucralose located around 624 and 775 cm^{-1} were both associated to

vibration of C-Cl and C-C_{alicyclic,aliphatic}. The medium bands located around 353, 536, 664, 710 and 745 were obtained. The band observed at 353 cm^{-1} was attributed to deformation of C-C_{aliphatic}, C-O-C and C-CH₃. The band observed at 536 cm^{-1} was assigned to deformation of C-C-O and C-CH₃. The bands located around 664, 710 and 745 cm^{-1} were associated to vibration of C-Cl and C-C_{alicyclic,aliphatic}.

The strong intensity band of saccharin observed at 704 cm^{-1} was attributed to vibration of C-S_{aliphatic} and C-C_{alicyclic,aliphatic}. The medium intensity bands located around 344, 384, 523, 1018 and 1179 cm^{-1} were obtained. The two bands observed around 344 and 384 cm^{-1} were assigned to deformation of C-C_{aliphatic}. The band located at 523 cm^{-1} was assigned to deformation of C-C-O. Another medium intensity band located at 1018 cm^{-1} was associated to deformation of C-C_{alicyclic,aliphatic}. The last medium intensity band observed at 1179 cm^{-1} was associated to deformation of C-H_{ring} and vibration of C-C_{alicyclic,aliphatic}. The weak intensity bands located around 777, 1595 and 1698 cm^{-1} were obtained. The band located at 777 cm^{-1} was attributed to vibrations of C-S_{aliphatic} and C-C_{alicyclic,aliphatic}. Another weak band observed at 1595 cm^{-1} was assigned to vibration of C-C_{alicyclic,aliphatic} and C=C.

The band observed at 1698 cm^{-1} was associated to vibration of C=C and C=O.

The sharp band of rebaudioside A observed at 355 cm^{-1} was attributed to deformation of C-C_{aliphatic}, C-O-C and C-CH₃. Other significant bands with medium intensity located around 432 and 739 cm^{-1} were assigned to deformation of C-C-O and vibration of C-C_{alicyclic,aliphatic}, respectively. The weak intensity bands located around 897 , 1123 and 1203 cm^{-1} were obtained. The band observed at 897 cm^{-1} was associated to vibration of C-C_{alicyclic,aliphatic} and C-O-C. The band located at 1123 cm^{-1} was associated to vibration of C-C_{alicyclic,aliphatic}. The band located at 1203 cm^{-1} was attributed to deformation of C-H_{ring} and vibration of C-C_{alicyclic,aliphatic}.

The strong intensity bands of glucose located around 406 and 542 cm^{-1} were assigned to deformation of C-C_{aliphatic} and C-C-O, respectively. The medium bands located around 841 and 915 cm^{-1} were both associated to vibration of C-O-C and C-C_{alicyclic,aliphatic}. The weak intensity bands located around 346 , 1074 , 1121 and 1345 cm^{-1} were obtained. The band observed at 346 cm^{-1} was attributed to deformation of C-C_{aliphatic}. The bands located around 1074 and 1121 cm^{-1} were both assigned to vibration of C-O-C_{asym} and C-C_{alicyclic,aliphatic}. The band observed at 1345 cm^{-1} was assigned to deformation of C-H_{ring}.

The most intense bands of fructose located at 627 cm^{-1} was associated to vibration of C-C_{alicyclic,aliphatic}. Other significant bands with medium intensity located around 421 , 525 , 818 and 872 cm^{-1} were obtained. The band observed at 421 cm^{-1} was attributed to deformation of C-C-O. The band located at 525 cm^{-1} was attributed to deformation of C-C-O and C-CH₃. Another medium intensity bands observed around 818 and 872 cm^{-1} were both assigned to vibration of C-O-C and C-C_{alicyclic,aliphatic}. The weak intensity bands located around 594 , 926 , 978 and 1081 cm^{-1} were obtained. The band observed at 594 cm^{-1} was associated to deformation of C-C-O. The band located at 926 cm^{-1} was associated to vibration of C-O-C and C-C_{alicyclic,aliphatic}. Another weak band located at 978 cm^{-1} was attributed to vibration of

C-C_{alicyclic,aliphatic}. The band observed at 1081 cm^{-1} was assigned to vibration of C-O-C_{asym} and C-C_{alicyclic,aliphatic}.

The sharp intensity band of galactose observed at 417 cm^{-1} was attributed to deformation of C-C_{aliphatic}. The medium bands located around 660 , 704 , 830 , 889 and 1068 cm^{-1} were obtained. The bands observed around 660 and 704 cm^{-1} were both associated to vibration of C-C_{alicyclic,aliphatic}. The bands located around 830 and 889 were both associated to vibration of C-O-C and C-C_{alicyclic,aliphatic}. The band located at 1068 was assigned to vibration of C-O-C_{asym} and C-C_{alicyclic,aliphatic}. The weak intensity bands located around 358 , 532 and 1248 cm^{-1} were obtained. The band located at 358 cm^{-1} was attributed to deformation of C-C_{aliphatic} and C-O-C. Another weak intensity band observed at 532 cm^{-1} was assigned to deformation of C-C-O. The band located at 1248 cm^{-1} was assigned to vibration of C-C_{alicyclic,aliphatic}.

The strong bands of sucrose located around 402 and 850 cm^{-1} were obtained. The band observed at 402 cm^{-1} was associated to deformation of C-C_{aliphatic}. Another strong intensity band located at 850 cm^{-1} was associated to vibration of C-O-C and C-C_{alicyclic,aliphatic}. The medium intensity bands located around 525 and 640 cm^{-1} were obtained. The band located at 525 cm^{-1} was assigned to deformation of C-C-O and C-CH₃. Another medium intensity band observed at 640 cm^{-1} was assigned to vibration of C-C_{alicyclic,aliphatic}. The weak intensity bands were located around 922 , 1039 and 1124 cm^{-1} were obtained. The band located at 922 cm^{-1} was attributed to vibration of C-O-C and C-C_{alicyclic,aliphatic}. Another band observed at 1039 cm^{-1} was attributed to deformation of C-H_{ring} and vibration of C-C_{alicyclic,aliphatic}. The band observed at 1124 cm^{-1} was associated to vibration of C-O-C_{asym} and C-C_{alicyclic,aliphatic}.

The usage of spectral data of various sweeteners and sugars has great potential for the development of novel, fast, reliable and low cost methods for the simultaneous detection of these sweeteners and sugars. The obtained spectra in the present study could serve as reference for the interpretation of spectral studies of various food

and beverage products containing sugars and/or sweeteners. In order to show the applicability of these data, the analysis of binary, triple and quaternary mixtures were also performed and specific bands were identified. The spectra of the binary mixture of rebaudioside A and neotame, and specific bands assigned to ATR-FTIR, NIR and Raman spectra were given in Fig. 4. The

graphs of two different triple mixtures (acesulfame K, neotame and sucralose) and (acesulfame K, rebaudioside A and glucose) were given in Fig.5 and Fig. 6, respectively. In Fig. 7, the graphs of the quaternary mixture of saccharin, glucose, galactose and neotame and band assignments were seen.

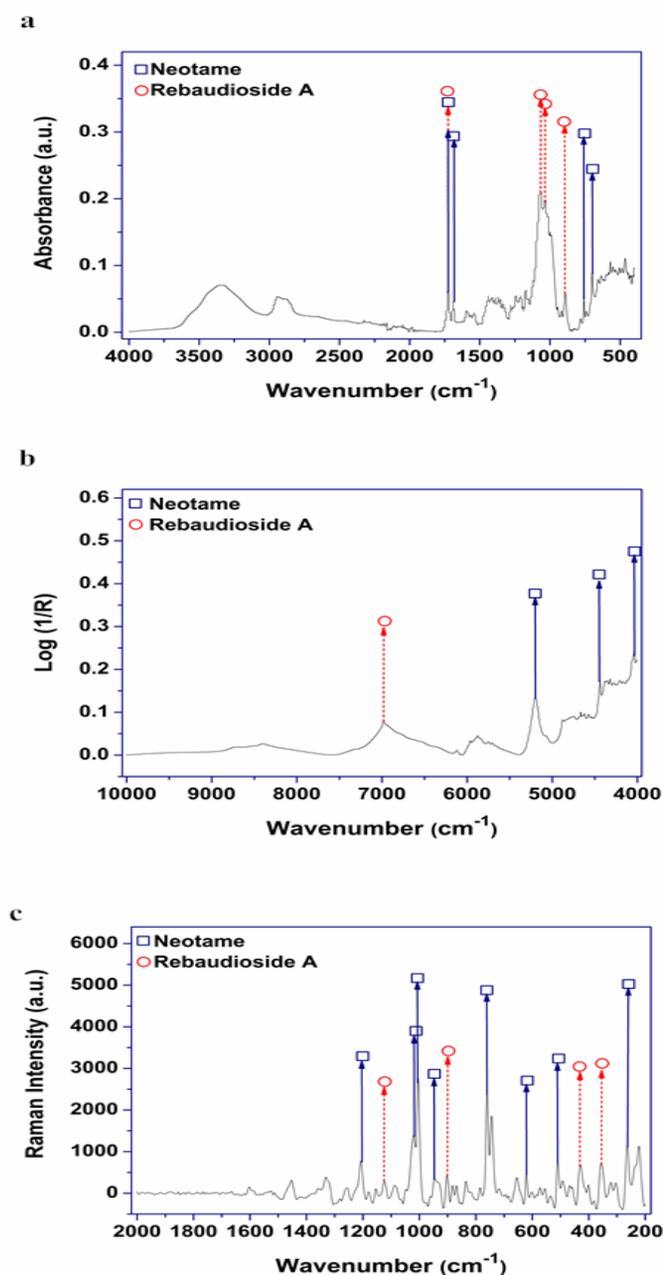


Fig. 4. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR), near-infrared (NIR) and Raman spectra of rebaudioside A and neotame mixture.

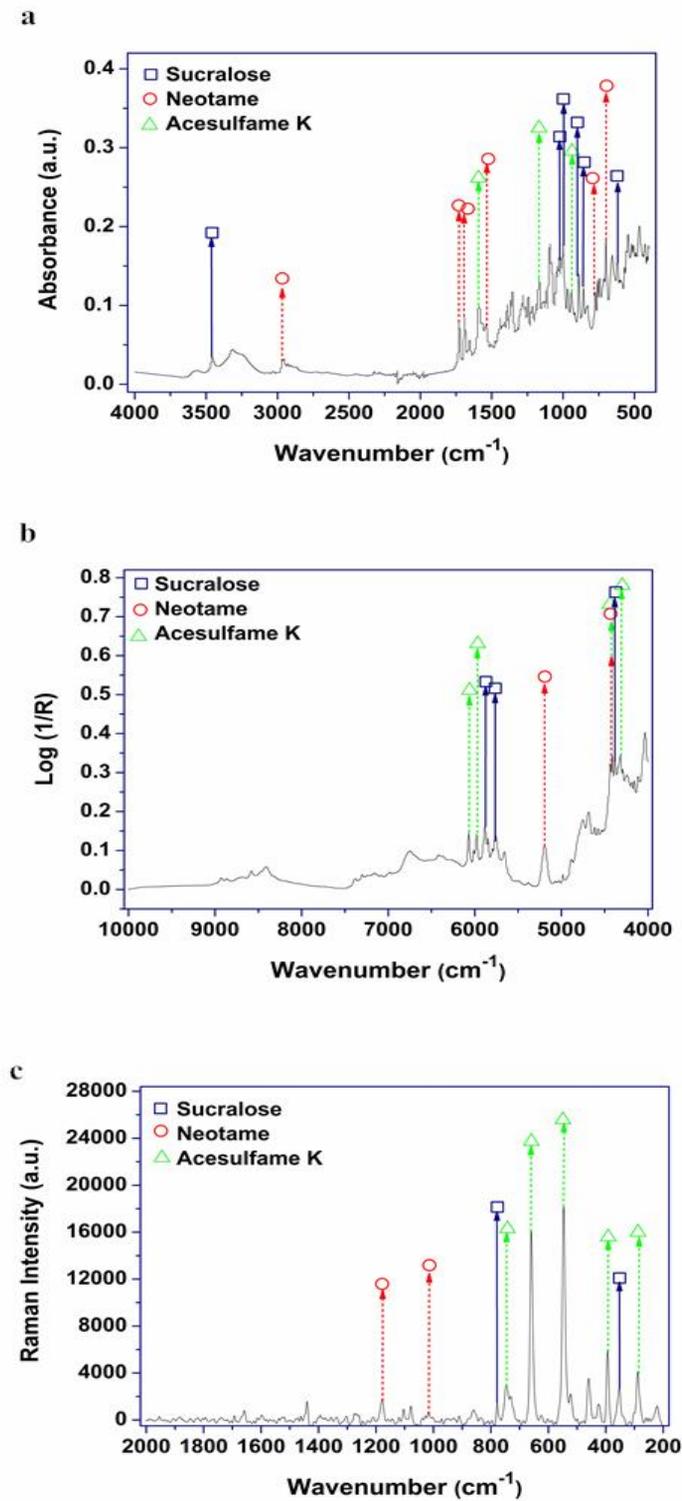


Fig. 5. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) (a), near-infrared (NIR) (b) and Raman (c) spectra of acesulfame K, neotame and sucralose mixture

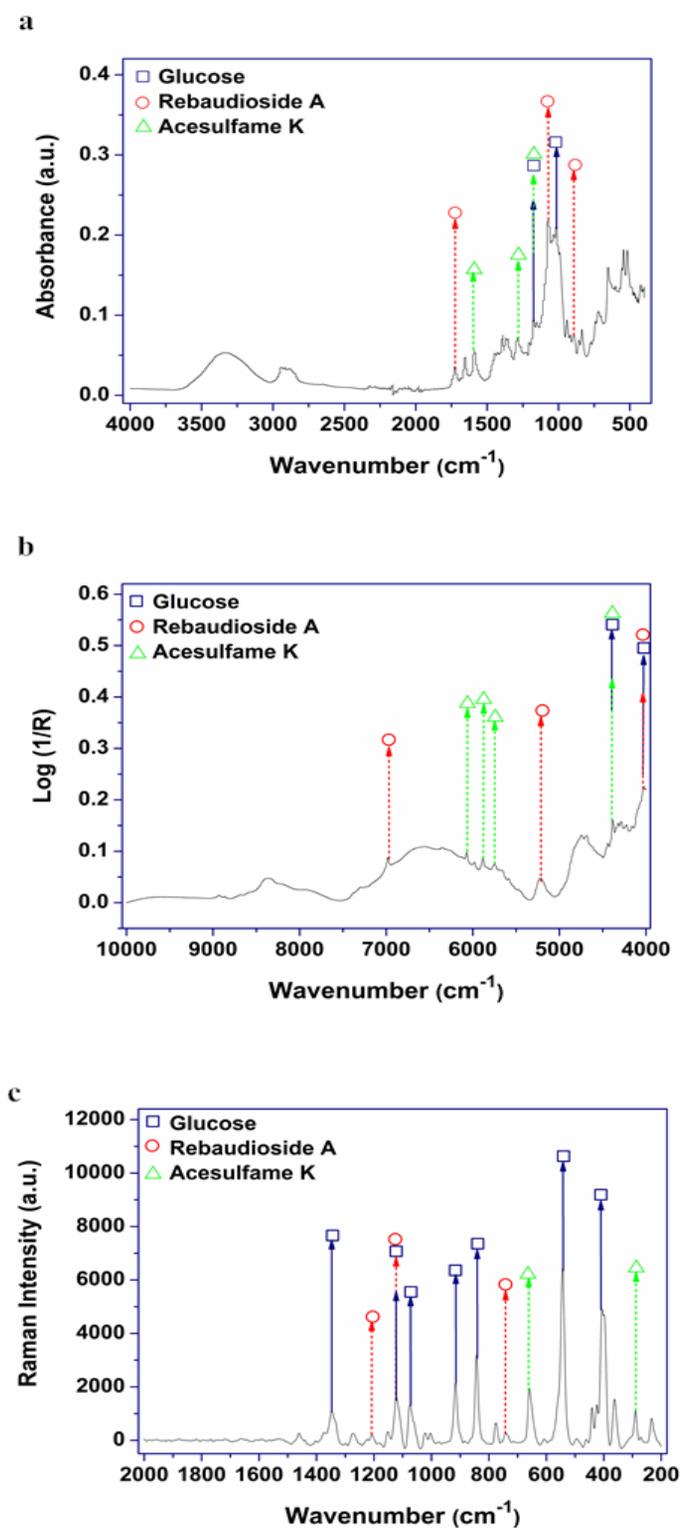


Fig. 6. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) (a), near-infrared NIR (b) and Raman (c) spectra of rebaudioside A, glucose and acesulfame K mixture.

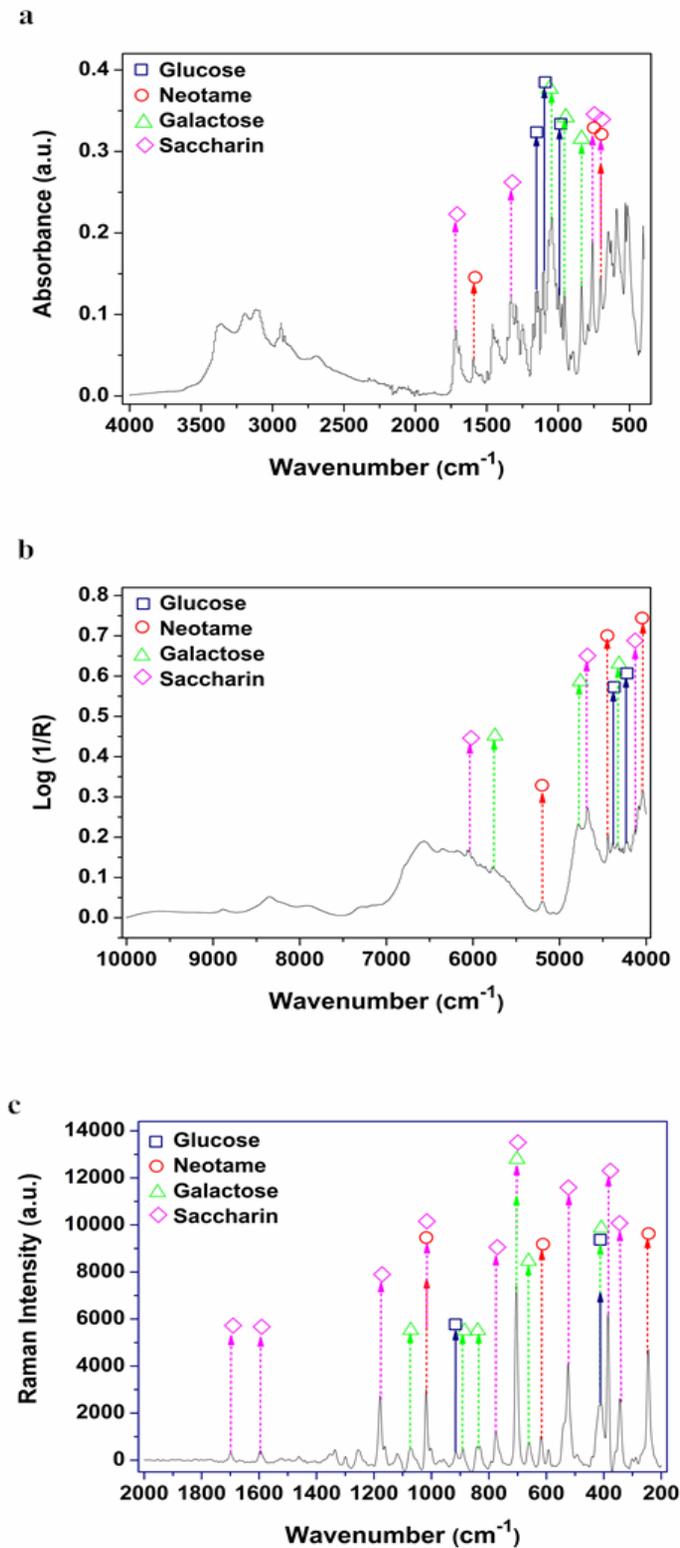


Fig. 7. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) (a), near-infrared (NIR) (b) and Raman (c) spectra of saccharin, glucose, galactose and neotame mixture.

CONCLUSIONS

This study gives an overview of ATR-FTIR, NIR and Raman spectra of sugars and sweeteners. The spectral data of sugars and sweeteners were generated and the differences among them were shown. These data can be used as reference for further spectral investigations in food analysis and in development of spectral methods which can make a significant contribution to the rapid discrimination of sweeteners and sugars, and their quantitative analyses for routine control. The band assignments in ATR-FTIR, NIR and Raman spectra of the gravimetrically mixed samples also demonstrated that these spectra could be useful in development of the methods of identification of sweeteners present together with other sweeteners or sugars.

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REFERENCES

- Anonymous, (2018). www.horiba.com/fileadmin/uploads/Scientific/Documents/Raman/bands.pdf (Accessed: 01 December 2018).
- Antiochia, R., Lavagnini, I., Magno, F., (2004). Amperometric mediated carbon nanotube paste biosensor for fructose determination. *Anal Lett*, 37(8): 1657-1669.
- Banos, J.L.G., Olano, A., Corzo, N., (2000). Determination of mono and disaccharide content of enteral formulations by gas chromatography. *Chromatographia*, 52(3-4): 221-224.
- Bearth, A., Cousin, M.E., Siegrist, M., (2014). The consumer's perception of artificial food additives: Influences on acceptance, risk and benefit perceptions. *Food Qual Prefer*, 38: 14-23.
- Bergamo, A.B., da Silva, J.A.F., de Jesus, D.P., (2011). Simultaneous determination of aspartame, cyclamate, saccharin and acesulfame-K in soft drinks and tabletop sweetener formulations by capillary electrophoresis with capacitively coupled contactless conductivity detection. *Food Chem*, 124(4): 1714-1717.
- Bernardez, M.M., Miguelez, J.D.M., Queijeiro, J.G., Queijeiro, J.G., (2004). HPLC determination of sugars in varieties of chestnut fruits from Galicia (Spain). *J Food Compos Anal*, 17(1): 63-67.
- Buyukgoz, G.G., Bozkurt, A.G., Akgul, N.B., Tamer, U., Boyaci, I.H., (2015). Spectroscopic detection of aspartame in soft drinks by surface-enhanced Raman spectroscopy. *Eur Food Res Technol*, 240(3): 567-575.
- Carvalho, A.Z., da Silva, J.A.F., do Lago, C.L., (2003). Determination of mono- and disaccharides by capillary electrophoresis with contactless conductivity detection. *Electrophoresis*, 24(12-13): 2138-2143.
- Cheng, X., Zhang, S., Zhang, H.Y., Wang, Q.J., He, P.G., Fang, Y.Z., (2008). Determination of carbohydrates by capillary zone electrophoresis with amperometric detection at a nano-nickel oxide modified carbon paste electrode. *Food Chem*, 106(2): 830-835.
- Dacome, A.S., da Silva, C.C., da Costa, C.E.M., Fontana, J.D., Adelman, J., da Costa, S.C., (2005). Sweet diterpenic glycosides balance of a new cultivar of *Stevia rebaudiana* (Bert.) Bertoni: Isolation and quantitative distribution by chromatographic, spectroscopic, and electrophoretic methods. *Process Biochem*, 40(11): 3587-3594.
- Feehley, T., Nagler, C.R., (2014). HEALTH The weighty costs of non-caloric sweeteners. *Nature*, 514(7521): 176-177.
- Fernandes, V.N.O., Fernandes, L.B., Vasconcellos, J.P., Jager, A.V., Tonin, F.G., de Oliveira, M.A.L., (2013). Simultaneous analysis of aspartame, cyclamate, saccharin and acesulfame-K by CZE under UV detection. *Anal Methods-Uk*, 5(6): 1524-1532.
- Ferrer, I., Thurman, E.M., (2010). Analysis of sucralose and other sweeteners in water and beverage samples by liquid chromatography/time-of-flight mass spectrometry. *J Chromatogr A*, 1217(25): 4127-4134.

- Goddu, R.F., Delker, D.A., (1960). Spectra-Structure Correlations for the near-Infrared Region. *Anal Chem*, 32(1): 140-141.
- Hanko, V.P., Rohrer, J.S., (2004). Determination of sucralose in splenda and a sugar-free beverage using high-performance anion-exchange chromatography with pulsed amperometric detection. *J Agr Food Chem*, 52(14): 4375-4379.
- Hannisdal, A., (1992). Analysis of Acesulfame-K, Saccharin and Preservatives in Beverages and Jams by Hplc. *Z Lebensm Unters For*, 194(6): 517-519.
- Hu, F.L., Xu, L.N., Luan, F., Liu, H.T., Gao, Y., (2013). Determination of neotame in non-alcoholic beverage by capillary zone electrophoresis. *J Sci Food Agr*, 93(13): 3334-3338.
- Hui, H.X., Huang, D.S., McArthur, D., Nissen, N., Boros, L.G., Heaney, A.P., (2009). Direct Spectrophotometric Determination of Serum Fructose in Pancreatic Cancer Patients. *Pancreas*, 38(6): 706-712.
- Ilaslan, K., Boyaci, I.H., Topcu, A., (2014). Rapid Analysis of Glucose, Fructose and Sucrose Contents of Commercial Soft Drinks Using Raman Spectroscopy. *Food Control*(0).
- Jentzsch, P.V., Ciobota, V., Salinas, W., Kampe, B., Aponte, P.M., Rosch, P., Popp, J., Ramos, L.A., (2016a). Distinction of Ecuadorian varieties of fermented cocoa beans using Raman spectroscopy. *Food Chem*, 211: 274-280.
- Jentzsch, P.V., Torrico-Vallejos, S., Mendieta-Brito, S., Ramos, L.A., Ciobota, V., (2016b). Detection of counterfeit stevia products using a handheld Raman spectrometer. *Vib Spectrosc*, 83: 126-131.
- Kaye, W., (1954). Near-Infrared Spectroscopy - Spectral Identification and Analytical Applications. *Anal Chem*, 26(2): 428-428.
- Kuhnle, G.G.C., Joosen, A.M.C.P., Wood, T.R., Runswick, S.A., Griffin, J.L., Bingham, S.A., (2008). Detection and quantification of sucrose as dietary biomarker using gas chromatography and liquid chromatography with mass spectrometry. *Rapid Commun Mass Sp*, 22(3): 279-282.
- Liu, Y.D., Ying, Y., Yu, H.Y., Fu, X.P., (2006). Comparison of the HPLC method and FT-NIR analysis for quantification of glucose, fructose, and sucrose in intact apple fruits. *J Agr Food Chem*, 54(8): 2810-2815.
- Lohumi, S., Lee, S., Lee, H., Cho, B.K., (2015). A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. *Trends Food Sci Tech*, 46(1): 85-98.
- Luo, L.Q., Zhu, L.M., Wang, Z.X., (2012). Nonenzymatic amperometric determination of glucose by CuO nanocubes-graphene nanocomposite modified electrode. *Bioelectrochemistry*, 88: 156-163.
- Mabood, F., Al-Harrasi, A., Bogue, R., Jabeen, F., Hussain, J., Hafidh, A., Hind, K., Ahmed, M.A.G., Manzoor, A., Hussain, H., Rehman, N.U., Iman, S.H., Said, J.J., Hamood, S.A., (2015). Determination of sucrose in date fruits (*Phoenix dactylifera L.*) growing in the Sultanate of Oman by NIR spectroscopy and multivariate calibration. *Spectrochim Acta A*, 150: 170-174.
- Mallikarjun, S., Sieburth, R.M., (2015). Aspartame and Risk of Cancer: A Meta-analytic Review. *Arch Environ Occup H*, 70(3): 133-141.
- O'Donnell, K., Kearsley, M.W., (2012). Sweeteners and sugar alternatives in food technology, 2nd ed. Wiley-Blackwell, Chichester, West Sussex, UK ; Ames, Iowa.
- Osborne, B.G., Fearn, T., Hindle, P.T., (1993). Practical NIR spectroscopy with applications in food and beverage analysis, 2nd ed. Longman Scientific & Technical; Wiley.
- Pane, D.D., Dias, C.B., Meinhart, A.D., Ballus, C.A., Godoy, H.T., (2015). Evaluation of the sweetener content in diet/light/zero foods and drinks by HPLC-DAD. *J Food Sci Tech Mys*, 52(11): 6900-6913.
- Peica, N., (2009). Identification and characterisation of the E951 artificial food sweetener by vibrational spectroscopy and theoretical modelling. *J Raman Spectrosc*, 40(12): 2144-2154.
- Periasamy, A.P., Chang, Y.J., Chen, S.M., (2011). Amperometric glucose sensor based on glucose

- oxidase immobilized on gelatin-multiwalled carbon nanotube modified glassy carbon electrode. *Bioelectrochemistry*, 80(2): 114-120.
- Pussa, T., (2008). Principles of Food Toxicology. CRC Press
- Rovio, S., Simolin, H., Koljonen, K., Siren, H., (2008). Determination of monosaccharide composition in plant fiber materials by capillary zone electrophoresis. *J Chromatogr A*, 1185(1): 139-144.
- Sakai, H., Yamashita, A., Tamura, M., Uyama, A., Mochizuki, N., (2015). Simultaneous determination of sweeteners in beverages by LC-MS/MS. *Food Addit Contam A*, 32(6): 808-816.
- Shim, J.Y., Cho, I.K., Khurana, H.K., Li, Q.X., Jun, S., (2008). Attenuated total reflectance-Fourier transform infrared spectroscopy coupled with multivariate analysis for measurement of acesulfame-K in diet food. *J Food Sci*, 73(5): C426-C431.
- Silveira, L., Moreira, L.M., Conceicao, V.G.B., Casalechi, H.L., Munoz, I.S., da Silva, F.F., Silva, M.A.S.R., de Souza, R.A., Pacheco, M.T.T., (2009). Determination of sucrose concentration in lemon-type soft drinks by dispersive Raman spectroscopy. *Spectrosc-Int J*, 23(3-4): 217-226.
- Silverstein, R.M., Bassler, G.C., Morrill, T.C., (1981). Spectrometric Identification of Organic Compounds. John Wiley and Sons, New York.
- Soffritti, M., Padovani, M., Tibaldi, E., Falcioni, L., Manservigi, F., Belpoggi, F., (2014). Effects of Aspartame: The Urgent Need for Regulatory Re-Evaluation. *Am J Ind Med*, 57(4): 383-397.
- Stroka, J., Dossi, N., Anklam, E., (2003). Determination of the artificial sweetener Sucralose (R) by capillary electrophoresis. *Food Addit Contam*, 20(6): 524-527.
- Torres, M.G., Fernández, N.G., del Toro, P.O., Paneque, M.R., (2007). Raman spectroscopy of poly (3-hydroxybutyrate) modified with poly (vinyl acetate) by radiation-induced copolymerisation. *Nucleus*, 42: 41-45.
- Workman, J., (2000). Handbook of Organic Compounds: NIR, IR, Raman, and UV-Vis Spectra Featuring Polymers and Surfactants. Academic Press.
- Yang, D.J., Chen, B., (2010). Determination of neotame in beverages, cakes and preserved fruits by column-switching high-performance liquid chromatography. *Food Addit Contam A*, 27(9): 1221-1225.
- Zell, M.T., Padden, B.E., Grant, D.J.W., Schroeder, S.A., Wachholder, K.L., Prakash, I., Munson, E.J., (2000). Investigation of polymorphism in aspartame and neotame using solid-state NMR spectroscopy. *Tetrahedron*, 56(36): 6603-6616.
- Zhu, G.Y., Zhu, X., Fan, Q., Wan, X.L., (2011). Raman spectra of amino acids and their aqueous solutions. *Spectrochim Acta A*, 78(3): 1187-1195.