

# Analysis of organic compounds by FT Raman spectrum in Thompson seedless Raisins.

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## ABSTRACT

Grape (*Vitis* sp.) belonging to Family Vitaceae is a commercially important fruit crop of India. Grapes are eaten as raw or they can be used for making wine, raisins, jam, and jelly, which are very nutritious and rich source of minerals like potassium, phosphorus, calcium, magnesium, other micronutrients and different vitamins. Raisins are dried fruits of certain varieties of grapevines with a high content of sugar and solid flash. Analysis of organic compounds by FT Raman spectrum of raisin. The Raman spectra of the films were recorded in the spectral range of 35–4000 cm<sup>-1</sup> using a Raman spectrometer. The Raman shift corresponds to the frequency of the fundamental IR absorbance band of the bond. It is known that specific chemical bonds (C-H, N-H, and C=O) generate specific peaks. Hence this technique proves to be a powerful technique which facilitates to determine molecular structure of spectral pattern of fingerprinting of sample.

**Keywords:** Raisin, Thompson seedless, FT Raman

## INTRODUCTION

Grape (*Vitis* sp.) belonging to Family Vitaceae is a commercially important fruit crop of India. Grapes are eaten as raw or they can be used for making wine, raisins, jam, and jelly, which are very nutritious and rich source of minerals like potassium, phosphorus, calcium, magnesium, other micronutrients and different vitamins. The dried grapes, commonly known as raisins, have a great importance in economy of the country and considered as one of the nutritious most popular dry fruits in the world. Raisins are dried fruits of certain varieties of grapevines with a high content of sugar and solid flash (Khair and Shah, 2005). The important raisin grape varieties are Thompson seedless and their selections like Tas-A-Ganesh, Sonaka and Manikchaman. The increased production of table grapes has a great potential to produce raisins with minimum losses of fresh fruits (Telis *et al.*, 2004). According to FAO data, grape production all over the world is about 62,348 million

tonnes (WHO and FAO, 2003). According to De Candolle (1886), the cultivation of grape goes back to 4000 BC in Egypt and the oldest wine was found in Armenia near the Caspian Sea in Russia. As per the report of Parker *et al.* (2007), the Thompson seedless grapes, were first introduced in 1876, accounted for 95% of the California crop used for golden raisin production.

Thapar (1960) indicated that grape was introduced in India in 1300AD by the Persian invaders in North and South India (Daulatabad in Aurangabad districts of Maharashtra) during the historic event of changing the capital from Delhi to Daulatabad by King Mohammed-bin-Tughlak and in South India districts Salem and Madurai by the Christian missionaries around 1832 A. D. From Daulatabad grape cultivation was spread to Hyderabad in Deccan during the Nizam's period. Nizam of Hyderabad has also introduced some grape varieties into Hyderabad from Persia in the early 20<sup>th</sup> century (Chadha and Shikhamany, 1999). There are two subgenera viz *Euvitis* and *Muscadania*. All commercially important varieties of grape belong to sub genus *Euvitis*, referred as true grape. More than 90% of cultivated grape varieties belong to this species which is referred as 'old world grape' the 'European grape' or 'California grape' (Shymal and Patel, 1953 and Shetty, 1959). India is a small producer of grapes, with a world share of less than 2 percent (Barrientos and Kritzing, 2004).

The total average cultivation of grape is near about 80,000 hectares in India and 28,000 hectares in Maharashtra. The total yield in India is about 15 to 18 lakh tons and in Maharashtra, it is about 7 to 9 lakh tons. Out of this annual production, 76% is used as table grapes, 0.3% in wine industry, 3.70% exported to Middle East and European countries as table fruit and 20% used for preparation of raisin. Recently, grape cultivation is increased more in Maharashtra and the major growing districts are Nashik, Sangli, Solapur, Ahmednagar, Pune and Osmanabad. Near about 80 % of grape production comes from Maharashtra followed from Karnataka and Tamil-Nadu. The commercial production of grapes started in India only after seedless varieties were introduced in Maharashtra during the 1960s. Maharashtra accounts for 70 percent of India's total grape acreage and 63 percent of production. Varieties grown are Thompson Seedless, Sonaka Seedless, Sharad Seedless and Tas-A-Ganesh. Harvesting starts in early February to early April. Within Maharashtra, the grape crop comprises 12 percent of the total fruit acreage, with 42,500 acres. Sangli, Solapur, Pune and Ahmednagar are the other locations. Due to the

higher water content, these fruits are spoiled, hence dehydration of such fruits is to avoid the spoilage. Grape is an important source of carbohydrates, minerals and vitamins but due to its low shelf life it is very difficult to fulfill the needs of the society. Over the last thirty years there has been an increasing trend towards raisin making in Maharashtra. The preparation of raisin was started long back, first known as "Manuka" (simply drying the grapes in open sunlight). Then after introduction of mutant Thomson seedless variety many grape growers turned to prepare yellowish Golden raisin and from last twenty five years many of grape growers from Sangli district are diverted towards the preparation of green raisin at Junoni [Solapur] and nearby areas which has a good market potential in cosmopolitan cities. Nashik, Sangli and Solapur are the leading grape producing districts of Maharashtra. The most of these regions are drought prone, so the area under grape cultivation is increasing day by day, which will create a problem of marketing of table grape. During last 20 to 32 years due to increase in yield, expanding area under cultivation and fluctuation in the market price the farmers are slowly turning towards raisin production. Hence, an attempt has been made to study some aspects of post-harvest physiology in relation to production of raisin in two varieties of grape, Thompson and Sonaka seedless growing in Sangli district.

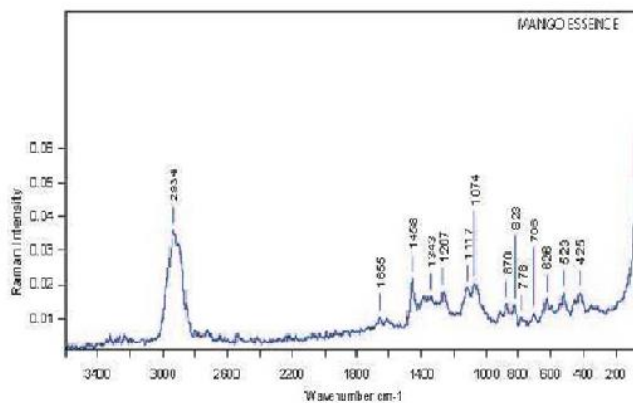
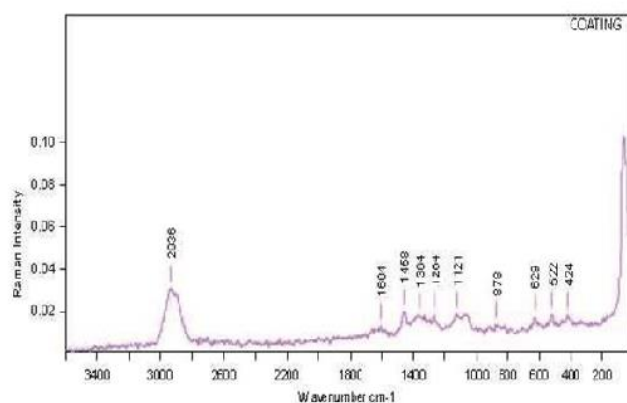
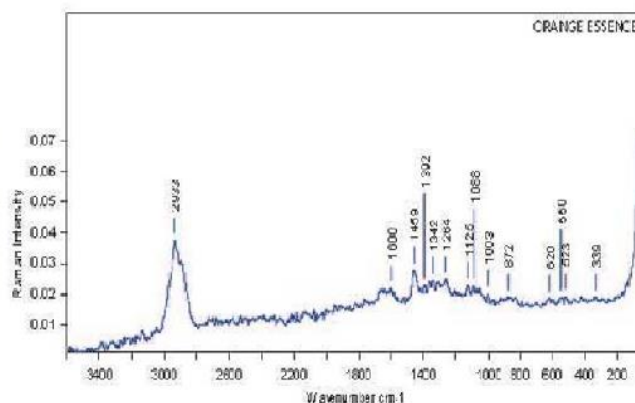
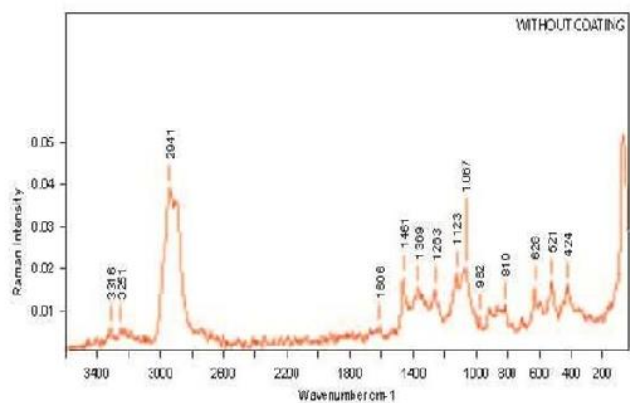
## MATERIAL METHODS

Analysis of organic compounds by FT Raman spectrum: The Raman spectra of the films were recorded in the spectral range of 35–4000  $\text{cm}^{-1}$  using a Raman spectrometer (Bruker Multi RAM, Germany Make) equipped with an Nd: YAG laser source with an excitation wavelength of 1064 nm and resolution 4  $\text{cm}^{-1}$ . In addition a CaF<sub>2</sub> beam splitter, an indium-gallium-arsenide (In Ga As) detector and 180° backscattering geometry were used in the spectrometer. Routine procedures such as bench alignment and fine tuning of the spectrometer were held before each batch of measurements. Sample cells were Wilmad WG-SM NMR tubes 4.97 mm outer diameter and 0.38 mm wall thickness. Optimal conditions (recording time, signal-to-noise ratio) were determined at 100 scans (3 min) with a resolution of 4  $\text{cm}^{-1}$ . Each Raman spectrum was automatically smoothed and baseline corrected using the proper functions from the built-in spectrometer software Omnic 3.1. Then the peak heights at 1636 and 1606  $\text{cm}^{-1}$ , from baseline, were measured and the height average of sample in triplicate was calculated.

## RESULTS

Analysis of organic compounds by FT Raman spectrum: The Raman shift corresponds to the frequency of the fundamental IR absorbance band of the bond (Thygesen *et al.*, 2003). It is known that specific chemical bonds (C-H, N-H, and C=O) generate specific peaks (Huang *et al.*, 2010). Hence this technique proves to be a powerful technique which facilitates to determine molecular structure of spectral pattern of fingerprinting of sample. Raman spectroscopy is a very effective technique for the study of molecular vibrations, thus providing a high content of molecular information multivariate analysis, and is a powerful tool capable of extracting quantitative chemical information. Due to Raman Spectroscopy it is very easy to study polymers, biomolecules, and molecules in gases and liquid. This system helps to understand the processes and kinetic changes on the surfaces and in the structures of these

molecules automatically within a very short time (Smith and Dent, 2005 and Owen *et al.*, 2006). It has been used for monitoring cell-wall extraction (McCann *et al.*, 1992), as well as to determine the cell-wall changes during processing and quality control of vegetable processing (Boeriu *et al.*, 1998). The advantage of Raman over other analytical techniques is its ability to work on water-rich samples such as food (Owen *et al.*, 2006 and Numata *et al.*, 2011). While infrared spectroscopy can also be applied to measure several properties in a wider range of products, e.g. dry matter in onions (Birth *et al.*, 1985), potatoes (Dull *et al.*, 1989) whole dates (Dull *et al.*, 1991) internal quality in peaches, nectarines (Slaughter, 1995), raisins in peaches (Peiris *et al.*, 1997), soluble solids in apple juice (Ventura *et al.*, 1998). According to Veronelli *et al.* (1995) and Withnall *et al.* (2003), strong bands of carotenoids are observed in the Raman spectrum within the 1500–1550 and 1150–1170  $\text{cm}^{-1}$  range due to in-phase C=C(n1) and C-C.



## DISCUSSION & CONCLUSION

An Analysis of organic compounds by FT Raman spectrum: The Raman spectrum of Thompson seedless raisins without coating shows the conjugated C-C bonds with their characteristic bands of carotenoids at 1531  $\text{cm}^{-1}$  wave number while the coated samples shows absence of this wave

number for carotenoids. The C(5) position is glycosylated the significant perturbations of spectral features between 640 and 750  $\text{cm}^{-1}$  were noticed. Anthocyanidin showed lower frequency range close to these 630  $\text{cm}^{-1}$  in Thompson seedless raisins with the zein protein coating mango and orange essence. The absorbance at wave number 1630  $\text{cm}^{-1}$  indicating carboxylate content of cell wall polysaccharides and the wave number

between 850 to 1200  $\text{cm}^{-1}$  indicates glycosidic linkages of c-o-c bonds of carbohydrates which were characterized by wave number 817, 865, 982, 1077, 1124, 1183  $\text{cm}^{-1}$  in case of Thompson seedless. While the IR wave number bands at 1152 to 1149  $\text{cm}^{-1}$  belongs to non-localized highly coupled vibrations of polysaccharides backbones. Thompson seedless variety the weak bands are 819, 865  $\text{cm}^{-1}$  also confirmed that pectin is major components. Thus, the characteristic wave number bands of polysaccharides can be observed in 1363, 1124, 1333  $\text{cm}^{-1}$  additional bands to ester and carboxylate group. The wave number in the range of 500  $\text{cm}^{-1}$  to 550  $\text{cm}^{-1}$  showed disulphide and sulphhydryl groups in Thompson seedless showing maintenance of amino acid profile, resulting in improvement of the protein quality of raisins. The polyphenolic content appearing with the intense bands of 1604, 1606, 1635, to 1652  $\text{cm}^{-1}$  wave numbers can be assigned to the benzene ring, while, the bands at 1185  $\text{cm}^{-1}$  are due to the stretching of C-O-C and C-OH vibration sharing intensity of wave number near 850  $\text{cm}^{-1}$  to 830  $\text{cm}^{-1}$  which can be assigned to tyrosine residues in Thompson seedless raisins. The wave number bands at 1601, 1653, 1606, 1601  $\text{cm}^{-1}$  of Thompson seedless varieties suggest presence of aromatic compounds showing the phenyl rings of polyphenolic substances. Stretching ( $n_2$ ) vibrations of the polyene chain. Schulz *et al.*, (2005) have shown that the wavenumber location of these bands, and in particular the  $n_1$  band, is strongly dependent on the length of the carotenoid chain, and generally, carotenoids with 11, 9, 8, 7 conjugated C-C bonds have their characteristic bands at about 1510, 1524, 1530, 1536  $\text{cm}^{-1}$  (Schulz *et al.*, 2005 and Baranski *et al.*, 2005).

Fresh-leaf Japanese tea produced strong bands at 1529  $\text{cm}^{-1}$ , 1161  $\text{cm}^{-1}$  and 1007  $\text{cm}^{-1}$  are assigned to the carotenoids (Ozaki *et al.*, 1992). The FTIR spectrum of Thompson seedless raisins are shown in Plate 7 and 8. The spectrum of Thompson seedless raisins without coating shows the conjugated carbon carbon bonds with their characteristic bands of carotenoids at 1531  $\text{cm}^{-1}$  wavenumber while the coated raisin samples show absence of this wave number for carotenoids. The C (5) position is glycosylated the significant perturbations of spectral features between 640 and 750  $\text{cm}^{-1}$  are visible. Anthocyanidin monoglycosides (in position 3 of the C ring) exhibit a strong RR signal close to 540  $\text{cm}^{-1}$  while 3,5-diglycosides have strongest feature in the lower frequency range close to 630  $\text{cm}^{-1}$ . In the present study of anthocyanidin has shown lower frequency range close to these 630  $\text{cm}^{-1}$  in Thompson seedless raisins with the zein protein coating mango and orange essence.

The cell-wall polysaccharides were quantified by absorbance at wavenumbers 3440  $\text{cm}^{-1}$  OH, 1750  $\text{cm}^{-1}$  ester, 1630  $\text{cm}^{-1}$  carboxylate and 1200–850  $\text{cm}^{-1}$  carbohydrate (the glycosidic

linkage C-O-C). The IR band at 1152–1149  $\text{cm}^{-1}$  is dominated by stretching of between 1100 and 700  $\text{cm}^{-1}$  belongs to non-localized, highly coupled vibrations of polysaccharide backbones (Engelsen and Norgaard, 1996). Pectic samples (positive values) are characterised mainly by the wavenumbers 1145, 1104, 1014 and 952  $\text{cm}^{-1}$ , which were recently identified as regions of high absorbance for pectic origin (Coimbra *et al.*, 1998). In the region of 1200–950  $\text{cm}^{-1}$  of both spectra, several intense bands at 1152 (1158), 1105 and 1083 (1080)  $\text{cm}^{-1}$  contributed to vibrations of glycosidic bonds and pyranoid rings. In addition, two weak bands at 850 and 833  $\text{cm}^{-1}$  gives confirmation that pectin is the major component (Copikova *et al.* 2001). The isolation portion of high molecules substance has significant bands of pectin (1742, 1152, and 1105  $\text{cm}^{-1}$ ) the spectra of pectin have additional bands at 1444, 1413 (1420), 1369 (1374), 1272 (1266) and 930 (925)  $\text{cm}^{-1}$ , which may be ascribed to ester and carboxylate groups (Filippov, 1978; Engelsen and Norgaard 1996 and Wellner *et al.* 1998). The band at 1333 (1338)  $\text{cm}^{-1}$  was assigned to C-H bending vibration of pyranoid ring. This band is very resistant to any changes of C-6 carboxyls (Filippov, 1978). Characteristic bands of polysaccharides can be observed at 1375, 1333, 1152, 1124, 929, and 378  $\text{cm}^{-1}$  Edwards *et al.*, (1997). The Raman spectra of Thompson seedless raisins (Plate 9) shows the absorbance at wavenumber 1630  $\text{cm}^{-1}$  the indicating carboxylate content of cell wall polysaccharides and the wavenumber between 1200 to 850  $\text{cm}^{-1}$  indicates glycosidic linkages of c-o-c bonds of carbohydrates which are characteristic by wave number 817, 865, 982, 1077, 1124, 1183  $\text{cm}^{-1}$  in case of Thompson seedless. While the IR bands at 1152 to 1149  $\text{cm}^{-1}$  belongs to non localized highly coupled vibrations of polysaccharides backbones. Among Thompson seedless varieties the weak bands are 819, 865  $\text{cm}^{-1}$  also confirm that pectin is major components as indicated by the above workers. Thus, the characteristic bands of polysaccharides can be observed in 1363, 1124, 1333  $\text{cm}^{-1}$  additional bands to ester and carboxylate group. Ozaki *et al.*, (1992) reported that intensity of bands 1462  $\text{cm}^{-1}$ , 1126  $\text{cm}^{-1}$  and 840  $\text{cm}^{-1}$  described to sugars, the band at 540  $\text{cm}^{-1}$  is also assigned to a  $d$  (C2–C1–O1) bending vibration in  $\beta$ -D-glucose (Söderholm *et al.*, 1999). Fructose has distinctive bands at 629, 710, 821, 870, 1065  $\text{cm}^{-1}$ .

The band present at 821  $\text{cm}^{-1}$  in fructopyranose and at 870  $\text{cm}^{-1}$  in fructofuranose was assigned to a  $\nu$  (C–C) vibration (Söderholm *et al.*, 1999). The band at 710  $\text{cm}^{-1}$  in fructofuranose was contributed by  $\delta$  (CCO) and the one at 629  $\text{cm}^{-1}$  was assigned to  $\delta$  (CCO)  $\nu$  (Tipson, 1987). Ethanol has a strong band at 879  $\text{cm}^{-1}$  and two small bands at 1030 and 1079  $\text{cm}^{-1}$ . The strong band had been assigned to (C–C) stretching, and two small bands present at 1030 and 1079  $\text{cm}^{-1}$  were contributed by C–O stretching and CH<sub>3</sub> rocking, respectively (Picard *et al.*, 2007). The spectrum of

sucrose presents the characteristic bands of a glucose ( $847\text{ cm}^{-1}$ ) and b-fructose ( $868\text{ cm}^{-1}$ ); in the spectrum of maltose besides a-glucose signals the band due to b-glucose ( $898\text{ cm}^{-1}$ ) is seen, whereas cellobiose shows only a signal at  $885\text{ cm}^{-1}$  for the b-anomer. (Corbett *et al.*, 1991). The spectral region between  $1200$  and  $850\text{ cm}^{-1}$  were applied for the detection of Uronic acid and neutral sugars of pectic origin from olive and orange cell walls (Coimbra *et al.*, 1998).

In the present study the several other sugars and their moieties are noticed with the wavenumber of  $1457, 1458, 1461, 1462, 1426$  and  $840\text{ cm}^{-1}$  in Thompson seedless raisins, as indicated by Ozaki *et al.*, (1992). Several Raman vibrational modes are useful for the interpretation of various amino acids and proteins occurring in plant tissue. Most characteristic bands are associated with the CONH group, referred to as amide A, amide B and amide I–VII (Miyazawa, 1962). The following three signals are of main interest for the identification of different protein backbone confirmations: amide I to be detected between  $1680$  and  $1600\text{ cm}^{-1}$  (stretching vibration of C O), amide II observed in the range between  $1580$  and  $1480\text{ cm}^{-1}$  and amide III to be found between  $1300$  and  $1230\text{ cm}^{-1}$  (both associated with coupled C–N stretching and N–H bending vibrations of the peptide group. Aliphatic amino acid residues show C–H stretching and bending modes near  $2800$ – $3000\text{ cm}^{-1}$  and  $1440$ – $1465\text{ cm}^{-1}$  respectively (Li-Chan, 1996).

The present study indicates that the wave number in the range of  $500\text{ cm}^{-1}$  to  $550\text{ cm}^{-1}$  shows disulphide and sulphhydryl groups in Thompson seedless. Showing maintenance of amino acid profile it results improves the protein of raisins. Phenolic compounds occur ubiquitously in most plant species. They comprise a considerable range of substances possessing an aromatic ring bearing one or more hydroxyl substituents derived from the shikimate and phenylpropanoid pathway. Recently special interest in fruit phenolics is related to their physiological activity which depends on their antioxidant properties, the ability to inhibit nitrosation and to chelate metal ions (Antolovich *et al.*, 2000). The most intense bands appearing at  $1630$  and  $1601\text{ cm}^{-1}$  can be assigned to the benzene ring, whereas bands at  $1185$  and  $965\text{ cm}^{-1}$  are due to COC and COH vibrations (Andreev *et al.*, 2001). The intensity ratio of a doublet near  $850\text{ cm}^{-1}$  and  $830\text{ cm}^{-1}$ , which is assigned to tyrosine residues, is also a good indicator of the environment as well as the hydrogen bonding of the phenolic hydroxyl groups (Li-Chan *et al.*, 1996). The polyphenolic content appearing with the intense bands of  $1604, 1606, 1635, 1652\text{ cm}^{-1}$  wavenumbers can be assigned to the benzene ring, while, the bands at  $1185\text{ cm}^{-1}$  are due to the stretching of C O C and C O H vibration sharing intensity of wave number near  $850\text{ cm}^{-1}$  to  $830\text{ cm}^{-1}$  which can be assigned to tyrosine residues in Thompson seedless raisins as

indicated by above scientist. According to the Himmelsbach and Akin (1998), Edwards *et al.*, (1997), Gierlinger and Schwanninger (2006), the bands at  $1601\text{ cm}^{-1}$  suggest the presence of aromatic compounds, so the presence of polyphenolics is plausible.

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