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Humeyra Yildirim , Isil Tulum , Ayse Erol , [Fahrettin Sarcan](#) *

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Article

Investigation of Molecular Compounds in Kumquat (*Fortunella Spp.*) Peel via Raman Spectroscopy

Hümeýra Yıldırım^{1,2}, Isil Tulum³, Ayse Erol¹ and Fahrettin Sarcan^{1,*}

¹ Department of Physics, Faculty of Science, Istanbul University, Vezneciler, Istanbul, 34134, Türkiye; hümeýra.yildirim@ogr.iu.edu.tr (H.Y.); ayseerol@istanbul.edu.tr (A.E.)

² TEBIP High Performers Program, Faculty of Science, Istanbul University, Vezneciler, Istanbul, 34134, Türkiye

³ Istanbul University, Faculty of Science, Department of Biology, Istanbul, Türkiye; iciltlm@gmail.com

* Correspondence: fahrettin.sarcan@istanbul.edu.tr

Abstract: In this study, Raman spectroscopy is employed to investigate the chemical compounds of kumquat peels and compared that of orange. Kumquat is a citrus fruit belonging to the genus *Fortunella* in family Rutaceae. It is observed that kumquat peel has 3 main Raman active characteristic vibration modes specified to carotenoids, which is dominated by β -carotene at 1007, 1158 and 1526 cm^{-1} , as it is well known for orange. The carotenoid distribution within the cross-section of kumquat is also investigated. The carotenoid-related Raman vibration modes are relatively stronger on the peel of kumquat, which is an important finding especially for the fruits that can be eaten with its peel. Our results pave the way to take an attention for the importance of kumquat as being a fruit that can be grown up in different climates compared to orange, which grow in warm climates.

Keywords: kumquat; citrus peels; functional components; Raman spectroscopy; spectral mapping; carotenoid

1. Introduction

Kumquat is a citrus fruit belonging to the genus *Fortunella* of the Rutaceae family. It is also known as golden orange in Türkiye. Up to date, Kumquat is classified into four main types: *Fortunella japonica*, *Fortunella margarita*, *Fortunella crassifolia* and *Fortunella hindsii* (Lou and Ho 2017). Kumquat fruits exhibit an orange-yellow hue, an elliptical shape and measuring approximately 2 cm in diameter, and it is regarded as one of the smallest citrus fruits (Chen *et al.*, 2017). Kumquats have numerous advantages, such as cold resistance, an eatable peel, small size, and adaptability to various soil types (Love *et al.*, 2007 ; Palma *et al.*, 2018). These advantageous make them easy to care for and ideal for smaller spaces, offering a versatile citrus option suitable for diverse environments, ranging from greenhouses to terraces, and as a cold-tolerant citrus fruit (Palma *et al.*, 2018). Compared to other citrus fruits, kumquat can be consumed whole with its peel, which provides an advantage in the intake of bioactive substances. While the pulp part of the fruit is sour, the peel part has a characteristic aroma due to flavonoids and terpenoids, which are important in the essential oil composition (Wang *et al.*, 2012 ; Yıldız Turgut *et al.*, 2015). Because of its acidic taste and soft peel, kumquat is used in products such as jams, pickles and sauces, in addition to its natural consumption (Choi *et al.*, 2005; Pawełczyk *et al.*, 2021). Besides, considering its use in the food industry, kumquat contains a variety of phytochemicals, including carotenoids, essential oils, ascorbic acid and flavonoids. These components are small molecules that are not essential for the survival of plants but represent pharmacological activity (Ogawa *et al.*, 2001). Kumquat is becoming increasingly important in traditional medicine because it contains many beneficial phytochemicals with diverse biological effects (Wang *et al.*, 2012; Lou *et al.*, 2016). Phytochemicals are known to have beneficial biological effects. These include antibacterial, anti-oxidative, anti-inflammatory, anti-cancer, as well as cardiovascular protective effects (Li X. *et al.*, 2022; Xinmiao Lv *et al.*, 2015; Al-Saman *et al.*, 2019). For

example, carotenoids, an important component in citrus peel, have the ability to detoxify free radicals in cells. An important feature of carotenoids is that they are precursors of vitamin A. The body can convert certain carotenoids into active vitamin A (Grune *et al.*, 2010). Carotenoids are used as nutraceuticals in various diseases such as eye diseases, cardiovascular diseases, neurodegenerative diseases and cancer (Saini *et al.*, 2022). It is crucial to find and analyse bioactive compounds in citrus peels. Many methods such as Gas Chromatography-Mass Spectrometry, High-Performance Liquid Chromatography, Fourier Transform Infrared Spectroscopy are used for biocomponent analysis of citrus peels (Yu *et al.*, 2022; Chen J. *et al.*, 2022; Niluxsshun MCD *et al.*, 2021).

These methods for component analysis have the advantages of high sensitivity and accuracy, but also disadvantages such as complexity and time-consuming for sample preparation (Yang *et al.*, 2017).

In this study Raman spectroscopy, which is a non-destructive method, is employed to determine and analyse the compounds of kumquat peel. Raman spectroscopy is an analytical technique where inelastic scattered photons are used to measure the vibrational energy modes of a molecule. When photons interact with a substance, the frequency of most of the scattered light does not change, which is called Rayleigh scattering. However, inelastic light scattering processes can also occur due to molecular vibrations, so-called Raman scattering (Sarcan *et al.*, 2014; Sarcan *et al.*, 2023; Serebrennikova *et al.*, 2021). The spectrum of scattered photons in Raman spectroscopy is fingerprint of the investigated material therefore allows easy identification of the molecule of interest. Raman spectroscopy has many advantages such as ease of sample preparation, non-destructive and the ability to work with aqueous samples. Because of these advantages, Raman spectroscopy has become a powerful alternative tool to other commonly used techniques. Accordingly, Raman spectroscopy has been used as a promising analytical tool in recent years as it provides a chemical fingerprint for molecular identification (Dodo *et al.*, 2022; Serebrennikova *et al.*, 2021). Raman spectroscopy is becoming increasingly popular in research on food, environment, medicine and many other fields. In various application areas such as pesticide detection (Chen *et al.*, 2016), pathogen detection in food (Zhu *et al.*, 2023), water pollution (Almaviva *et al.*, 2022) and neurodegenerative disease diagnosis (Devitt *et al.*, 2018).

In 2017, Yang *et al.* used Raman spectroscopy for chemical mapping of functional compounds in citrus peels. The relative amount and distribution of essential oils, carotenoids and flavonoids in citrus peels at different locations (flavedo, albedo and longitudinal section) were studied (Yang *et al.*, 2017). To the best of our knowledge, there is no study that uses Raman spectroscopy for the determination of functional components on kumquat fruit.

In this study, carotenoids were determined in different locations (flavedo, albedo and cross-section) of kumquat fruit peel without any extraction process and a comparison was made between two kinds of citrus fruits (orange and kumquat). The fact that kumquat fruit can be consumed with its peel unlike other citrus fruits thanks to the terpenoids and flavonoids in the peel composition makes our research valuable. Kumquat is becoming increasingly important in food and pharmacology due to its nutritional and phytochemical content (Li X. *et al.*, 2022). Therefore, a rapid and non-destructive determination of shell composition is very critical. The results obtained in our research are valuable in terms of advances in the use of Raman spectroscopy for the detection of biocomponents in citrus peels.

2. Materials and Methods

Sample Preparation

Kumquats and oranges from Verita (Verita, Istanbul, Türkiye) were soaked in saline for 10 min. The fruits were then washed three times with distilled water to remove the chemicals in the peel. Citrus peels were peeled. Pieces were cut for the Flavedo and Albedo parts. Cross-sectional pieces were also taken to understand the component distribution. Samples were adhered to the slide with double-sided tape and measured.

Raman Spectroscopy

Raman spectroscopy measurements were carried out using a free space custom modular micro-spectroscopy set-up equipped with a thermoelectric cooled CCD (Newton BEX2-DD, Andor) with a 1800 grooves/mm of grating in a spectrometer (Shamrock 500i, Andor). To excite the samples, a 532 nm CW laser (Gem532, Novanta Photonics) was used, and the excitation laser beam is focused to a spot of $\sim 1.2 \mu\text{m}$ in diameter and on the devices placed on a XYZ sample stage. Raman spectra from the samples were collected *via* a 50x objective (NA = 0.42).

3. Results and Discussion

Results

The flavedo and albedo structures of kumquat and orange fruits were observed by optical microscope (Figure 2). It was noted that the flavedo layer of the kumquat ($N \sim 1.5 \text{ mm}$) was 2.5 times thinner than that of the orange ($N \sim 4 \text{ mm}$).

The thinner flavedo part makes the kumquat fruit eatable with its peel. In addition to that the thinner flavedo layer of kumquat compared to orange is important for obtaining a higher concentration of beneficial components from a smaller part of the fruit.

Figure 3 shows the Raman spectra of kumquat and orange peel. The strong Raman peaks were observed at 1007 , 1158 and 1526 cm^{-1} as well as the relatively weak peaks were observed at 872 , 963 , 1188 , 1212 and 1263 cm^{-1} (Figure 3). Peaks at 1007 , 1158 and 1526 cm^{-1} are assigned to carotenoids, a vitamin A precursor according to previous literature data (Yang *et al.*, 2017). β -carotene containing 9 conjugated double bonds (Figure 1d) has been detected by Raman spectroscopy in foods such as tomatoes, carrots and pumpkin in previous studies. The bands attributed to carotenoids in the studies are shown in the Table 1.

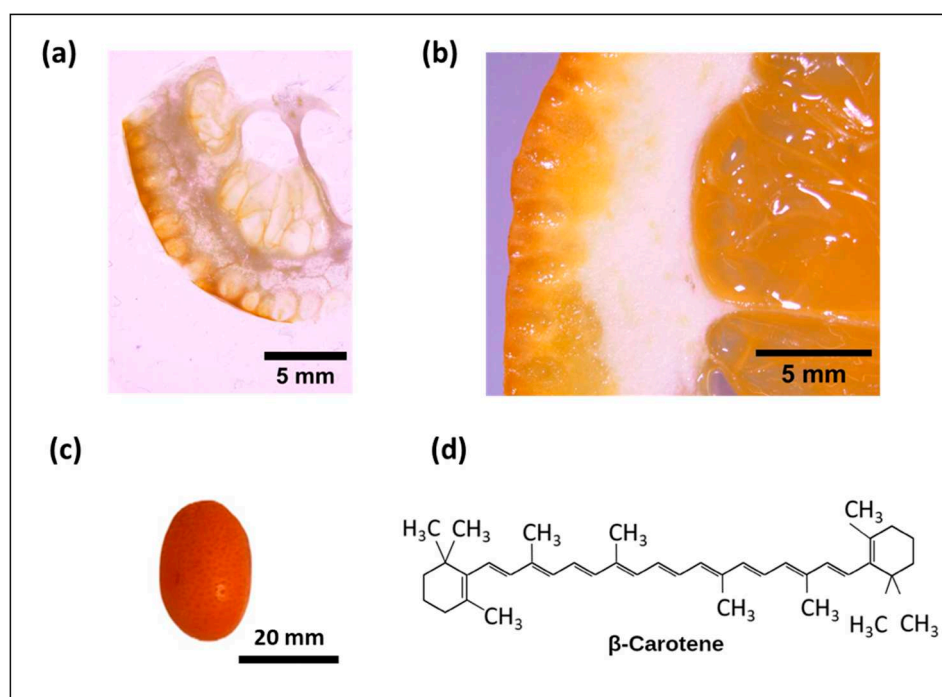


Figure 1. Optical microscope image of **a)** kumquat and **(b)** orange. **c)** Image of kumquat and **d)** Chemical structures of β -carotene.

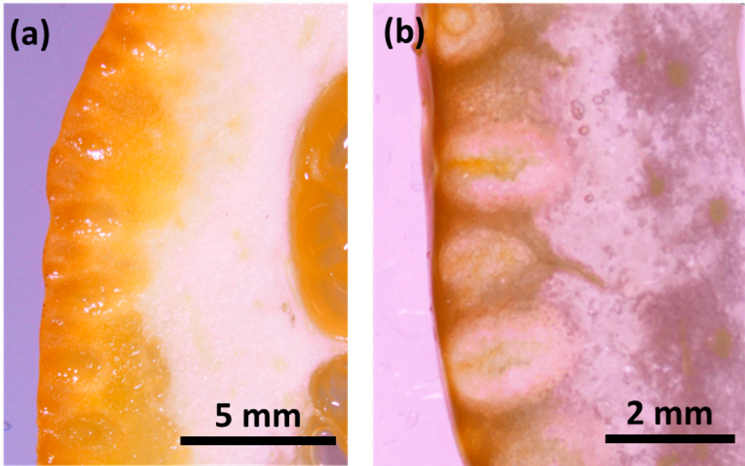


Figure 2. Cross-sectional optical microscope images of **a)** orange and **b)** kumquat.

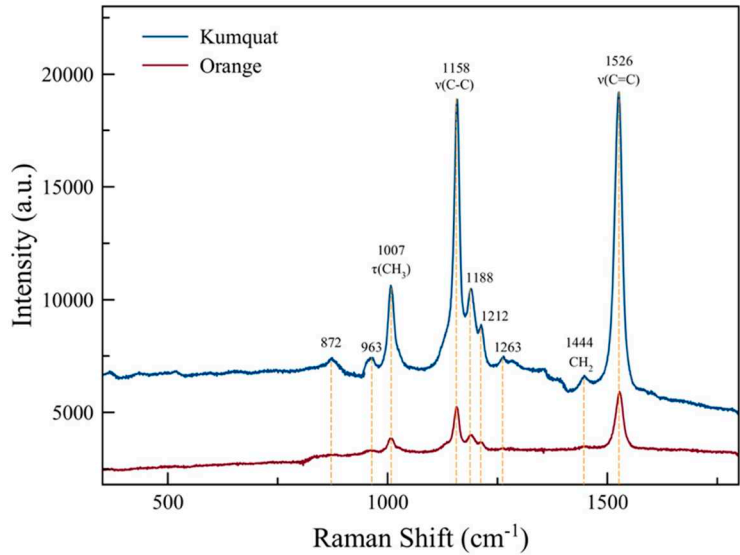


Figure 3. Raman Spectra of Orange and Kumquat.

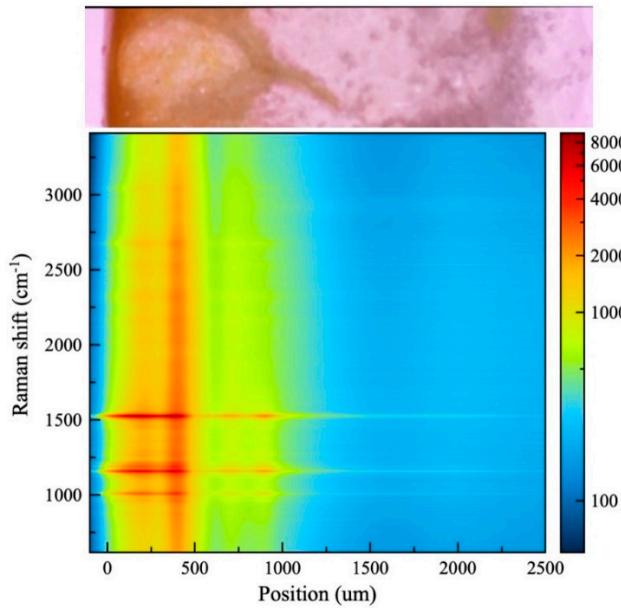


Figure 4. Raman spectrum mapping of transverse section of kumquat peel.

Table 1. Wavenumbers of ν_1 , ν_2 and ν_3 modes (cm^{-1}) of the predominant carotenoids obtained from several products by Raman spectroscopy.

Sample	ν_1 (C=C)	ν_2 (C–C)	ν_3 (C–CH ₃)	
Citrus	1528	1156	1010	Yang <i>et al.</i> 2017
Tomato	1510	1156	1005	Baranska <i>et al.</i> 2006
Carrot	1520	1156	1007	Schulz <i>et al.</i> 2005
Pumpkin	1527	1157	1008	Oliveira <i>et al.</i> 2009
Kumquat	1526	1158	1007	This work

Raman spectrum of carrot showed strong bands at 1520, 1156 and 1007 cm^{-1} assigned to $\nu(\text{C}=\text{C})$, $\nu(\text{C}-\text{C})$ and $\tau(\text{CH}_3)$ of β -carotene, respectively; in the Raman spectrum of tomato puree, β -carotene was observed with three intense bands at 1510 (ν_1), 1156 (ν_2) and 1005 cm^{-1} (ν_3) (Schulz *et al.* 2005; Baranska *et al.* 2006).

According to the reported values in the literature, the results obtained confirmed the characteristic Raman bands at 1526 cm^{-1} and 1158 cm^{-1} assigned to the in-phase C=C and C-C stretching vibrations of the polyene chain and 1007 cm^{-1} assigned to the in-plane rocking modes of CH_3 groups attached to the polyene chain joined by C-C bonds (De Gelder *et al.* 2007; Park *et al.* 2023; Yang *et al.* 2017). In addition, the peak at 1444 cm^{-1} is assigned to the $\text{CH}_3\text{-CH}_2$ bending modes indicates essential oil (Schulz *et al.* 2002; Vargas Jentzsch *et al.* 2014).

It is observed that the Raman peak intensities of both carotenoid and essential oils are 6 times stronger on the kumquat peel compared to that of orange. This makes kumquat a strong alternative for vitamin A intake beyond its advantageous properties such as cold resistance, compatibility with various soil types and edibility with its peel compared to other citrus fruits.

In the cross-sectional piece taken from kumquat fruit, the carotenoid content was investigated from the albedo layer to the flavedo layer. Looking at the distribution of carotenoids, it was found that the carotenoid density decreased when going from the albedo layer to the flavedo layer (Figure 4).

4. Conclusion

In conclusion, we have used Raman spectroscopy for a rapid and non-destructive detection of carotenoids in kumquat and compared that of orange peels. Raman spectrum analysis of kumquat peel have revealed distinct characteristic bands at 1007, 1158 and 1526 cm^{-1} associated with carotenoids, particularly dominated by β -carotene. This result indicates the potential of kumquat to be a valuable alternative among citrus fruits for obtaining vitamin A. It has been observed that the concentration of carotenoids is more pronounced in the flavedo part compared to the albedo part. The findings of this study address the differences in the biochemical profiles of citrus fruit peels and highlight the potential health benefits associated with kumquat peel consumption. The information obtained from our study will contribute to the quantitative analysis of the materials in the fruits. Furthermore, the successful application of Raman spectroscopy as a robust analytical tool for non-destructive compositional analysis in the field of food science and nutrition research.

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Conflicts of Interest: The authors declare no competing interests.

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