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Assessment of Sugar and Mineral Components in Taif Pomegranate Granules Using Spectral and Microanalytical Analyses

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ABSTRACT

For the first time this article was focused on the use of spectral and microanalytical tests to investigate the sugar and mineral contents in Taif Pomegranate Granule Ingredients (TPGIs). Deionized water was used to extract "trace elements" and "sugars" *via* an ultrasonic bath as well as the chemical composition of the Taif pomegranate fruit granules was studied and discussed in terms of dry weight. The functional groups of sugars, carbohydrates and kind of mineral salts were identified using the analytical techniques such as scanning electron microscopy (SEM), infrared spectroscopy (FTIR), "High-Performance liquid chromatography" (HPLC) system equipped with a "Sugar Pak-1" column, atomic absorption equipment and flame photometry. The optimal conditions were studied for several factors (column temperature, mobile phase types, mobile phase flow rate and injection loop size). The results of the analyzes conducted demonstrated that the components of Taif pomegranate granules (TPGIs) contained sugars (such as fructose, glucose, and sucrose), some essential elements (selenium, sodium, potassium, calcium, magnesium, and iron) and chlorine ions. The components of TPGI are an important source of many nutrients that are rich in monosaccharides, antioxidants and flavonoids and can contribute to a healthy diet.

Keywords: HPLC; minerals; sugars; infrared spectroscopy; Taif pomegranate granule ingredients.

INTRODUCTION

Although pomegranate fruit is cultivated on a commercial level, pomegranate trees grow and adapt to tropical or temperate climates and are limited to areas with these environments' fruits can be eaten raw or processed to make juices, jams, smoothies, and sauces (Chandra, *et al.*, 2010 & Tehranifar, *et al.*, 2010). About 12 to 20% of pomegranate seeds are oil, and 80% of this oil is octadecatetraenoic fatty acid (Hornung, *et al.*, 2002). Triglycerol makes up 99% of the fatty acids, with lignin and hydroxycinnamic acids were the remaining portions (Wang, *et al.*, 2004). Punctate acids are among the oil's conjugated fatty acids; linoleic, oleic, palmitic, and stearic acids are among its unconjugated fatty acids. The following are also present: "ursolic acid", "tocopherols (γ-tocopherol)," "sterols like (daucosterol, campesterol, stigmasterol, beta-sitosterol and cholesterol)", and sex steroids like "17-α-estradiol, estrone, testosterone, and estriol". Reports have also identified gallic acid, hydroxybenzoic acids, and ellagic acid (Wang, *et al.*, 2004).

Pomegranates have also been found to contain "Ellagitannin, punicalagina and punicalin, corelagina, Casuarenin, Pedoncolagina, Tilemagrandin, Granatin A and B, punicavulin" (Vidal, *et al.*, 2003 & Tanaka, *et al.*, 1986), in addition to other polyphenolic compounds, hydroxybenzoic acids (ellagic acid and gallic acid), hydroxycinnamic acids (chlorogenic acid, caffeic acid, and p-coumaric acid), antioxidants, and epicatechin (de Pascual-Teresa, *et al.*, 2000).

Anthocyanins and flavonoids generate the distinctive color of pomegranate juice, it shows antioxidant activity and increases fruit color intensity at different ripeness levels (Hernández, *et al.*, 1999). The other significant components of pomegranate juice include antioxidants (Qian, *et al.*, 2005), "ellagitannins, quercetin, and proanthocyanidins," such as "punicalagina and punicalin," and "hydroxycinnamic acids," such as "caffeic acid, chlorogenic acid, and coumaric acid" (Adams, *et al.*, 2006). In addition to essential minerals like iron, manganese, magnesium, calcium, sodium, strontium, potassium, and zinc, juice also contains glucose, fructose, sucrose, and aliphatic organic acids like citric acid, malic acid, tartaric acid, fumaric acid, and succinic acid (Poyrazoglu, *et al.*, 2002; Lansky, & Newman, 2007).

Flavonoids, including "flavonols, kaempferol, and quercetin", glycosylated flavonols e.g. "rutin, kaempferol-3-O-glycoside, flavonoids (luteolin), glycosylated flavones (naringin), and anthocyanins (delphinidin)". The color of the fruit is generated by cyanidin and pelargonidin, in addition to the glycosylated forms of these compounds (Hernández, *et al.*, 1999). Tannins are exhibited in the leaves and peel, and in some glycosides, such as "flavone named apigenin", which is found in the leaves. Naringin has also been studied for its anxiolytic properties (Zand, *et al.*, 2000) and anticancer effects. Additional polyphenols include glycosylated flavones (luteolin), punicalaginases, punicalaginases, corylagina and punicavulina (Nawwar, *et al.*, 1994).

Pomegranate compounds that reduce oxidation and dichlorodihydrofluorescein diacetate were found. Dichlorofluorescein is highly fluorescent, which is largely due to fluorescence specifically and the presence of ellagic acid; therefore, dichlorofluorescein was used to test the dichlorodihydrofluorescein method, as hypothesized, and was compared to this phenolic compound. This acid generates

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the antioxidant activities of pomegranate (Sánchez, *et al.*, 2008).

Mineral content in the leaf changes during growth stages, as the nitrogen concentration is greater in the intermediate stage. Potassium is one of the main elements found in abundance in the early stage, while both calcium and iron are elements found in mature and large leaves. Crucially, the percentages of the three minerals—magnesium, iron, and zinc—decline considerably with fruit ripening (Munde, *et al.*, 1980). The study aims to explore the sugar and mineral contents of Taif pomegranate seeds.

MATERIALS AND METHODS

Materials

Raw materials:

Taif pomegranates (Taif red cultivars which belong to the Punica granatum species of the family Lythraceae) were purchased from the local market (Taif, Kingdom Saudi Arabia).

Chemicals:

The following standard solutions were purchased from Sigma-Aldrich: 1000 mg/L of glucose standard solution in water ($C_6H_{12}O_6$; Mwt. 180.16), 1000 mg/L of fructose standard solution in water ($C_6H_{12}O_6$; Mwt. 180.16), and 1000 mg/L of sucrose standard solution in water ($C_{12}H_{22}O_{11}$; Mwt. 342.30). The standard solutions of "ethylenediaminetetraacetic acid calcium disodium salt hydrate; Mwt. 374.27 on anhydrous basis). Sigma-Aldrich provided standard solutions for the following: iron ($Fe(NO_3)_3$ in HNO_3 0.5 mol/l 1000 mg/l Fe), calcium ($Ca(NO_3)_2$ in HNO_3 0.5 mol/l 1000 mg/l Ca), magnesium ($Mg(NO_3)_2$ in HNO_3 0.5 mol/l 1000 mg/l Mg), and selenium (SeO_2 in HNO_3 0.5 mol/l 1000 mg/l).

Instruments:

A Waters HPLC system with a 515 stainless steel filter pump (Milford, MA, USA) and a Nova Pak–C18 (4 µm, 3.9 × 20 mm) water column heater unit with a Waters 410 Differential Refractometer were used to identify the sugar components (glucose, fructose, and sucrose). Throughout the investigation, a 300 × 6.5 mm Waters Sugar Pak–1 column was employed. There are many distinctive elements in Taif pomegranates (Table 1), including selenium, sodium, potassium, calcium, magnesium, and iron were determined using PYE-UNICAM SP 1900 atomic absorption spectrometer with the appropriate lamps. Using a Shimadzu FT-IR Spectrometer with 30 scans and 2 cm^{-1} resolution, infrared spectra were recorded for the fine powder sample from KBr discs within the 4000-400 cm^{-1} range. Energy dispersive X-ray detection (EDX) and scanning electron microscopy (SEM) images were produced using Joel JSM-6390 equipment that had an accelerating voltage of 20 kV.

Methods

Technical Methods:

- **Preparing of Fruit Samples:**

For HPLC and atomic absorption analyses: Pomegranate granule samples (250 grams) were separated, washed under distilled water, and then dried. Following a one-hour sonication and heating to 60 °C with magnetic stirring, the samples were ground using 100 milliliters of distilled water. To separate the ground seeds from the suspension solution, stainless steel mesh sieves were used. A Buchner funnel fitted with filter paper was also used. After being diluted to a final 75 ml, the sample was refrigerated.

For Infrared and SEM spectroscopy: Taif pomegranate samples were collected from a local market, and only the pomegranate granule ingredients with the best qualities were used in this experiment. The collected pomegranate granule ingredient sample was cleaned with water to remove foreign particles. The components of the pomegranate granules were dried completely over several days in the sun at various ambient temperatures. After that, they were dried for 24 hours at 80 °C in a hot air oven (MEMMERT UFB 400 UNIVERSAL OVEN). A fine powder was created by grinding (Mortar Grinder RM 200, Retsch Laboratory Mills, Crushers and Sieve Shakers) the dried pomegranate granule samples. For additional examination (Richa, *et al.*, 2023), the crushed samples were kept in amber bottle and subjected to analytical methods.

Analytical Methods:

- **Separation of Sugars**

The following concentrations were used to test the improvement in the separation of sugars and the aqueous solution of calcium disodium salt hydrate containing ethylenediaminetetraacetic acid: 100 mg/L for the third mobile phase, 50 mg/L for the second, and water alone for the first. During the ideal working phase, the flow rate of freshly prepared 50 mg/mL Ca-EDTA was 0.3 mL/min. An injector model 712 from Rheodyne (Rohnert Park, USA) was used to optimize the injection loop for 5 µl. Using Empower software, product concentrations were calculated from the peak area under the curve. Hettich Republic Germany provided the Mikro 22R centrifuge. Barnstead International supplied the water purification system and ultrasonic bath.

Peaks identifications were carried out using "tR retention times," and the three sugars were distinguished based on established standards that were each injected through an HPLC apparatus separately. The retention times for fructose, glucose, and sucrose are 14.86 min, 17.33 min, and 26.97 min, in that order. The injection volume was 5 µL, and the flow rate for 50 mg/L was 0.3 mL/min.

RESULTS AND DISCUSSIONS

HPLC chromatography analysis

To guarantee accurate injection, typical chromatograms of the standards for glucose, fructose, and sucrose were injected one at a time. When the retention times for the three sugars were analyzed, A very good coverage of the concentration of our pomegranate granule samples ingredients (TPGI) have good peak area response, and very good retention time reproducibility. After completing condition optimization to obtain the best signal and the best baseline for stability and separation, the three sugars (sucrose, glucose, and fructose) under investigation showed a typical chromatographic separation pattern of the industrial standard in the chromatographic analysis using "Sugar Pack–1" (Figure 1). According to chromatographic profiles, the three sugars were clearly separated (El Kersh, *et al.*, 2023; Abdel Hameed, *et al.*, 2022 & Wagner, *et al.*, 2023). The success of food quality assessment techniques for nutritional and regulatory applications will depend on their dependability and maturity. Under working conditions, Figure 1 showed that this preparation method produced excellent chromatograms of the test mixture and the real pomegranate grain sample.

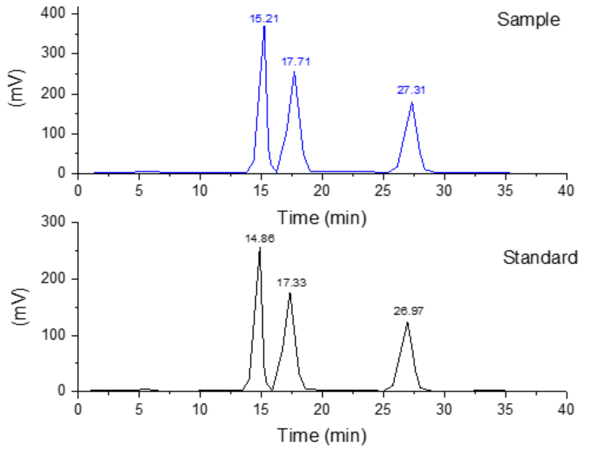

Figure 1. HPLC profile of fructose, glucose, and sucrose standard compounds and pomegranate granules ingredients sample

Mineral contents of Taif pomegranate fruits

There are many distinctive elements in Taif pomegranates (Table 1), including selenium, sodium, potassium, calcium, magnesium, and iron. As previously observed, selenium is present in a clear percentage in Taif pomegranates compared to pomegranate fruits in other places. This undoubtedly occurs because the climate, difference in environmental location, soil type, and agricultural procedures strongly influence the contents, these items have been described previously (Awadallah, *et al*., 1986).

Table 1. Percentages of minerals in pomegranate granules ingredients sample

Elements	Percentage (ppm)
Sodium	172
Potassium	285
Calcium	45
Magnesium	20
Iron	4
Selenium	61

Identification of active groups in Taif pomegranate

To map infrared frequencies, band positions and intensities observed in infrared spectra were compared with wave numbers and intensities from the dried pomegranate granule ingredient samples, which were then ground into a fine powder (Tirado-Kulieva, *et al*., 2022). As seen in Table 2 & Figure 2, the vibrational spectra of fructose, glucose, and sucrose exhibit similarities. The spectrum can generally be assigned as follows: The stretching vibration motion of OH group is in the suitable region of 3400–3220 cm^{-1}, CH stretching vibrations are presence at 2930 cm^{-1}. Water molecules are responsible for the bending vibration motion at 1620 cm^{-1}, and the deformation of the OCH and COH bands is computed between 1500 and 1390 cm^{-1}. Then, in case of in-plane CH and OH deformation bands are exhibited at 1330 to 1145 cm^{-1}. The stretching vibrations bands of CO and CC are regarded at 1087, 1015, 898, 831, and 680 cm^{-1} frequency.

Table 2. FTIR spectral bands of dried pomegranate granules ingredients solid fine powder sample

Infrared bands	Assignments	Suggested component
3400, 3220	ν(O–H)	Glucose & Fructose & Sucrose & H_2O
2930	$\nu(CH_2) + \nu(CH)$	
1620	$\delta(H_2O)$	
1500, 1390	δ(OCH) + δ(COH)	
1330, 1276, 1145	In-plane δ(OH) + CH	
1087, 1015, 898, 831, 680	ν(CO) + ν(CC)	

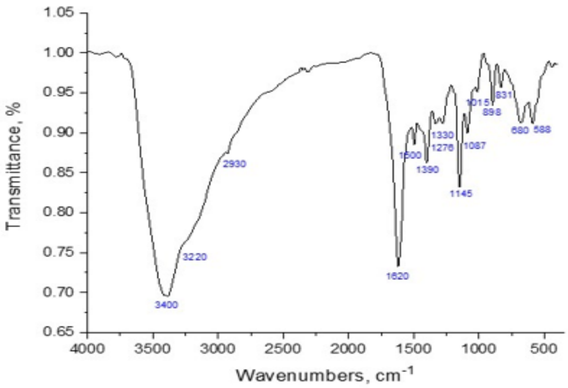

Figure 2. FT-IR spectrums of dried pomegranate granules ingredients solid fine powder sample

Examination of SEM and EDX analyses

SEM scanning was used to examine the sample's morphology (Sharma & Bhardwaj, 2019). The surface morphology of the dried pomegranate granule solid fine powder sample was evaluated by SEM at 500X magnification, and the obtained images are shown in Figure 3. The SEM images showed that the particles were agglomerated with a controlled morphological structure. Based on the SEM study, the solid fine powder sample in the dried pomegranate granules was distributed throughout the sample and showed different particle sizes in the microstructure. In particular, the particles showed different shapes and diameters, and some cracks were observed in the shapes of the block walls.

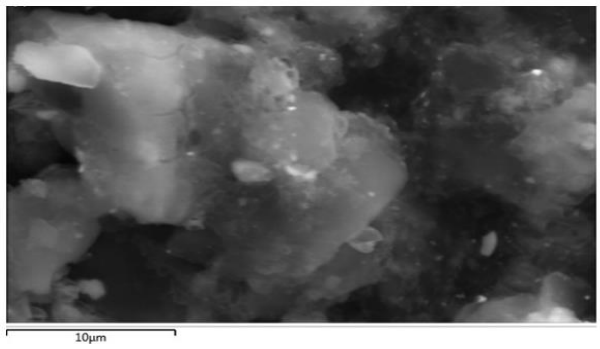

Figure 3. SEM micrograph of dried pomegranate granules ingredients solid fine powder sample.

Chemical analysis results obtained by EDX (Table 3 & Figure 4) for the solid fine powder sample containing dried pomegranate granule ingredients showed the presence of some elements, such as sodium, potassium, calcium, magnesium, iron, selenium, chlorine, carbon, nitrogen, and oxygen, at spectral peaks 1.04, 3.31, 3.69, 1.52, 6.39, 11.21, 2.62, 0.28, 0.39 and 0.53 keV, respectively. The types and concentration of minerals depend on the origin of the fruits and the cultivation sites.

Table 3. EDX spectra of minerals in pomegranate granules ingredients sample

Elements	Spectral peak keV	Elements	Spectral peak keV
Sodium	1.04	Selenium	11.21 & 1.38
Potassium	3.31	Chlorine	2.62
Calcium	3.69	Carbon	0.28
Magnesium	1.52	Nitrogen	0.39
Iron	6.39	Oxygen	0.53

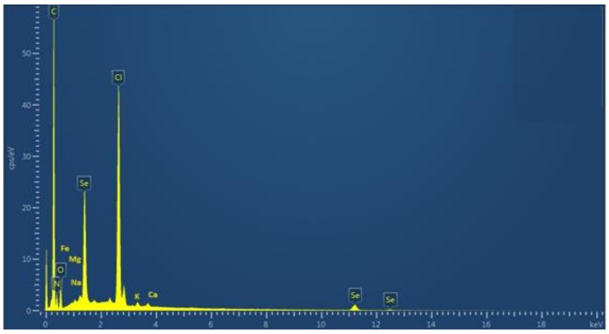

Figure 4. EDX spectrum of dried pomegranate granules ingredients solid fine powder sample

CONCLUSIONS

Taif pomegranates are generally considered an interesting and healthier fruit because of consists of three essential sugars (glucose, fructose, and sucrose), and an important mineral. Through further research into the chemical composition of fruits, food technologists could produce fruits with improved nutritional quality. In this study, an analytical HPLC method was developed to simultaneously analyze and qualitatively determine the main sugars in Taif pomegranate granules. The results obtained indicate that this method is suitable for separating and identifying the three sugars, namely, fructose, glucose, and sucrose, without derivatization. The results of the analysis with a sample of Taif pomegranate corresponded well with those obtained by other authors, except for the presence of selenium, which is an antioxidant element that gives Taif pomegranate its medicinal and nutritional importance. The chemical composition and nutritional value of the pomegranate fruit granules were studied and discussed in terms of dry weight. Using energy dispersive X-ray spectroscopy (EDX), scanning electron microscopy (SEM), and infrared spectra, the functional groups of sugars and carbohydrates were determined. The FT-IR spectrum of the dried pomegranate granule solid fine powder sample showed peaks at 3400–3220, 2930, 1620, 1500–1390, 1330–1145, 1087, 1015, 898, 831, and 680 cm^{-1}, these peaks are assigned to CH vibrations, H_2O bending vibrations, OCH and COH deformation, in-plane deformation of CH and OH, and stretching of both CO and CC. The research findings hold significance for fruit processors that produce pomegranate juice. This study also contributes to improved consumer interest in nutrition and nutritional labeling.

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تقييم المكونات السكرية والمعادن في حبيبات رمان الطائف من خلال التحاليل الطيفية والتحليلية الدقيقة

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الملخص

تهدف الدراسة الى استكشاف المحتويات السكرية والمعادن لحبيبات رمان الطائف، حيث تحتوي مكونات حبيبات الرمان الطائفي (TPGIs) على السكريات (مثل الفركتوز والجلوكوز والسكروز) وبعض العناصر الأساسية (السيلينيوم والصوديوم والبوتاسيوم والكالسيوم والمغنيسيوم والحديد) وأيونات الكلور. باستخدام الاختبارات الطيفية والتحليلية الدقيقة، تم في هذه الدراسة استكشاف المحتويات السكرية والمعادن لحبيبات رمان الطائف. حيث استخدم نظام "تحليل كروماتوغرافي سائل عالي الأداء" (HPLC) ومجهز بعمود "-Sugar Pak 1" ومقياس انكسار. أظهرت النتائج بالبحث ان مكونات TPGI مصدرًا مهمًا للعديد من العناصر الغذائية الغنية بمضادات الأكسدة والفلافونويد ويمكن أن تساهم في اتباع نظام غذائي صحي. كما تُعد السكريات الأحادية واحدة من العناصر الأكثر أهمية، والتي تستخدم لتقييم الدرجة التجارية للفواكه لاستخدامها في الصناعة.