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Determination of Fructose, Glucose and Sucrose in Taif Grape using High Performance Liquid Chromatography and analysis of mineral salts

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ABSTRACT

The major components in grape are water followed by sugars mainly fructose, glucose and sucrose. Grape is an excellent source of many nutrients able to contribute to a healthy diet. It is a good source of organic acid, sugars, elements and phenolic compounds. The monosaccharide is one of the most important parameters for the assessment of the commercial quality of the fruits for human and industrial used. Taif is the first leading grape producers in Saudi Arabia. In our knowledge there has been no research on the Taif grape. In this study we will assessed for the first time fructose, glucose, sucrose as well as some elements such as potassium, sodium and iron in Taif grape. High Performance Liquid Chromatography HPLC will be used combined with Sugar Pak-1 column and Refractometer as detector. Optimized conditions for several parameter (temperature of the column, kinds of the mobile phase, flow rate of the mobile phase and the size of the injection loop were studied. We used the deionized water the method of extraction of sugars and trace elements by means of an ultrasonic bath. The mineral elements such as Fe, Cu, K and Na were analyzed by atomic absorption and flame photometry. This paper describes a detailed procedure for the analysis of fructose, glucose, sucrose and mineral salts in Taif grape. The results can be confirmed the three sugars separated successfully after optimization of the condition of the instrument using univariate method of optimization.

Keywords: Sugars, HPLC, grapes, Sugar Pak1, Mineral analysis.

INTRODUCTION

The major components in the grape are water followed by sugars (fructose and glucose) [1]. This monosaccharide is one of the most important parameters for the assessment of the commercial

quality of the grape. The analysis of sugars and sugar mixtures is of considerable important to food and beverage industry.

Carbohydrates, in general, do not absorb UV light. Thus chemical attachment is often required to provide UV absorptive at selected wavelengths. The current protocols in food analytical chemistry 2002 reported High Performance Liquid Chromatography (HPLC) method for determination of mono and disaccharides using Refractive Index Detector (RID) for nutritional production [2]. The HPLC equipped with an aminopropyl column so all the sugar sample are extracted, dried, reconstituted in an aqueous acetonitrile solvent as a mobile phase. On the other hand the current protocols used calcium- load caution – exchange column to overcome the difficulties for using acetonitrile as a media for analysis and converted to more green chemistry by using deionized filter water as the mobile phase.

At present the determination of mineral and trace elements has taken on considerable important in both grape and their derivatives (grape juices, wines and other alcoholic beverages) not only from nutritional, but also from the technological point of view. These elements can have a considerable influence on the production process with, in some cases, negative effect such as oxide - reduction reaction, precipitation, colloidal alterations, formation of gel [3, 4]. **Susana *et al.*, 2004 [5]** studied the sugar contents (glucose and fructose) on grape skin for five Portuguese grape varieties and compared in grape juice and whole grape using liquid chromatography (LC) with RID.

Glucose and fructose were analyzed in wine using HPLC with modified polar-bonded NH-Silica phase column and a RID performed by Enzo *et al.*, 2007 [6]. The HPLC separation of standard monosaccharide and disaccharides were successfully achieved by Takako *et al.*, 2001 [7] using new prepared stationary phase. Manuel *et al.*, 2004 [3] determined the Cu and Zn levels in samples of the main varieties of (white and red) table grape consumed in Spain, as well as in samples of commercial grape juices. The analytic technique used was atomic absorption spectroscopy AAS with electro thermal atomization after acid digestion of the samples. Trajce and Irina, 2009 [8] reviewed and discussed the main approaches preliminary sample digestion and direct instrumental aspiration measurements combined with AAS for trace element determination in wine. Mineral elements were extracted and analysis AAS and FES according to the methods described by Roos and Price, 1970 [9]. The metals determined by direct aspiration of the sample, suitably diluted with water, after removal of suspended matter by centrifugation. In this method and the method we presented in this paper it is not necessary to ash the samples prior to analysis. Nutritional study of Copper and Zinc in grape and commercial grape juices from Spain was presented by Manuel *et al.*, 2004 [3].

There are several methods reported in the literature for determining components in grape fruit and their derivative. Quantitative analysis organic acid extracted from grape was performed by HPLC coupled to photodiode array detection and electrospray ionization mass spectrometry [10-12]. Fateh *et al.*, 2007 [13] were used HPLC and AAS to determine sugars and mineral salts in Tunisian fresh figs. Sugar, such as glucose, fructose and sucrose is an important component in nature and also a main element in grape. However, sugar and the minerals elements in Taif grape has not investigated by the researchers using the classical methods or modern instrument methods, such as HPLC. HPLC method has been developed to extract and quantity individual

sugars present in fresh Taif grape and imported grape from the local market. Sugars were extracted from fresh grape by heating the samples to 80 °C with deionized water and sonicated by the ultrasonic bath for 30 minutes. The chromatographic conditions for the optimal separation of the sugars were established, and the method was validated using Sugar Pak-1. The results of selectivity, linearity, precision and accuracy are presented. The method can be used for the routine determination of glucose, fructose and to some extent also of sucrose in different kinds of grapes. The study on both qualitative and quantitative analysis of sugars were achieved

MATERIALS AND METHODS

2.1. Reagents

Glucose and sucrose were obtained from Techno Pharmachem, Bahadurgarh, India. Fructose and Calcium- EDTA were obtained from Riedel- deHaen and Fluke respectively. Three stock standards sugars solutions were prepared. The first one contained glucose 100 mg/ml, the second one fructose 100 mg/ml, the third one is sucrose 50 mg/ml, the dilution was carried on to make a suitable dilution for doing the working calibration curve as it need. The prepared standard solutions of sugars were stored at 4 °C. all the samples before injection in the HPLC sonicated for at least 15 minutes in ultrasonic bath to remove air bubbles and passed through filtration desk 0.45 µm. Sodium, Potassium, Copper and Iron standard solution obtained from a Buck Scientific. The stock solutions of these elements at a contraction of 1000 mg/L were already prepared in water and 5% HCL. Working solution prepared in suitable concentration used for direct determinate were made up in water.

2.2. HPLC System of Analysis

Sugars composition (Glucose, Fructose and sucrose) were determined by HPLC Waters system equipped with 515 pump from (Milford, MA, USA) stainless steel filter, guard column Nova Pak C₁₈ (4 µm, 3.9x 20 mm) Waters column heater module with Waters Temperature control module, Waters 410 differential refractometer, Waters Automated Gradient Controller associate with Dell computer system using Empower software to run and control all the calculations for the instrument. A Waters Sugar Pak-1 column (300x 6.5 mm) was used through this study. The optimizations of the separation of sugars, aqueous solution of Ca-EDTA in three different concentrations were tested: mobile phase one water only, mobile phase two (50 mg/L) and mobile phase three (100 mg/L). The flow rate of the 50 mg/ml Ca-EDTA eluent (freshly prepared) was 0.3 ml/ minute as the optimized working phase. The temperature of the oven was optimized to 90 °C. The univariate method of optimization was used. The loop of the injection was optimized for 5 µl with Rheodyne injector (Rohnert Park, USA) model 712. The concentrations of the products were determined from the peak area under the curve using Empower software. Centrifuge kind Mikro 22R was supplied from Hettich, Republic of Germany. Water purification system and the ultrasonic bath supplied by Barnstead International.

Before the quantitative and qualitative determination of sugars in the samples, we prepared standard solutions of different sugars: sucrose, glucose and fructose. With those standard solutions of different sugars we made calibration lines for each one of the sugar, which later used for assessing the concentration corresponding to the different peaks in the chromatograms

2.3. Sample Preparation

White grape fresh fruit from Taif production (*Vitis vinifera*) and imported grape (*Vitis labrusca*) were purchased from local market. The two grapes were kindly identified by Professor Yassin Al-Sodany, Professor of Taxonomy, Faculty of Science, Taif University, Kingdom of Saudi Arabia. The grapes were washed under distilled water then dried. Triplicate fresh grapes from each kind were exactly weight (100 gm). The grapes skin was removed from the fresh fruit as well as the seeds. The pulp cut up into small pieces and ground with 20 ml portion of deionized water two times heated to 80 °C under magnetic stirring and sonicated for 30 minutes. The suspension solution were passed through a stainless steel mesh sieves before filtering by Whitman filter paper using Buchner funnel. The sample made up to final volume of solution sample 50 ml. Centrifuge and stored in refrigerator. An aliquot of the samples was diluted with purified water to bring the concentration of the samples within the range of the calibration curves. All standards and solutions ready to analysis were passed through the 0.45 um desk filter and sonicated for 15 minutes in ultrasonic bath.

2.4. Analysis of Mineral Elements

Mineral compositions of the grape for measurements (K, Na, Fe & Cu) were carried out by extracted into deionized water (the same extract as mention above). The solution diluted within rang of the calibration curve of the elements by deionized water. Janway Flame Emission Spectrophotometer for evaluation of sodium and potassium with air propane burner. Buck scientific atomic absorption spectroscopy (AAS) was used to evaluate the Copper and Iron using air / Acetylene burner. A triplicate samples were extracted for each products (Taif grape and imported grape).

2.5. Spike of Grape Sample

In order to evaluate possible matrix effects and the accuracy validation of the method used in this study. Two ml batches of the grape sample were prepared to examine the recovery yield and the validation of the process were applied to determined fructose. One batch was spike with zero concentration of fructose. The second batch was spike with an amount of fructose, so the final concentration of the sample is 10mg/ml fructose spiked. As well as a standard solution of final concentration of 10 mg/ml was directly injected for spiking chromatographic analysis. Sugars concentration was measured based on the peak area using Empower software, for the three injections as mention above. The same manner were carry out to monitor recovery for the process to determination of glucose.

RESULTS AND DISCUSSION

3.1. Chromatographic Performance

Peak identification was based on the retention times t_R , Identification of the three sugars were confirmed with known standards injected individually through the HPLC and the retention time for sucrose ($t_R = 17.45$ minutes), glucose ($t_R = 21.98$ minutes) and fructose ($t_R = 25.96$ minutes). The flow rate for 50 mg/L was 0.3 ml/min, the column temperature was 90°C and the injection volume 5 μ l (figures 1-3) shows a typical overlay chromatograms for standards of glucose, fructose and sucrose were inject separately to confirm the proper check for the retention time as well as presented the calibration curves for the three sugars, it shows very good reproducibility of the retention time, nice response for the peak area, excellent linearity and it is covers the

concentration for our samples of the Taif grape and the imported grape. In the chromatographic analysis, with Sugar Pak1 figure 4, shows a typical chromatographic separation pattern of the synthetic standard of the three sugars under study (sucrose, glucose and fructose) after the optimization of the conditions were carried out for the best signal, best baseline stability and separation. The chromatographic profiles showed very good separation of the three sugars, and sucrose was the first eluent and this agreed with the theory of the column that we are used [14]. In the chromatographic analysis, with Sugar Pak-1 and RID, each run was performed in about 25 minutes with great resolution. Regarding the calibration, the liner rang was, on average from 0.5 to 50 mg/ml for all sugars, with correlation factors between 0.985 to 0.997.

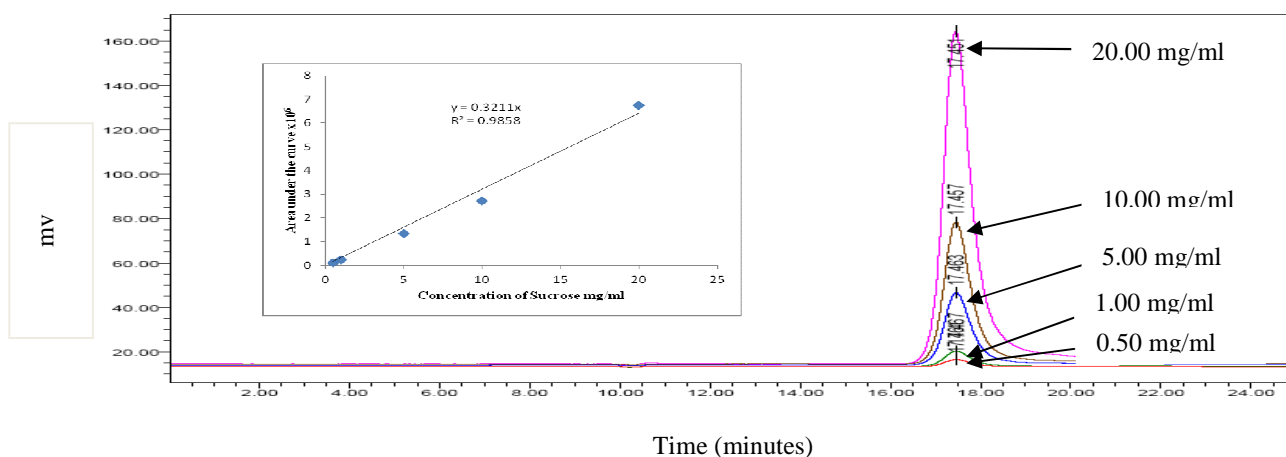


Figure (1): Overlay Chromatograms and calibration curve for individual sucrose at working conditions using Sugar Pak-1 and Refractive index detector (flow rate 0.2 ml/min, column temperature 90°C).

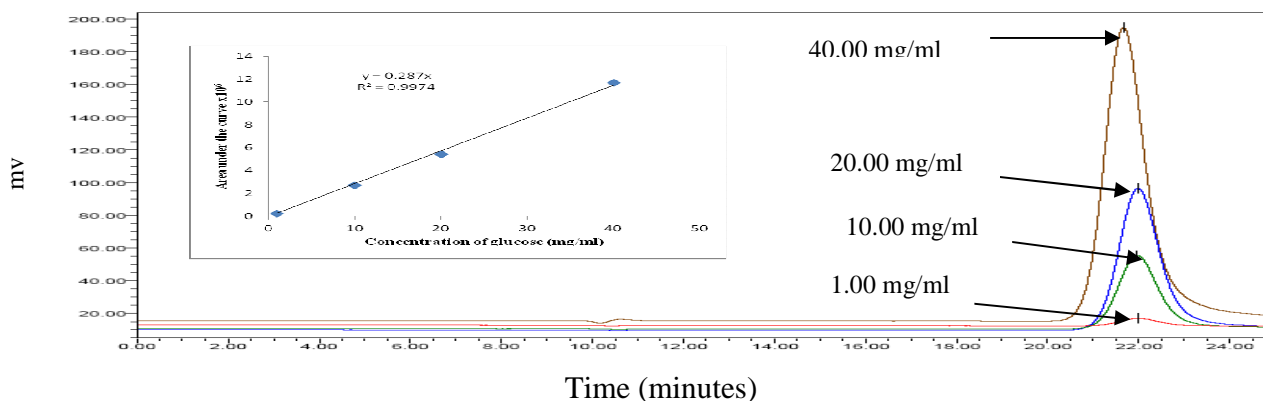


Figure (2): Overlay Chromatograms and calibration curve for individual glucose at working conditions using Sugar Pak-1 and Refractive index detector (flow rate 0.2 ml/min, column temperature 90°C).

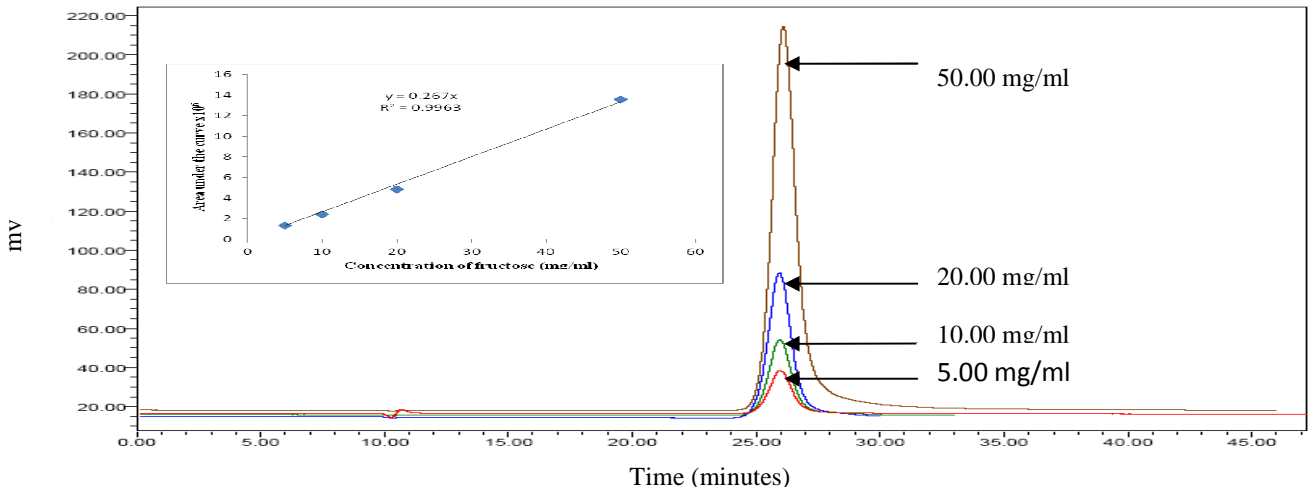


Figure (3): Overlay Chromatograms and calibration curve for individual fructose at working conditions using Sugar Pak-1 and Refractive index detector (flow rate 0.2 ml/min, column temperature 90°C).

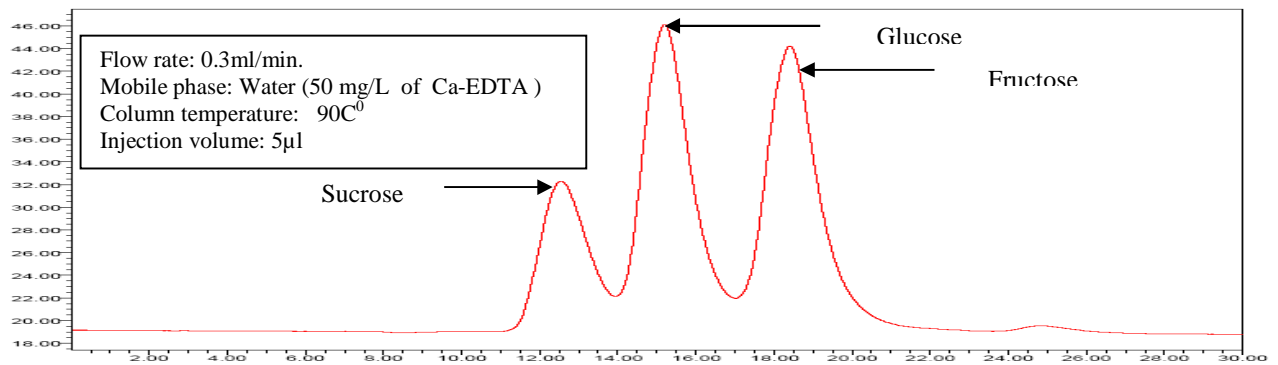


Figure (4): Separation chromatogram for mixture of sucrose, glucose and fructose using Sugar Pak-1 and RID.

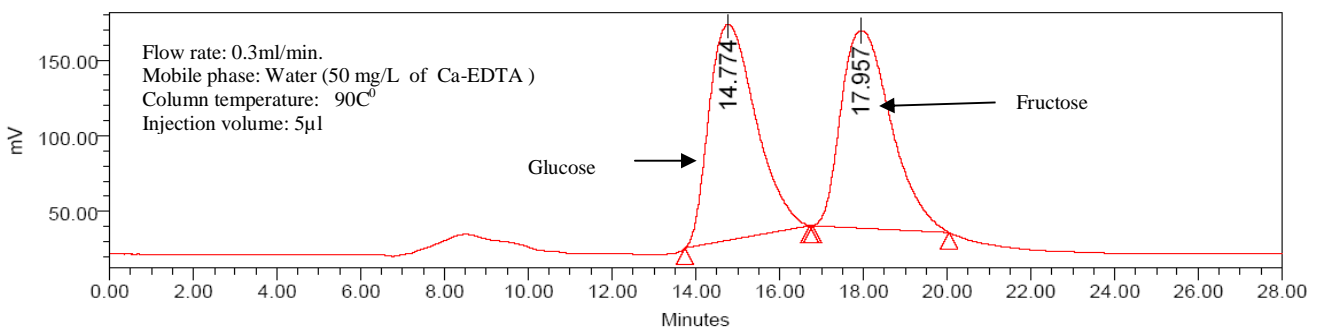


Figure (5): Separation chromatogram for Taif grape using Sugar Pak-1 and RID.

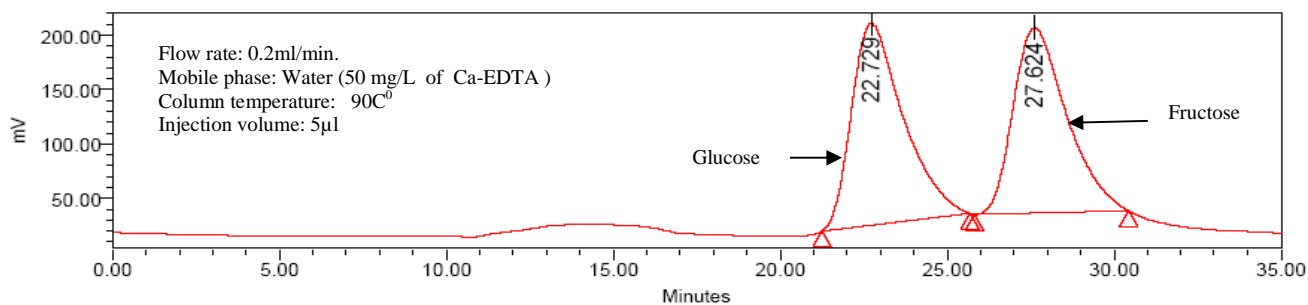


Figure (6): Separation chromatogram for imported grape using Sugar Pak-1 and RID.

3.2. Grape Sample Analysis

There is an interest in developing reliable and ripe methods to assess the quality of foods for nutritional and regulatory purposes. The common procedure for preparing the grape juice consists of two steps, sample extraction and cleaning sample extract using the centrifuge and disk filter 0.45 µm. Figures [4,5 & 6] showed that this preparation method yield excellent chromatograms for the test mixture and the real grape sample at the working conditions.

Figure (6): Separation chromatogram for imported grape using Sugar Pak-1 and RID. Six grape samples, including three Taif grape and three import grape were analyzed using the samples preparation method described in this study. No sucrose was detected in all the samples under study (figure 5 & 6 table 1, summarize the composition of sugars in the fresh grape). The mean values for glucose and fructose showed a significant difference among the Taif grape and the imported grape. The glucose and fructose in Taif grape were higher in value then in imported grape. While the elements potassium and sodium higher in imported grape then in Taif as shown in table 2, undoubtedly due to the strong influence of the climate of the place, different environmental place, type of the soil and agricultural procedures on the contents of these elements in the fruit which is study by **Angelva, 1999 et al., [4]**.

In this study, we investigated the composition of sugars (glucose, fructose and sucrose) in Taif grape and imported grape from the local market. The values obtained for glucose and fructose were promising as expected, we found no reference for the concentrations of these sugars as natural components of the Taif grape in bibliography. The value for the glucose to fructose ratio, which is near one. In addition, sucrose was not detected in both of grapes under study whereas the potassium were detected in significant amounts see table 1 and figure 5 & 6.

Table 1. Composition of sugars in fresh grape fruit in 100 grams weight samples

Composition	Taif grape	R.S.D	Imported grape	R.S.D.	Recovery %	Linearity mg/ml	R ²
Glucose (%)	3.79 ±0.32	7.92%	2.28±0.24	10.52%	97.4	1.0-40.0	0.998
Fructose (%)	3.77±0.12	2.99%	2.33±0.29	12.45%	101	5.0-50.0	0.997
Sucrose	nd	-	nd	-		0.5-20.0	0.988

Values expressed as mean of triplicate determinations ± standard deviation.

R.S.D. = Relative standard deviation.

R² = Square of correlation coefficient.

nd= not detected

Table 2. Composition of minerals elements in fresh Grape fruit in 100 grams weight samples

Composition	Taif Grape	Imported Grape	Linearity µg/ml	R ²
Potassium (mg)	1806.7±2.89	626.7±11.50	0-1.2	0.995
Sodium (mg)	51.7±2.02	150±6.92	0-2.5	0.995
Copper (µg)	55±NG	400± NG	0-5	0.998
Iron (µg)	35± NG	10± NG	0-10	0.997

Values expressed as mean of triplicate determinations ± standard deviation.

R.S.D. = Relative standard deviation.

R² = Square of correlation coefficient.

NG = Negligible

CONCLUSION

The methods used in this project for determination of sugars and mineral elements were simple suitable and rapid procedure. HPLC , Flame emission and AAS respectively were used to measure the contents of glucose , fructose and sucrose, potassium, Sodium, Copper and Iron in the Taif grape and the imported grape. In general Taif grape fruit is an interesting fruit. The pulp is rich in glucose and fructose and useful mineral elements. Sucrose was not detected in Taif grape as well as the imported grape. That is mean it is more health and more appreciated fruit for the people which suffering from diabetes. The information data from this research are important for fruit processors looking for grape juice industry. Moreover, with nutrition labeling and increased consumers interest in nutrition. Sugar Pak-1 columns are well suited to the analysis of sugars glucose, fructose and sucrose in grape fruit. The separation and detection of fructose, glucose and sucrose are readily achieved using 50 mg/L EDTA-Ca as the mobile phase with Sugar Pak-1 column and RID . This system avoids the use, high cost and disposal implications of toxic acetonitrile when separation is performed on amino silica columns.

The results showed that, within our expectations, there were same difference in the composition of sugars and minerals elements in Taif grape and imported grape fruit collected from different market around Taif. Glucose and fructose appeared as the major sugars contributing to the sweet taste of the fruit. Further research on the chemical composition in the fruit should enable food technologists to select with improved nutritional quality.

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