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Study of the Physicochemical Properties/Gas Chromatography Profile of Adulterated Pomegranate Juice by Nano-Composite-Fiber

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ABSTRACT

In this work, pomegranate juice was adulterated manually by adding sour cherry and red grape juices. Physicochemical properties (brix, acidity, formalin index, reducing sugar, and total sugar) and Volatile Organic Compounds (VOCs) gas chromatography profile (extracted by PPy-Ag nano-fiber) of pure pomegranate juice and adulterated pomegranate juice were evaluated and used to find adulteration. PPy-Ag nano-fiber as a Head-Space Solid Phase Micro Extraction-Gas Chromatography (HS-SPME-GC) method was used to extract and detect the VOCs profile (total peak area and total peak height) of different juices. The Box-Behnken Design (BBD) was used to study the effects of Pomegranate Juice (PJ), Sour Cherry Juice (SCJ) and Grape Juice (GJ) percent (V/V%) in the adulterated mix juice physicochemical properties and GC-profile. Results showed that the percent of pomegranate juice, sour cherry juice, and grape juice affect the juice physicochemical property, and there are relations between the physicochemical property of the juice and VOCs gas chromatography profile (total peak area and total peak height) that can help to find adulteration. The presented research makes it possible to detect adulteration of sour cherry and grape juice and their percent in the adulterated mix by analyzing the juice physicochemical properties and GC-profile.

Keywords: Fruit juice, Red grape juice, Sour cherry juice, Volatile organic compounds.

INTRODUCTION

One of the most important agricultural businesses is fruit juice industry. The fruit juices and concentrates have some beneficial effects on human health and are valuable semi-finished products. These juices are the main sources of energy and nutrients, and can affect human nutrition. Some fruits like pomegranate, apple, and peach are used in the fruit juice industry [1-2]. The fruit juice industry has the largest share in the food industry. Some technology like cryoconcentration, thermal concentration, and membrane concentration are used to provide fruit juice and fruit concentration. One of the most famous, valuable, and extensively cultivated worldwide fruit is pomegranate [11-9]. Several products of pomegranate include pomegranate juice, pomegranate flavoring, flavored waters, and smoothies are presented in the markets. Pomegranate and its juice have some usage in the world because of their beneficial health properties like, abortifacient and contraceptive. This fruit also is used to treat intestinal parasite infestations. The sensory quality and health benefits of pomegranate are two factors that are responsible for the increased value of pomegranate juice compared to other fruit juices. This value also makes concord pomegranate juice a likely target for adulteration by less valuable juices [11-10]. Adulteration in fruit juice is

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one of the problems that affect the quality of fruit juices and it is necessary to find some ways to decrease frauds in this industry. Some frequent types of adulteration in fruit juice that are used include: 1- simple dilution with water, by the addition of natural constituents from juices, 2- addition of sugar syrup, which decreases the total amino acid value, 3- use of constituents not naturally present in the juice such as colorants, and 4- addition of cheap juice from other types of fruits. The most detected pomegranate adulteration is (1) addition of sugars or sweet juices, e.g. peach juice and (2) addition of cheap and widely available juices, e.g. grape and sour cherry juice [17-3]. Recently, several methods were posed to improve quality of fruit juice and prevent adulteration and frauds that threats the health of consumers. There are several analytical methods to determine authenticity of fruit juices like carotenoids, phenols, volatile organic acid, and amino acid profile using different spectroscopic/chromatographic methods. In addition to the mentioned methods, there are some other chemical analysis methods that can be used to detect adulterations; many specific chemical parameters can be used to detect adulterations, mainly those based on acidity, brix, formalin index, reducing sugar, total sugar, and VOCs profile in gas chromatography [6-18].

Conducting polymers and their derivatives and composites with differential metal and metal oxide have had phenomenal growth in the fields of absorbing materials, molecular electronics, membranes, filters, chemical and biochemical sensors, super capacitors, separation, extraction and so. These polymers have some character like high surface area, electrical conductivity, adsorption-desorption character, and environmental stability that cause using of these materials in different industries. Conductive polymers also have many potential applications due to their combined electrical conductivity and polymeric properties such as flexibility, low density, and facile structural modifications [15-9].

A new Micro Extraction method based on Solid Phase (including polymers like polypyrrole, polyaniline, and so) called SPME is successfully used for extraction of VOCs from different materials like food, water, wastewater, and blood samples, and for injection to different analytical instruments like Gas Chromatography (SMPE-GC) and Super critical Fluid Chromatography (SPME-SFC). In the SPME, the Solid Phase is present directly in the sample (direct-SPME) or in the Head Space of the sample (HS-SPME) and analytes are absorbed on an extracting phase. The latter consists in a very thin polymeric film immobilized on a substrate [9 and 16].

The present work is in continuation of our researches on the production of conducting polymer (application as SPME fiber and gas sensors), differential fruit juices and concentrate adulteration using physicochemical and GC-profile analysis. In this work, we aimed to adulterate pomegranate juice manually by adding sour cherry and red grape juices and evaluate the physicochemical properties (brix, formalin index, reducing sugar, and total sugar) and VOCs gas chromatography (extracted by PPy-Ag SPME fiber) of pomegranate juice and adulterated pomegranate juice to find adulteration.

MATERIALS AND METHODS

Reagents and Chemicals

Pyrrole (provided from Fluka, Switzerland) was distilled in a vacuum, before use. It was stored in deep-freezer in sealed condition to avoid photo-polymerization. Ferric chloride ($FeCl_3$) as oxidant and silver nitrate were purchased from Aldrich. All organic compounds were purchased from Merck. The pomegranate, sour cherry, and grape concentrate were provided from Urum-Narin Company, Urmia, Iran. The pomegranate, sour cherry and grape juices were obtained from their concentrate.

Apparatus

The PPy-Ag fiber was prepared by a chemical polymerization. An SPME fiber holder for manual sampling was designed and fabricated by Dr. Sajad Pirsa and Dr. Mohammad Alizadeh research group in Urmia University (Iran) [15]. The GC apparatus used in this study was from Agilent 7890 A, Wilmington, DE, USA. The Scanning Electron Micrographs (SEM) using an SEM instrument (Philips XL30, Holland) was used to evaluate the morphology of PPy-Ag fiber.

Scanning Electron Microscopy (SEM) Study

The scanning electron microscope (SEM) (HITACHIS-4160, Japan) was used to study the size and morphology of PPy-Ag particles and PPy-Ag fiber. The surface of PPy-Ag particles and PPy-Ag fiber were coated with gold particles, then, the SEM images were obtained by collecting the samples on an aluminum SEM disk.

Fruit Juice Preparation

To provide 1,300 mL pomegranate juice, 137.93 g pomegranate concentrate and 65 g sugar were mixed; then, adequate water was added to the mix. To provide 1,300 mL sour cherry juice, 131.885 g sour cherry concentrate and 65 g sugar were mixed, then adequate water was added to the mix. In the case of 1,300 mL sour red grape juice, 236.3634 g sour cherry concentrate and 26 g sugar were mixed; then, sufficient water was added to the mix. After standardization of the brix and acidity of fruit juices, these three different juices were mixed with each other in different percent according to the experimental design (Table 1).

Analysis Methods

Apparent Viscosity Measurement

The apparent viscosity was measured by using a Brookfield viscometer (Brookfield DVII +, America, LV2) after 30 seconds rotation (30 rpm).

Acidity Determination

Measuring acidity was performed with titration of 10 g fruit juice sample (5 g sample and 5 g of distilled water) by NaOH solution (0.1 M) in the presence of phenolphthalein.

Formalin Index, Reducing Sugar and Total Sugar Determination

The reducing sugar (g 100 g^{-1} juice) in the fruit juices was determined by titration method where clarified fruit solution was titrated against mixed Fehling's solution using methylene blue as an indicator. Total sugar (g 100 g^{-1} juice) was also determined by titration, but before titration, citric acid was added and the solution was boiled in order to complete the inversion of sucrose. Formalin index (g 100 g^{-1} juice), total sugars, and reducing sugars in the fruit juices were determined according to Iran's Standard based on Lane-Inon measurement [7].

Fiber Preparation, Chromatographic Conditions and Extraction Procedure

The PP-Ag nano-fiber was fabricated by chemical polymerization method at room temperature under atmospheric condition, according to our previous work [13]. HS-SPME extraction procedure was done like previously reported works [15]. A gas chromatography instrument (Agilent7890 A, Wilmington, DE, USA) with flame ionization detector (GC-FID) at our previous research work condition was used [15]. The gas chromatographic profile of fruit juice VOCs at the optimum condition is shown in Figure 1.

RESULTS AND DISCUSSION

The morphology, size, and porosity of the synthesized PPy-Ag film were studied by Scanning Electron Microscopy (SEM). Figure 2 shows the SEM micrographs of the PPy-Ag fiber. Results show that PPy-Ag particles are seed and spheral like and in the nano-sized between 50-100 nanometers.

Experimental Design

The sour cherry, pomegranate, and red grape percentages (% Wt) were the three factors that affected physicochemical properties of the mixed fruit juice including acidity, brix, formalin index, reducing sugar, and total sugar. To study the effect of these factors on the responses, a Box Behnken Design was used. Three variables, including sour cherry, pomegranate, and red grape

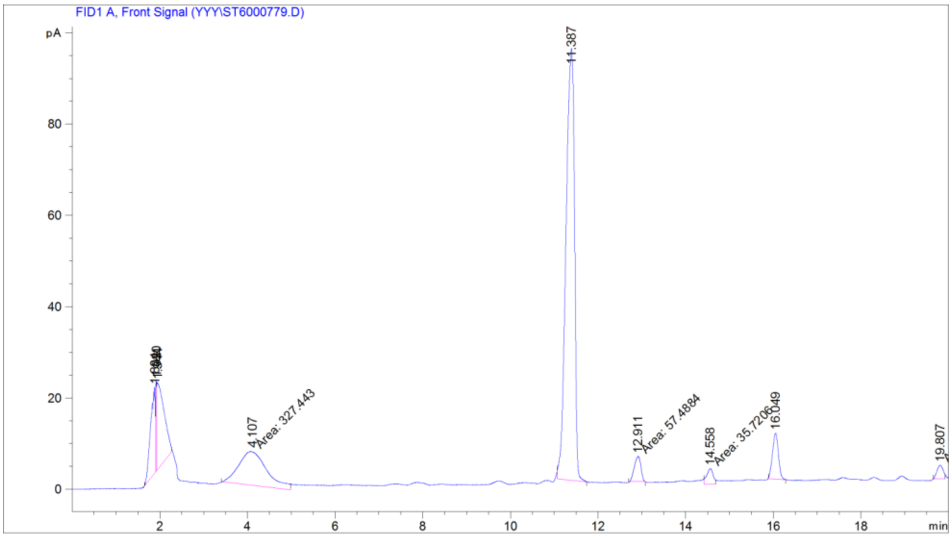

Figure 1. GC-FID chromatogram of juice VOCs extracted by PPy-Ag fiber.

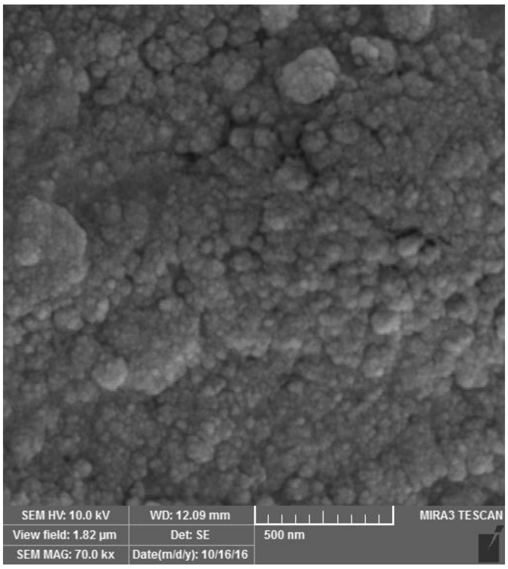

Figure 2. SEM images of PPy-Ag fiber.

percentage (all at five levels) were investigated. In Table 1, the 3 processing variables as factors, levels, and experimental design are given. Table 1 also presents the evaluated responses including, acidity, brix, formalin index, reducing sugar and total sugar. The Design-Expert software (version 7) was used for analysis of sour cherry, pomegranate, and red grape percentage effect on the physicochemical properties. Minitab version 17 was used to study the relations between physicochemical properties and gas chromatography profile. Initially, the full term second-order polynomial response surface models were fitted to each of the response variables, according to the following equation:

$$Y=b0+b1\times F1+b2\times F2+b3\times F3+b4\times F1\times F1+b5\times F2\times F2+b6\times F3\times F3+b7\times F1\times F2+b8\times F1\times F3+b9\times F2\times F3 \quad (1)$$

Where, *Y* is the responses (acidity, brix, formalin index, reducing sugar and total sugar); *F1*, *F2* and *F3* are sour cherry, pomegranate, and the red grape percent (% Wt). *b* values are the coefficient values obtained through multiple linear regressions. Where possible, stepwise deletion of terms was applied to remove the statistically non-significant terms, so as to simplify the model. However, when the exclusion of such terms from the model decreased R^2_{adj} and increased the estimator of the variance *S*, the term was included in the model. The statistically non-significant linear terms also remained in the model when the respective quadratic or interactive effects were statistically significant. The quadratic polynomial models for three response functions accompanied by *F* values and corresponding R^2 was used; the estimated regression coefficients are summarized in Table 2.

Contour Plot of Juice Physicochemical Properties Based on Variables

The contour plots based on the model

Table 1. List of experiments in the Box-Behnken Design (BBD) and the responses of each run.

Run order	Factors			Responses (Physicochemical) property				
	F1: Sour cherry (%)	F2: Pomegranate (%)	F3: Red grape (%)	Brix	Acidity (g 100 g^{-1})	Formalin index (g 100 g^{-1})	Reducing sugar (g 100 g^{-1})	Total sugar (g 100 g^{-1})
1	16.66	16.66	67.7	14	0.448	6.5	11.077	12
2	50	50	0	12	0.464	7.5	6.73	9.89
3	0	50	50	13.5	0.4832	6	10.2857	11.8
4	16.66	66.7	16.66	12.5	0.4864	6	8.276	11.077
5	0	0	100	14.5	0.525	8	12.4138	13.1
6	0	0	100	14.5	0.52125	7	12.2	12.857
7	50	0	50	13.5	0.432	9	9	10.91
8	0	50	50	13	0.4864	5.5	8.78	11.25
9	0	100	0	12	0.512	4.5	8.276	9.73
10	66.7	16.66	16.66	12	0.4288	8	7.5	9.863
11	100	0	0	12	0.42545	10	5.39	9.114
12	0	100	0	12	0.5056	4.5	7.06	10.588
13	100	0	0	12	0.43215	11	6.154	9.1
14	33.3	33.3	33.3	12.5	0.4576	8	9.351	10.435

Table 2. Some characteristics of the constructed models for responses.

Response type	Regression equation[a]	Model summary
pH	*pH= 3.71×F1+3.66×F2 +3.27×F3*	*R-sq*= 0.976 *R-sq(adj)*= 0.972
Total acidity (g 100 mL^{-1})	*Acidity= 0.42×F1+0.50×F2+0.52 g 100 g^{-1} F3-0.17×F1×F3-0.11×F2×F3*	*R-sq*= 0.989 *R-sq(adj)*= 0.984
Formalin index (g 100 mL^{-1})	*FI= 10.33×F1+4.46×F2+7.26×F3*	*R-sq*= 0.934 *R-sq(adj)*= 0.922
Reducing sugar (g 100 mL^{-1})	*RS= 5.95×F1+7.63×F2+12.36×F3*	*R-sq*= 0.940 *R-sq(adj)*= 0.929
Total sugar (g 100 mL^{-1})	*TS= 9.10×F1+10.29×F2+12.93×F3*	*R-sq*= 0.948 *R-sq(adj)*= 0.938

[a] F1: Sour cherry percent; F2: Pomegranate percent, F3: Grape percent.

function were used to predict responses to survey influence of each variable on the analyzed physicochemical properties.

Figure (3-a) shows a contour plot of juice acidity versus sour cherry juice, pomegranate juice, and grape juice percentages. Results show that all variables (sour cherry, pomegranate and grape juice percentage) have affected the juice acidity. There is a linear relation between acidity and variables, and some interactions between sour cherry, pomegranate, and red grape percentages that affect the juice acidity. The pure pomegranate and red grape have almost the same acidity, but the mixed juice acidity is decreased by increasing sour cherry percent. Therefore, acidity study in the mixed juice can help us to find pomegranate adulteration.

Figure (3-b) shows a contour plot of formalin index versus sour cherry juice, pomegranate juice, and grape juice percentages. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the formalin index, but there is no interaction between variables. The pure pomegranate juice has the lowest and the pure sour cherry juice has the highest formalin index. The formalin index of the mixed juice is increased by increasing of both sour cherry and grape percentages. It is clear that by studying the formalin index in the fruit juice, one can find purity or adulteration of different juices.

Figure (3-c) shows a contour plot of reducing sugar versus sour cherry juice, pomegranate juice and grape juice percentages. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the reducing sugar and there is no interaction between variables. The red grape has the highest reducing sugar and the pomegranate and sour cherry have the lowest formalin index. By studying the reducing sugar, it is impossible to determine sour cherry adulteration in the pomegranate, but it is easily possible to find grape adulteration in the pomegranate juice.

Figure (3-d) shows a contour plot of total sugar versus sour cherry juice, pomegranate juice and grape juice percentages. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the total sugar and there is no interaction between variables. The pure grape juice has the highest total sugar and the pure sour cherry juice has the lowest total sugar. Adulteration of sour cherry and grape in the pomegranate have different effects on the total sugar, the pomegranate juice total sugar is increased by increase in grape juice percentage, and decreases by increase in sour cherry juice percentage.

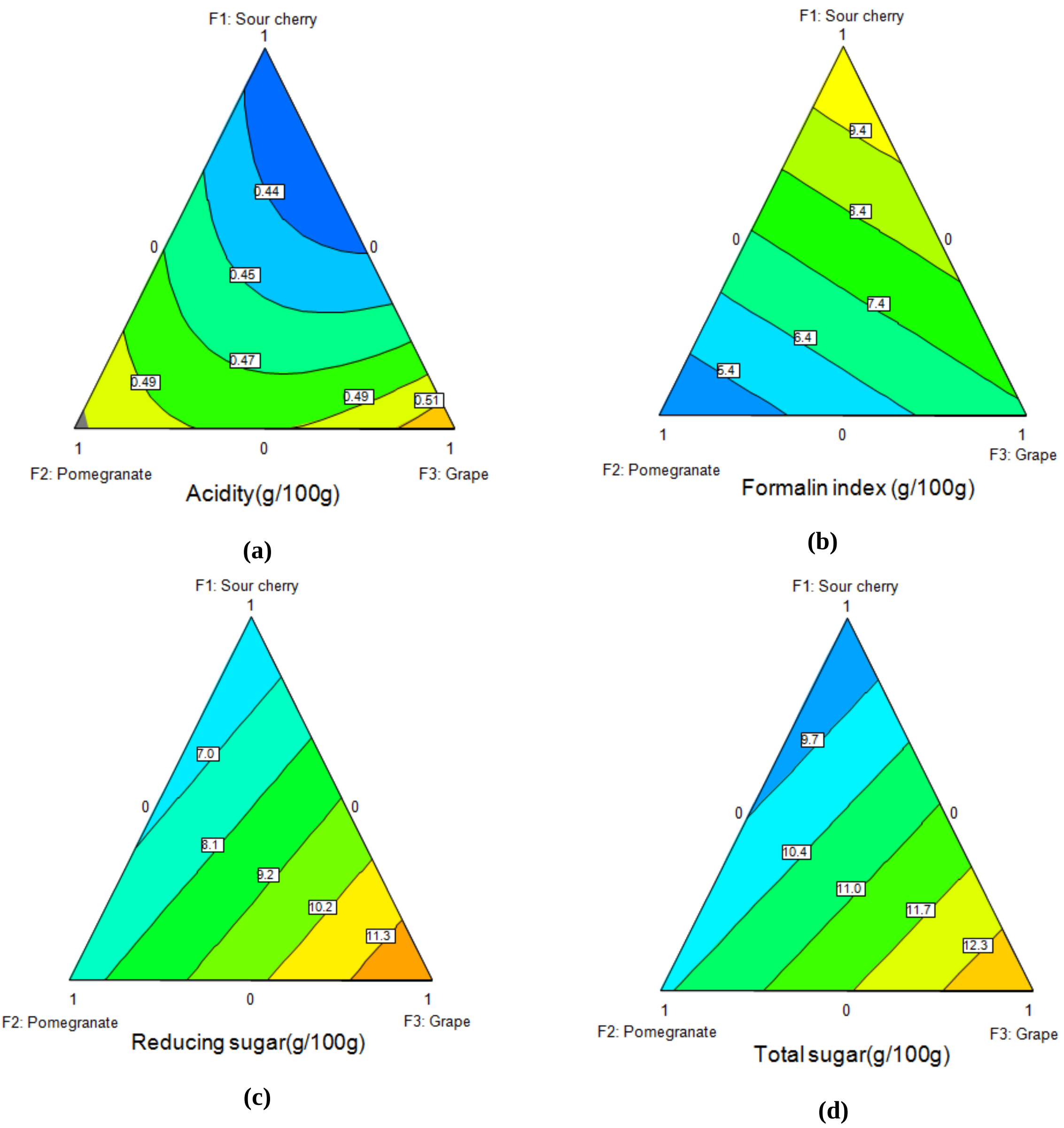

Figure 3. Contour plot of (a) juice acidity based on variables, (b) formalin index based on variables, (c) reducing sugar based on variables, and (d) total sugar based on variables.

Relation between Physicochemical Properties of Fruit Juice and Volatile Compounds Chromatogram

To evaluate the relation between physicochemical properties of fruit juices and volatile compounds GC-chromatograms (total peak area and total peak height), the acidity, formalin index, reducing sugar, and total sugar of juices were considered as variables and total peak area and total peak height were considered as resposes. Therefore, the full term second order polynomial response surface models were fitted to each of the response variables, according to Equation (1), where Y is the responses (total peak area and total peak height); factors (F) are acidity (F1), formalin index (g 100 g^{-1}) (F2), reducing sugar (g 100 g^{-1}) (F3), and total sugar (g 100 g^{-1}) (F4), and *b* values are the coefficient values obtained through multiple linear regressions. Table 3 also presents the evaluated factors (acidity, formalin index, reducing sugar and total sugar) and responses (total peak area and total peak height). The quadratic polynomial models for two response functions accompanied by *F* values and corresponding R^2 was used, and the estimated regression coefficients are summarized in Table 4. The surface plots of the total peak area and total peak height based on two variables (while the other variables were kept in the center levels) are shown in Figures 4 and 5, respectively. Results show that the total peak area and total peak height of juice volatile organic compounds are affected by the physicochemical properties of juices. For example, (1) In the interaction of formalin index and total sugar, the total peak area is increaded slightly by increse in formalin index (F2) to 8%, then it decreases to 12%, but increase in total sugar (F4) causes increase in total peak area; and 2) In the interaction of acidity and total sugar, the total peak area is decreased slightly by increase in acidity, while increase in total sugar (F4) cause increase in total peak area. Finally, there are good relations between physicochemical properties of juice samples and volatile compound peaks in GC, which can help in determination of physicochemical properties of juice samples

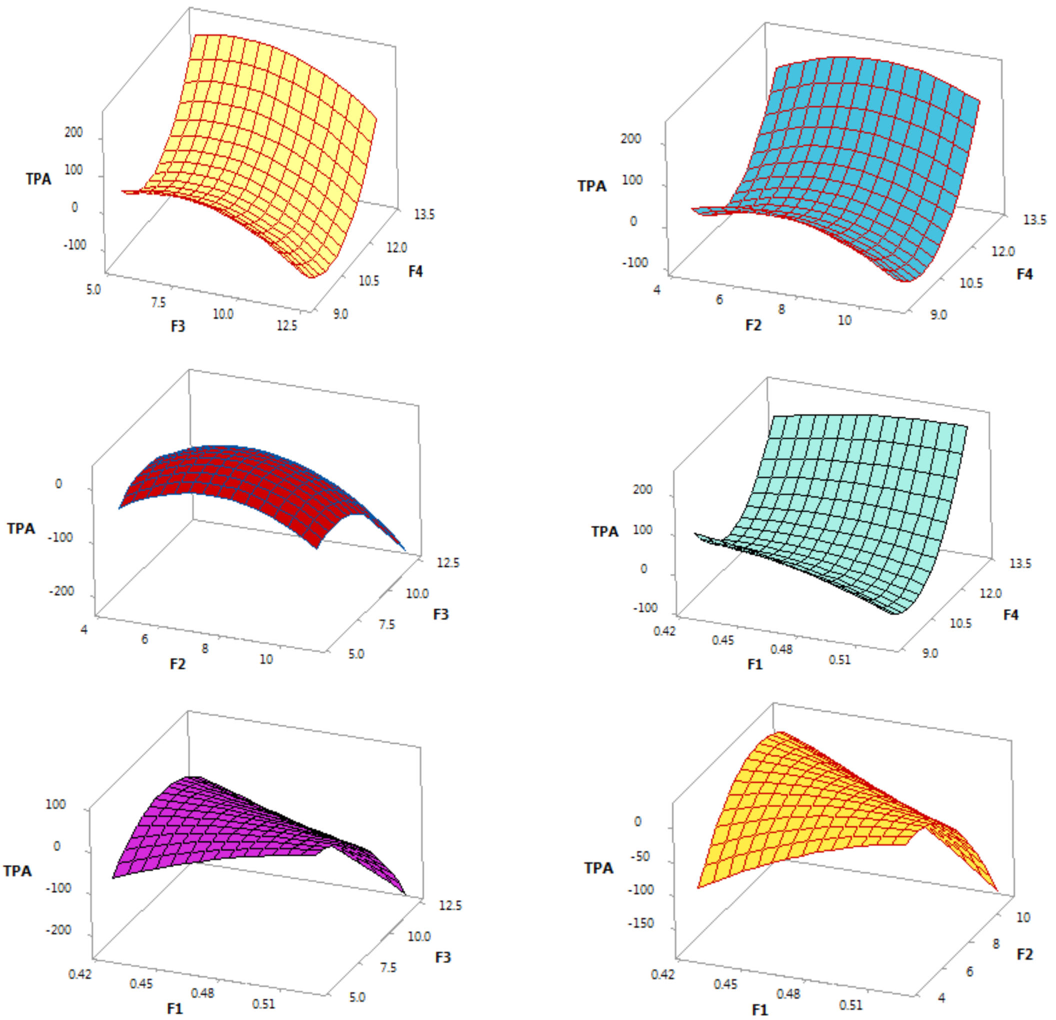

Figure 4. Surface plots of total peak area based on variables.

Table 3. List of experiments in the Box-Behnken Design (BBD) based on physicochemical properties and GC-profile character (total peak area and total peak height).

Run order	Factors (Physicochemical properties)				Responses (GC-profile character)	
	F1: Acidity (g 100 g^{-1})	F2: Formalin index (g 100 g^{-1})	F3: Reducing sugar (g 100 g^{-1})	F4: Total sugar (g 100 g^{-1})	Total peak area	Total peak height
1	0.448	6.5	11.077	12	20	10.9
2	0.464	7.5	6.73	9.89	19.5	14.2457
3	0.4832	6	10.2857	11.8	18.667	12.563
4	0.4864	6	8.276	11.077	22.5	15.7038
5	0.525	8	12.4138	13.1	21.5	15.48
6	0.52125	7	12.2	12.857	23	12.5
7	0.432	9	9	10.91	18.5	12.76
8	0.4864	5.5	8.78	11.25	16.5	12.956
9	0.512	4.5	8.276	9.73	20.667	11.337
10	0.4288	8	7.5	9.863	23	9.13
11	0.42545	10	5.39	9.114	16.5	11.74
12	0.5056	4.5	7.06	10.588	24	9.844
13	0.43215	11	6.154	9.1	20.833	13.184
14	0.4576	8	9.351	10.435	0	0

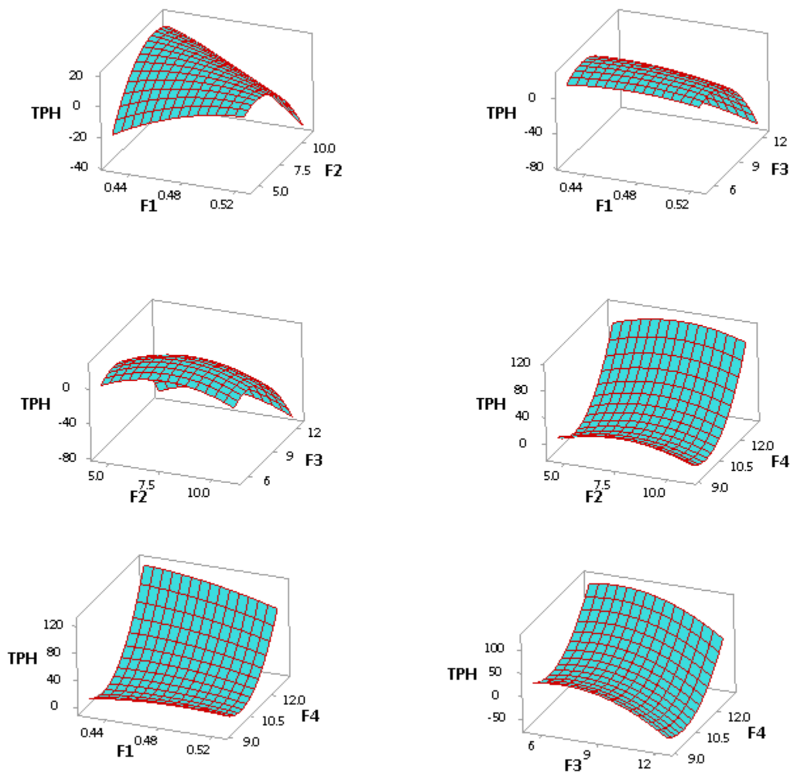

Figure 5. Surface plots of total peak height based on variables.

Table 4. Some characteristics of the constructed models for responses (total peak are and total peak height) versus some physicochemical properties.

Response type	Regression equation[a]	Model summary
Total peak area	*TPA= 592.2+8702 F1+274.3 F2+316.0 F3-931.3 F4-6521 F1×F1-5.690 F2×F2-5.456 F3×F3 +33.14 F4×F4-464.5 F1×F2-477.8 F1×F3 +448.7 F1×F4-1.372 F2×F3+3.558 F2×F4*	*R-sq*= 99%
Ttoal peak height	*TPH= -336.5+4257 F1+76.51 F2+51.46 F3-222.8 F4-2128 F1×F1-1.634 F2×F2- 2.599 F3×F3+13.19 F4×F4-117.5 F1×F2-31.38 F1×F3-105.5 F1×F4-0.08131 F2×F3 +0.4335 F2×F4*	*R-sq*= 98%

[a] F1: Acidity; F2: Formalin index; F3: Reducing sugar, F4: Total sugar.

and different adulteration used in the juice sample.

CONCLUSIONS

The adulterated pomegranate juice by sour cherry and red grape juice was evaluated by studying the physicochemical properties and VOCs gas chromatography profile (total peak area and total peak height). The followings were evaluated: (1) Relations between sour cherry, pomegranate, and red grape percentages (as variable factors) with juice physicochemical properties; and (2) Relations between acidity, formalin index, reducing sugar and total sugar (as variable factors) with GC-profile of juice VOCs (total peak area and total peak height). Results showed that GC-profiles of juice VOCs were changed by changing the juice compounds percentages and there were good relations between physicochemical properties of juice samples and VOCs GC-profile. Some other important results include:

1) The pure pomegranate and red grape have almost the same acidity but the mix juice acidity is decreased by increase in sour cherry percent,

2) The formalin index of mixed juice is increased by increase in both sour cherry and grape percentages,

3) By studying reducing sugar, it is impossible to determine sour cherry adulteration in the pomegranate, but it is easily possible to find grape adulteration in the pomegranate juice, and

4) Adulteration of sour cherry and grape in the pomegranate have different effects on the total sugar, i.e. the pomegranate juice total sugar is increased by increase in grape juice percentage, and decreases by increase in sour cherry juice percentage.

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ف. قاسمی، م. علیزاده، س. پیرسا و ف. محترمی

چکیده

در این مطالعه، آب انار تقلبی به صورت دستی با افزودن آبمیوه های آلبالو و انگور قرمز تهیه شد. خصوصیات فیزیکوشیمیایی (بریکس، اسیدیته، شاخص فرمالین، شکر احیا کننده و قند کل) و مشخصات کروماتوگرافی گازی آب انار خالص و آب انار تقلبی، به منظور مشخص کردن تقلبات استفاده شده بررسی شد. نانوفیبر PPy-Ag به عنوان یک فاز جامد ریز استخراج جفت شده با کروماتوگرافی گاز (HS-SPME-GC) برای استخراج و شناسایی پروفیل ترکیبات فرار (سطح زیر پیک کلی و ارتفاع پیک کلی) آب میوه های مختلف مورد استفاده قرار گرفت. برای بررسی تأثیر آب انار، آب آلبالو و آب انگور در خواص فیزیکوشیمیایی و ویژگی های کروماتوگرافی گازی آب میوه های مخلوط از طرح آماری باکس بنکن استفاده شد. نتایج نشان داد که درصد آب انار، آب آلبالو و آب انگور بر خصوصیات فیزیکوشیمیایی آب میو تاثیر می گذارد و بین خواص فیزیکیو شیمیایی آب میوه و پروفیل کروماتوگرافی گازی ترکیبات فرار آبمیوه های تقلبی (سطح زیر پیک کلی و ارتفاع پیک کلی) ارتباط معنی داری وجود دارد که میتواند در تشخیص تقلبات استفاده شده در آب انار به ما کمک کند. تحقیق حاضر، امکان شناسایی تقلبات استفاده از آبمیوه انگور و آبمیوه آلبالو و درصد آنها در آبمیوه انار با استفاده از بررسی خواص فیزیکو شیمیایی و نیز پروفیل کروماتوگرافی گازی را نشان می دهد.