Extraction and determination of volatile organic acid concentration in pomegranate, sour cherry and red grape juices by PPy-Ag nanocomposite fiber for authentication

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Abstract:

A solid phase microextraction fiber based on nano-sized polypyrrole-Ag (PPy-Ag) was fabricated. The size and morphology of PPy-Ag particles was characterized by scanning electron microscopy (SEM). A method based on PPy-Ag-SPME-gas chromatography (GC) was developed for determination of sour cherry and grape juice adulteration in pomegranate juice samples by determination of volatile organic acids (VOAs) concentration. The VOAs of pomegranate juice (Valeric, Isovaleric, Lactic and Acetic acids) were extracted by fabricated polypyrrole-Ag fiber and the concentration of VOAs was determined by GC analysis. The effects of pomegranate juice (PJ) percent, sour cherry juice (SCJ) percent and red grape juice (GJ) percent as three variable factors on the concentration of VOAs were studied. Box Behnken Design (BBD) was applied to design the experiments that study the factors effect on the VOAs concentration. The results showed that sour cherry and grape juice adulteration in pomegranate juice can affect the VOAs concentration and there is a good relation between pomegranate, sour cherry and grape juice percent and VOAs concentration. So the presented analytical method is a suitable and fast method for determination of pomegranate juice authentication.

Keywords: Pomegranate Juice, Adulteration, Polypyrrole-Ag, Nanocomposite, Volatile Organic Acid, Solid Phase Microextraction.

1. INTRODUCTION

The fruit juices have differential beneficial compounds like phenolic compounds, carotene, vitamin C and E. Some juices like raspberries, strawberries, cherries, purple grapes, pomegranates and blueberries are the major sources of anthocyanins [1]. The consumption of fruit juices can prevent certain diseases such as cardiovascular diseases, and cancer. The beverage industry recently is growing on the branch of fruit juices because of the fruit juice beneficial and the effects [2-4]. Some juices like pomegranate, orange, and various types of berries are produced and consumed in the largest volume worldwide because of positive health effects and high levels of antioxidants. The fraud and adulteration were done in the juices because of large-scale production and economic value. Some popular frauds and adulterations that are done in the juices including 1- addition, pulp or sugar, 2- uses cheaper alternatives in the main product, 3- dilution with water and so. The punicalagin is the most ellagitannins of pomegranate that has antioxidant activity and found almost exclusively in pomegranate juice. The ellagitannins can also be obtained from the peel, so the authenticity of PJ cannot be established by presence of punicalagin and other ellagitannins, so it is necessary to analysis other chemical characteristics of PJ to determine pomegranate juice authenticity [4-8].

The pomegranate juice depending on its ripeness has sour or sweet taste and is a tonic juice for throat and heart. Thus, pomegranate is considered a healthful counterbalance to a diet high in sweet-fatty components. Among the different fruit juices the PJ has several times more total polyphenol concentrations and antioxidant activity compared to grape, grapefruit, orange, and so. Recently, clinical studies on the pomegranate juice have been done. These researches showed that consumption of PJ has positive health benefits so consumer demand to the pomegranate juice has increased [9-12]. The beneficial effect of pomegranate juice and some other interesting features of pomegranate juice like a high price, short harvest season and high product demand, cause to increase adulteration in PJ.

Some pomegranate juice adulteration and fraud are: 1- Mixing with water and sugar, 2- the typical intense astringency of juice prepared with carpellar membranes or with extended maceration of the juice with the fruit rind or peel, and 3- Some other fruit juices like sour cherry and red grape juices that are used to adulterate pomegranate juice are cheaper than PJ [13-15]. These juices have chemical composition, color and volatile profile similar to pomegranate juice. There are some methods to identify adulteration in pomegranate juice. If the chemical composition of adulterated juice differs significantly from pure juice, it will be able to identify frauds. Substitution of PJ with cheaper ingredients like sour cherry and red grape juices is common practice and has a potential for financial gain. Some techniques like microbiological, physical and chemical methods can be used to test pomegranate juice adulteration [13]. The some fruit juices adulteration were detected by different analytical methods like near-infrared transflectance spectroscopy, liquid chromatographic pulsed amperometric detection and capillary-gas chromatographic [5, 10 and 12]. Conducting polymers such as polypyrrole (PPy) have been intensively studied because of their remarkable chemical, physical and mechanical properties, relatively high environmental stability and easily synthesized which are used in absorbents and filters. However, the PPy (prepared by chemical polymerization) is appropriate for use on a non-conducting substrate surface. The main advantage of chemical polymerizations is that it allows conducting polymer films to be easily designed and optimized by the incorporation of molecular species into the polymer structure on a non-conducting substrate surface [16-18].

A solid phase micro-extraction (SPME) is a solvent-free extraction technique ideally suited to VOAs extraction. SPME can be coupled to gas chromatography-Flame Ionization Detection (GC-FID) and high-performance liquid chromatography (HPLC), however, SPME-GC-FID is considered one of the most popular methods for the analysis of VOAs emitted from fruit juices. Some parameters that affect the SPME performance like fiber type, the extraction temperature, extraction time, and the sample volume are optimize in the SPME methods [19 and 20].

In this work, nano-sized PPy-Ag fiber was used as SPME fiber to extract VOAs from pomegranate juice and analysis by GC-FID. The pure PJ was adulterated with sour cherry and red grape juices manually and the effects of these juices on the VOAs (Valeric, Isovaleric, Lactic and acetic acids) concentration and pH of PJ were studied. Results showed that VOAs concentration and pH of PJ are changed by mixing of sour cherry and red grape juices to the pure pomegranate juice, so the presented method is a good technique to determine pomegranate juice authentication

2. EXPERIMENTAL

2.1. Reagents and Chemicals

Pyrrole (provided from Fluka, Switzerland) was distilled in vacuum, before use. It was stored in deep-freezer in sealed condition for avoiding photo-polymerization. Ferric chloride ($FeCl_3$) as oxidant and silver nitrate were purchased from Aldrich. The stock solution of analytes (Valeric, Isovaleric, Lactic and acetic acids) was prepared at a concentration of 5000 $\mu g \cdot ml^{-1}$ in deionized water. To provide a model solution of analytes, the standard solution was diluted with double distilled water. The model solutions were used at different concentration to study the calibration curve of all analytes and calculation analyte concentration in pomegranate juice samples. All organic compounds were purchased from Merck. The pomegranate, sour cherry and grape concentrates were provided from orum-narin company, Urmia, Iran. The pomegranate, sour cherry and grape juice were provided from their concentrate.

2.2. 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) [21]. The GC apparatus used in this study was from Agilent 7890 A, Wilmington, DE, USA. The composition and morphology of PPy-Ag fiber were evaluated by scanning electron micrographs (SEM) using an SEM instrument (Philips XL30, the Netherlands). The pH values were determined using a digital pH meter (pH 114, Snail Instruments),

2.3. Fruit juice preparation

To provide 1300 ml pomegranate juice, 137.93 g pomegranate concentrate and 65 g sugar were mixed then the need water was added to the mix, to provide 1300 ml sour cherry juice, 131.885 g sour cherry concentrate and 65 g sugar were mixed. Then the need water was added to the mix. To provide 1300 ml sour red grape juice, 236.3634 g sour cherry concentrate and 26 g sugar were mixed then the need 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).

2.4. Preparation of PPy-Ag fiber

PPy-Ag nanocomposites were polymerized on the surface of polyester fibers by the chemical polymerization method under atmospheric condition as is described following: 20 ml double distilled water containing 5 g of $AgNO_3$ prepared and for 1 h solution was stirred in a magnetic stirrer at room temperature, 130 μL of pyrrole was added to the aqueous solution gradually and stirred at room temperature for 30 min. 50 cm of polyester fiber was submerged to the above solution. 20 ml double distilled water containing $FeCl_3$ (0.2 M) was dissolved in the above solution. After 1 h, PPy-Ag coated polyester fiber was taken out from the bath and rinsed in double distilled water for about 5 min in an ultrasonic bath.

2.5. Chromatographic Conditions

A gas chromatography instrument (Agilent7890 A, Wilmington, DE, USA) with flame ionization detector (GC-FID) at the following condition was used for separation, detection, and analysis of analytes: capillary column, silica, 30 m length, 0.25 µm phase thickness and 320 µm i.d; N_2 used as carrier gas with a flow rate of 2 ml/min; the column pressure was set at 8.8913 psi. Splitless mode injection was 50 ml/min splitting ratio in 0.75 min. The initial column temperature was 50ºC and then the temperature was increased to 80ºC at the 2ºC/min and kept in 80 ºC for 5 min. The detector temperature was 250 ºC. Heater temperature 200 ºC, H_2 flow 27 ml/min and air flow 20 ml/min.

2.6. Headspace Extraction Procedure

The fruit juice sample (2 ml) was extracted with PPy-Ag fiber using headspace solid phase microextraction (HS-SPME). PPy-Ag fiber connected to the needle of designed syringe was used. To the condition of provided PPy-Ag fiber, it was injected into GC injection port for 1 h at 100 °C prior to use. A glass (10 ml) with a polytetrafluoroethylene silicon septum containing a magnetic stir bar and 2 ml of juice sample was provided. An aluminium cap was used to seal the vial to prevent sample loss due to evaporation. A hot plate was used to heat vials contained some fruit juice samples during the extraction process. When the stirring liquid sample in the sealed vial is heated on the hotplate, the PPy-Ag fiber by designing syringe was exposed to the headspace of it. After completing of extraction of the analyte to PPy-Ag fiber, the fiber was withdrawn into the designed syringe needle and removed from the vial, then immediately inserted into the injection port of the GC. Some parameters that affect extraction efficiency like, extraction time (10 min) and temperature (80 °C) were optimized experimentally. The chromatographic separation is shown in Fig. 1.

3. RESULTS AND DISCUSSION

3.1. PPy-Ag film Morphology

Scanning electron microscopy shows morphology, size and porosity of synthesized PPy-Ag film. Fig. 2 shows the SEM micrographs of the PPy-Ag particles. Results show that the structure of the PPy–Ag nanocomposites on the fiber surface are slightly agglomerated in the range of 30-120 nm and are mostly in seed-like form. It seems that Ag particles are disturbed between polypyrrole particles.

3.2. Experimental design

The GC chromatogram of pomegranate juice VOAs was affected by three variables, including pomegranate juice (PJ) percent (F1) (in five levels), sour cherry juice (SCJ) percent (F2) (in five levels) and grape juice (GJ) percent (F3) (in five levels). The effects of these three variables on the VOAs concentration (Valeric, Isovaleric, Lactic and acetic acids) were investigated. To study the effect of these factors on the responses an expermental design based on Box Bhneken design (BBD) was used. A 5-level-3-factor experimental design was used for study, pomegranate, sour cherry and grape juice percents on the VOAs concentration. Table 1 shows the 3 processing variables as factors, levels and experimental design are given in terms of coded and uncoded. For each of the three studied variables, high (coded value: +2) and low (coded: -2) set points were chosen to construct an orthogonal design as tabulated. It helps for the estimation of the significant factors affecting VOAs concentration. Table 2 shows the list of experiments in the BBD (Coded Values) and the responses (Valeric, Isovaleric, Lactic and acetic acid concentration). Polynomial equations and contour plots for a particular response were created using Design-Expert software version 7. The model response was defined by the following Eq. (1):

$$Y = \sum_{i=1}^{3} \beta_i F_i + \sum_{i=1}^{3} \sum_{j=i+1}^{3} \beta_{ij} F_i F_j + \sum_{i=1}^{3} \beta_{ii} F_{ii} \quad (1)$$

Where Y is the response (Valeric, Isovaleric, Lactic and acetic acid concentration); F symbols are the variable parameters, and β values are the coefficient values obtained through least square method. The Design-Expert software (version 7) was used to perform statistical analysis. Initially, the full term quadratic*quadratic response surface models were fitted to each of the response variables, according to the equation (1). Where, possible stepwise deletion of terms was applied to remove the statistically non-significant terms, so simplifying the model. However, when the exclusion of such terms from the model decreases R^2 (adjusted) and increases 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 summarized in Table 3.

3. 3. Study the variables effect on the VOAs concentration by contour plot

The contour plots based on the model function were used to predict responses to survey influence of each variable (sour cherry, pomegranate and grape juice percent) on the volatile organic acid concentration.

3.3.1. Contour plot of valeric acid concentration based on variables

Fig. 3 shows a contour plot of valeric acid concentration versus sour cherry juice, pomegranate juice and grape juice percent. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the valeric acid concentration. There is a linear relation between valeric acid concentration and variables. The pure pomegranate juice has the lowest valeric acid concentration and sour cherry has the highest valeric acid concentration. Valeric acid concentration is increased by increasing of both sour cherry and grape juice percent in the mix juice, so it is possible to determined sour cherry and grape juice adulteration in the pomegranate juice by valeric acid concentration analysis.

In the similar research Yang & Chong reported that the concentration of valeric acid in the grape juice is very low and is not detectable [22]. In the results of our study (this work) the valeric acid concentration in grape juice is detectable by SPME-GC-FID method and higher than the reported works, the difference of valeric acid concentration obtained in this work and the similar works may be in the result of difference in the grape variety, environmental condition, microextraction method and chromatographic methods.

3.3.2. Contour plot of isovaleric acid concentration based on variables

Fig. 4 shows a contour plot of isovaleric acid concentration versus sour cherry juice, pomegranate juice and grape juice percent. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the isovaleric acid concentration. Isovaleric acid concentration is increased by increasing of sour cherry percent in the mix. The pure grape and pomegranate have the lowest isovaleric acid concentration and pure sour cherry juice have the highest concentration of isovaleric acid. It is impossible to determine grape adulteration in the pomegranate juice by studying of isovaleric acid concentration, but sour cherry adulteration in pomegranate juice can be detected by studying of isovaleric acid concentration.

In the similar research Yang & Chong reported that the concentration of isovaleric acid in the grape juice is 0.48 mg/ml of red grape juice [22]. In this work the isovaleric acid concentration in grape juice is higher than the reported work. This different in the isovalric acid concentration may be in the result of difference in the grape variety, environmental condition, microextraction method and chromatographic methods.

3.3.3. Contour plot of lactic acid concentration based on variables

Fig. 5 shows a contour plot of lactic acid concentration versus sour cherry juice, pomegranate juice and grape juice percent. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the lactic acid concentration and there are some interactions between variables. Different percents of pomegranate, grape and sour cherry juice show the different lactic acid concentration, so it is possible to determine sour cherry and grape adulteration in the pomegranate juice (in the different mixes) by studying of lactic acid concentration.

In the similar research Gundoglu & Yilmaz reported that the concentration of lactic acid in the 11 varieties of pomegranate juice were between 4.516-33.115 mg/l of juice [23]. The lactic acid concentration in pomegranate juice is higher than the reported work. This different in the lactic acid concentration may be in the result of difference in the analytical method and variety of pomegranates.

3.3.4. Contour plot of acetic acid concentration based on variables

Fig. 6 shows a contour plot of acetic acid concentration versus sour cherry juice, pomegranate juice and grape juice percent. Results show that all variables (sour cherry, pomegranate and grape juice percent) have affected the lactic acid concentration and there are some interactions between variables. The pure pomegranate, pure grape and pure sour cherry juice have the lowest acetic acid concentration that mixing of three fruit juices cause to increase acetic acid concentration. It is possible to detect sour cherry and grape juice adulteration in the pomegranate juice by studying acetic acid concentration.

In the similar work Melgarejo and coworkers in 2000 reported that the concentration of acetic acid in the two variety of ….pomegranate was 0.2 g/100g and in the one another variety of …. Pomegranate was 0.13g/100g [24] and the concentration of acetic acid in the other varieties of pomegranate juice was not detectable. The obtained concentrations for acetic acid in this work were according to the reported work [24].

3.4. Study the effects of VOAs concentration on the pH of juices

For studying of VOAs concentration on the juice pH, the concentration of valeric acid, isovaleric acid, lactic acid and acetic acid were considered as variables and juice pH was considered as respose. So the full term second order polynomial response surface models were fitted to each of the response variables, according to equation (1) by Minitab version 17 soft ware.Where Y is the response (pH); factors (F) are valeric acid (F1) , isovaleric acid (F2), lactic acid (F3), and acetic acid (F4) cocentration, and b values are the coefficient values obtained through multiple linear regressions. The model response was observed by the following Eq. (2):

$$pH = -2.226 - 0.001316\ F1 + 0.009155\ F2 - 0.000054\ F3 + 0.000006\ F4 \qquad (2)$$

The equation 2 shows the effects of different VOAs on the pH of juice, according to this equation all acid concentration has the significant effect on the juice pH. The surface plots of pH based on two variables (while the other variables were kept in the center levels) are shown in the fig. 7. Results show that juice pH is affected by VOAs concntration. In the mix juice that VOAs concentration is affected by pomegranate, red grape and sour cherry percent, the mix juice pH strongly depends on the VOAs concentration. Generally, according to the equation 2 and interactions between different acids (fig.7), the juice pH is increased by increasing of isovaleric acid and acetic acid concentration and is decreased by increasing of valeric acid and lactic acid concentration. Different organic acids have different acidity constant (K_a) so according to the K_a in the fifferent conditions can increase or decrease the pH.

4. CONCLUSIONS

The VOAs of pomegranate Juice (Valeric, Isovaleric, Lactic and acetic acids) were extracted by fabricated polypyrrole-Ag fiber (as extraction agent) and the concentration of VOAs was determined by HS-SPME-GC-FID method. Box Behnken Design (BBD) was applied to design the experiments that study the effect of pomegranate, sour cherry and grape juice percent on the VOAs concentration. Results showed that 1- Valeric acid concentration increased by increasing of both sour cherry and grape juices percent in the mix juices, so it is possible to determined sour cherry and grape juices adulteration in the pomegranate juice, 2- The pure grape and pomegranate have the lowest isovaleric acid concentration and pure sour cherry juice has the highest concentration of isovaleric acid, 3- It is possible to determined sour cherry and grape adulteration in the pomegranate juice (in the different mixes) by studying of lactic acid concentration, and 4- The pure pomegranate, pure grape and pure sour cherry juices have the lowest acetic acid concentration that mixing of three fruit juices causes to increase acetic acid concentration. Finally the presented analysis method as a fast and suitable method could be easily used for determination of pomegranate authentication.

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Fig.1. GC-FID chromatogram of pomegranate juice VOAs extracted by PPy-Ag fiber

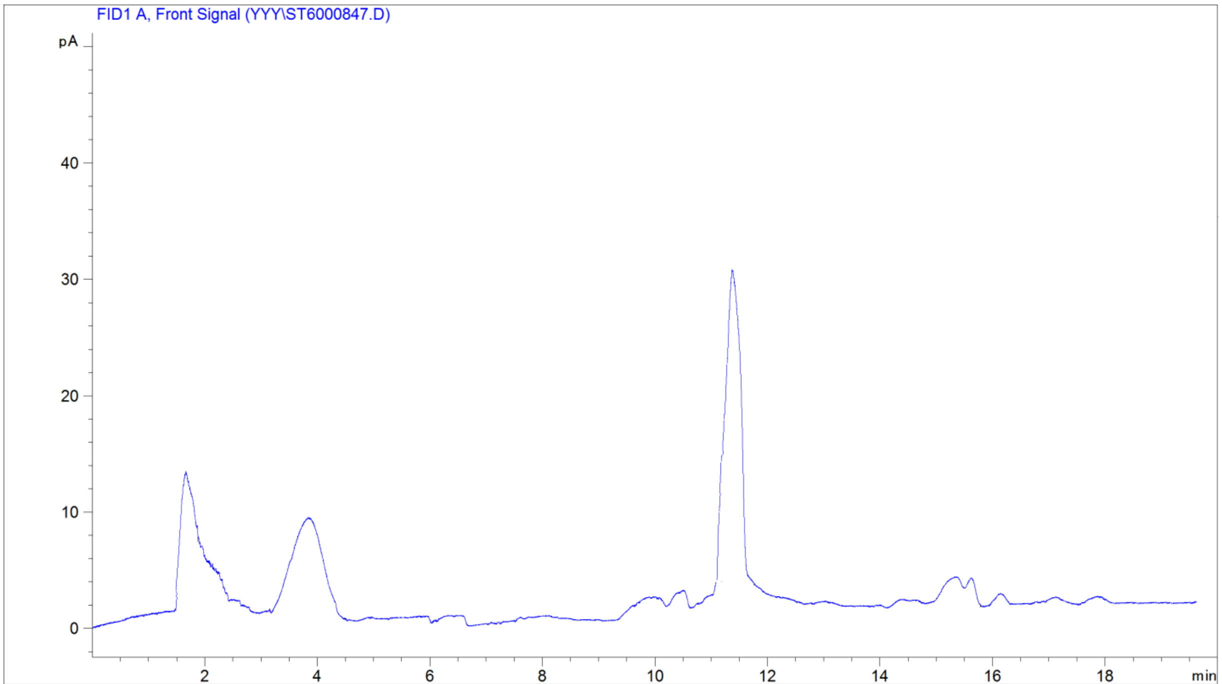

Fig.2. SEM image of PPy-Ag fiber

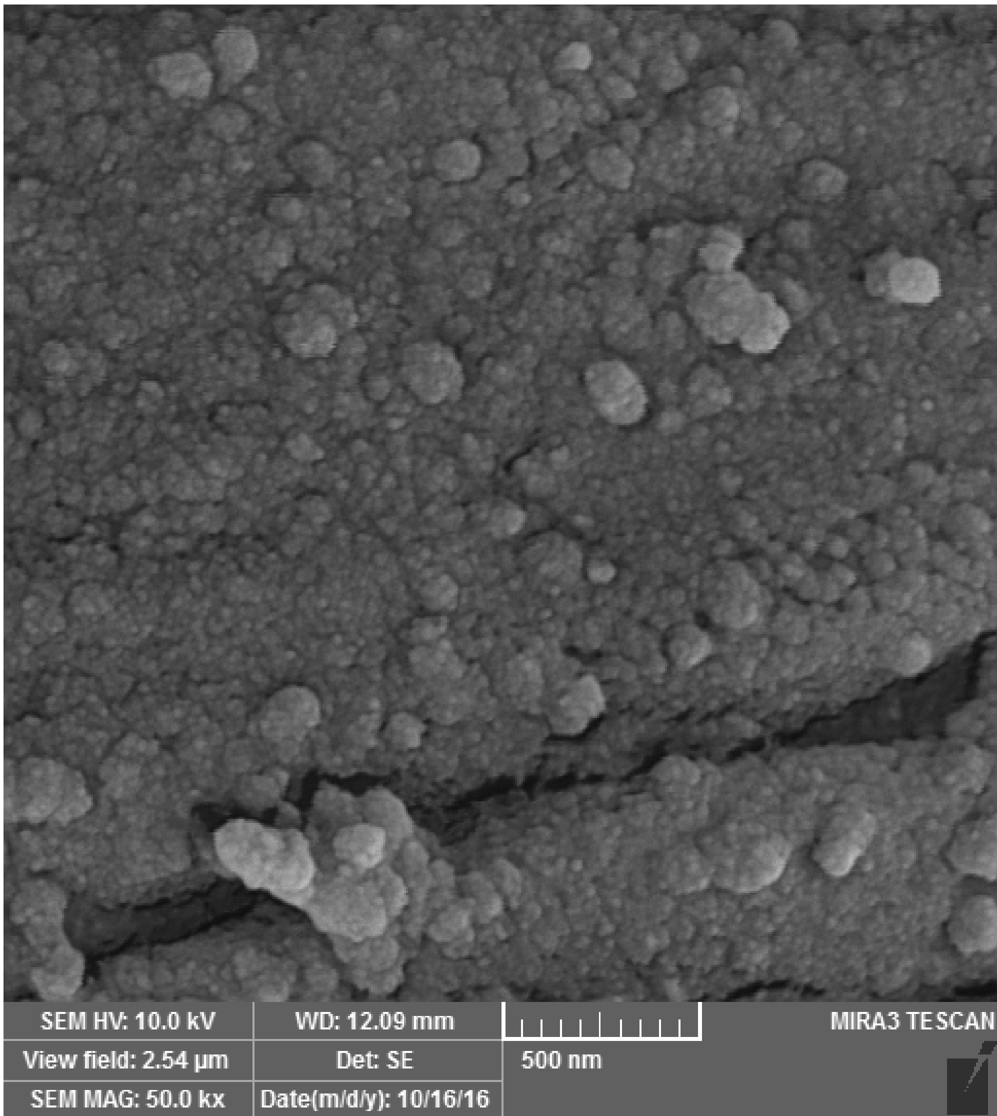

Fig.3. Contour plot of valeric acid concentration based on pomegranate, sour cherry and grape juice percent.

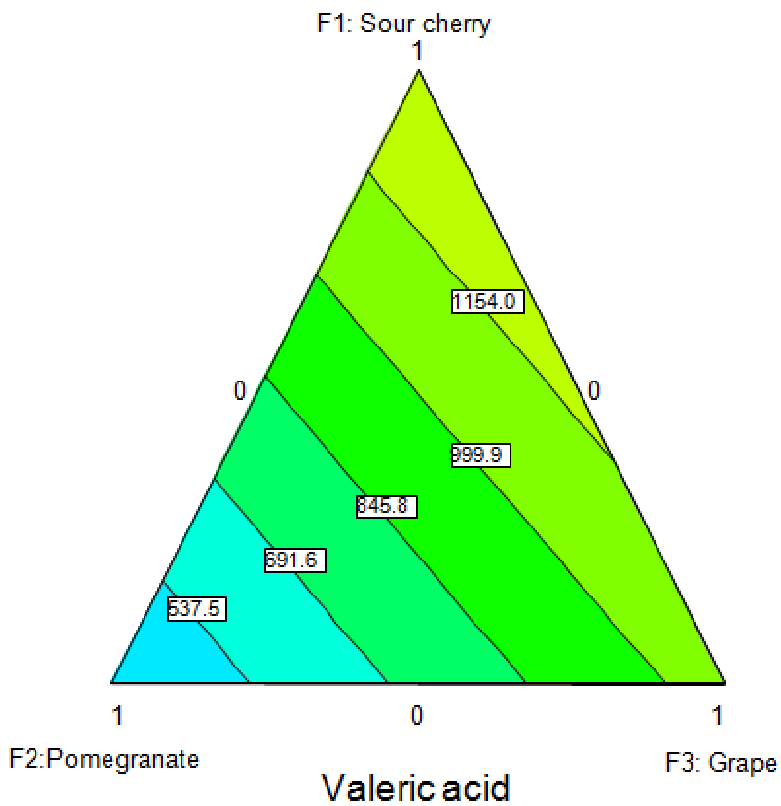

Fig.4. Contour plot of iso-valeric acid concentration based on pomegranate, sour cherry and grape juice percent.

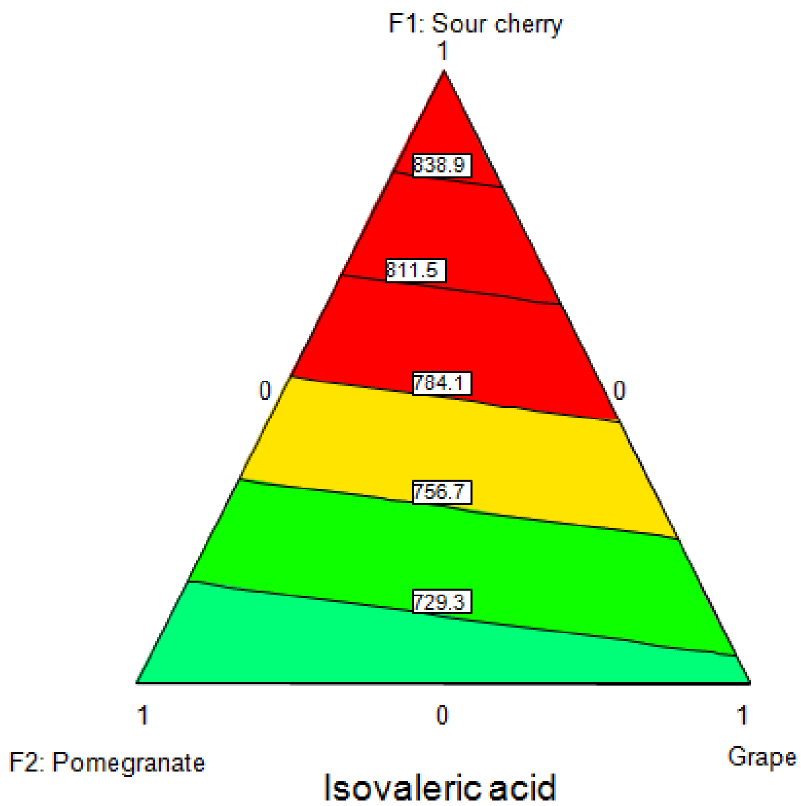

Fig.5. Contour plot of lactic acid concentration based on pomegranate, sour cherry and grape juice percent.

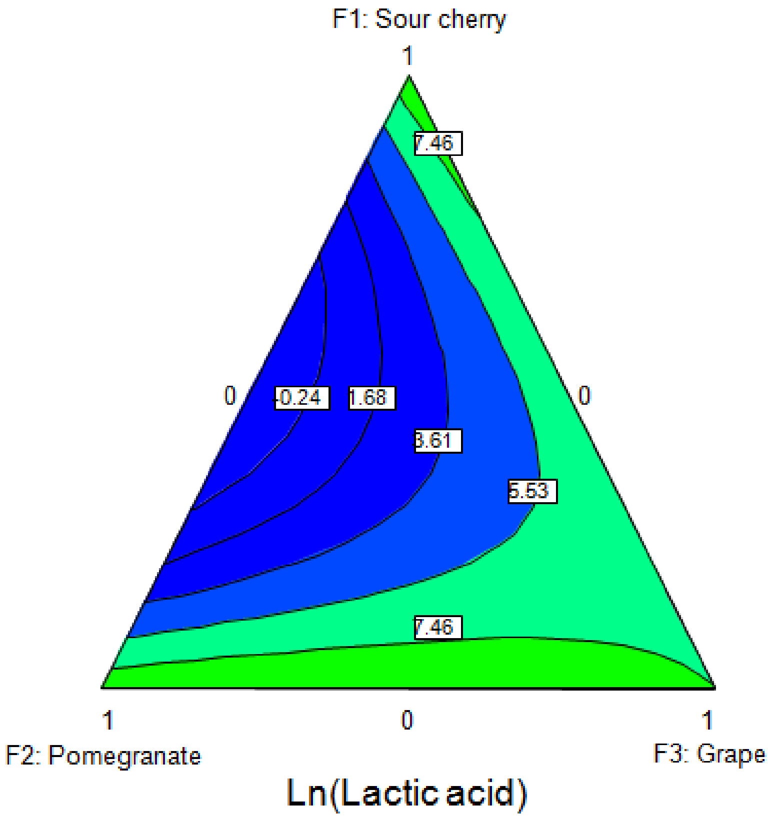

Fig.6. Contour plot of acetic acid concentration based on pomegranate, sour cherry and grape juice percent.

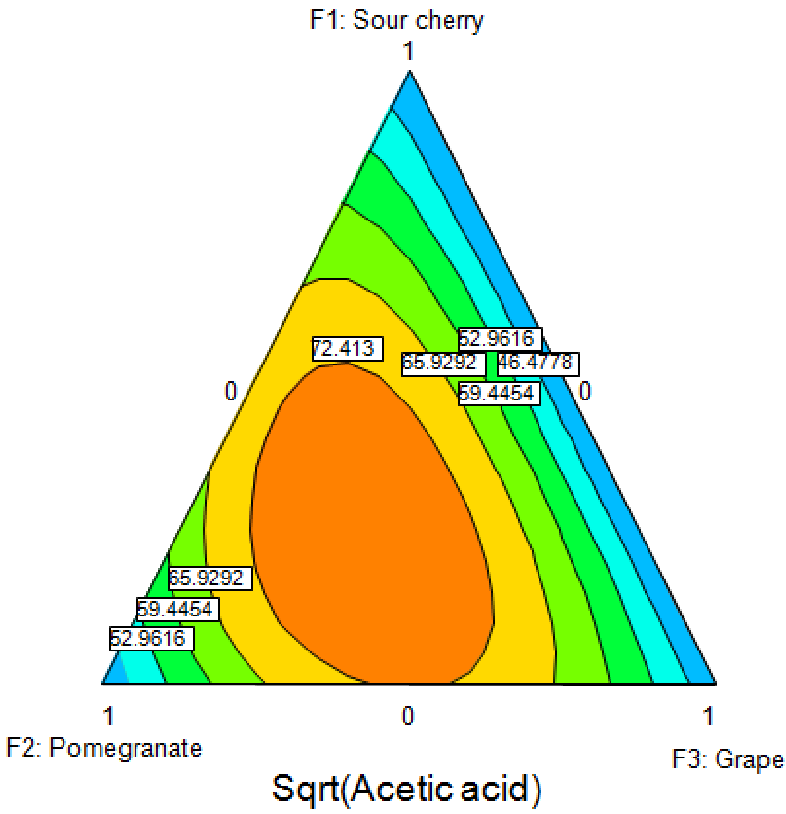

Fig.7. Surface plots of juice pH based on valeric acid, iso-valeric acid, lactic acid and acetic acid concentration.

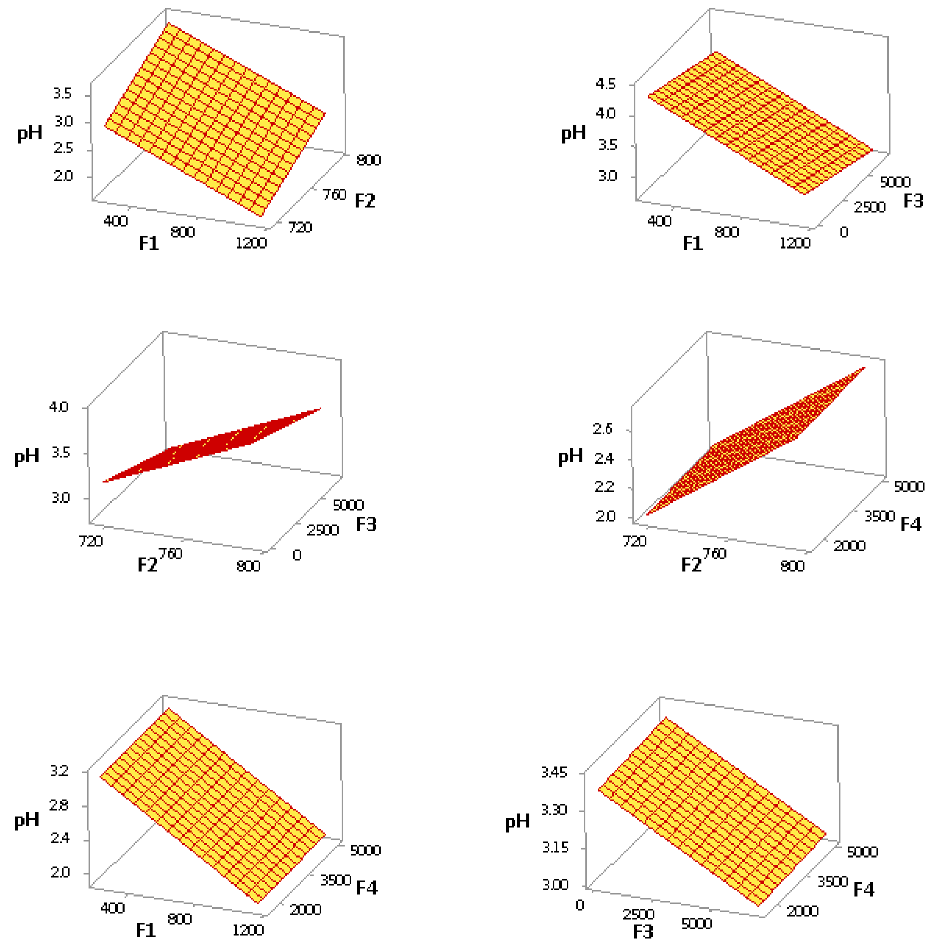

Table 1. Box behnken design used to evaluate the effects of three factors.

Run	Factors					
	F1: Sur cherry (% W/W)		F2: Pomegranate (%W/W)		F3: Grape (%W/W)	
	coded	uncoded	coded	uncoded	coded	uncoded
1	-1	16.66	-1	16.66	1	67.7
2	0	50	0	50	-2	0
3	-2	0	0	50	0	50
4	-1	16.66	1	66.7	-1	16.66
5	-2	0	-2	0	2	100
6	-2	0	-2	0	2	100
7	0	50	-2	0	0	50
8	-2	0	0	50	0	50
9	-2	0	2	100	-2	0
10	1	66.7	-1	16.66	-1	16.66
11	2	100	-2	0	-2	0
12	-2	0	2	100	-2	0
13	2	100	-2	0	-2	0

Table 2. List of experiments in the BBD and the responses of each run

Run order	Factors			Responses (concentration)				pH
	Sur cherry (% W/W)	Pomegranate (%W/W)	Grape (%W/W)	Valeric acid	Iso Valeric acid	Lactic acid	Acetic acid	
1	-1	-1	1	976.8	754.7	564.0	3827	3.39
2	0	0	-2	ND*	ND	ND	ND	3.67
3	-2	0	0	694.2	689.6	ND	5382	3.46
4	-1	1	-1	617.3	724.4	60.3	5008	3.62
5	-2	-2	2	230.4	707.8	NR	1680	3.29
6	-2	-2	2	918.8	743.4	1867	1760	3.28
7	0	-2	0	1140.5	793.5	865.9	1590	3.5
8	-2	0	0	1086.9	ND	11412	5362	3.48
9	-2	2	-2	227.5	716.8	6780	1657	3.68
10	1	-1	-1	124	ND	425	382	3.

				9.2		74	7	67
11				158		821	169	3.
	2	-2	-2	2.6	ND	9	1	74
12				376.		715	174	3.
	-2	2	-2	8	ND	3	5	68
13				975.		581	156	3.
	2	-2	-2	3	ND	5	1	69

*ND: Not Detected

Table 3. Some characteristics of the constructed models for responses

Response type	Regression equation	Model Summary
Valeric acid	Valeric acid (ppm)= 1308.145× F1+383.36× F2+1066.81× F3	R-Squared=0.749 Adj R-Squared=0.686
Iso-Valeric acid	Iso Valeric acid (ppm)= 866.33× F1+701.85× F2+722.81× F3	R-Squared=0.775 Adj R-Squared=0.663
Lactic acid	Ln(Lactic acid (ppm))= 8.84× F1+8.86×F2 +7.48 F3-44.09F1×F2-5.94F1×F3+4.40F2×F3	R-Squared=0.994 Adj R-Squared=0.986
Acetic acid	Sqrt(Acetic acid (ppm))= 40.5× F1+41.04×F2 +41.36 F3+111.1F1×F2-3.56F1×F3+127.1F2×F3+254.74 F1×F2×F3	R-Squared=0.997 Adj R-Squared=0.995

F1: Sour cherry juice, F2: Pomegranate juice, F3: Grape juice