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DETERMINATION AND COMPARISON OF MAJOR POLYPHENOL OF FOUR RED FRUITS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) WITH DIODE-ARRAY DETECTION

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ABSTRACT

Polyphenols are ubiquitous compounds in plants which are abundant micronutrients in our diet. They got more attention in recent years due to their bioactive functions and health effects on many diseases such as cancer. These components are secondary plant metabolites that function as antimicrobial, antiviral and anti-inflammatory compounds. Extraction of these compounds from plants and fruits and in vitro and in vivo study of their various health effects has been subject of many researches. The objective of this study was to investigate the profiles of polyphenolic compounds in apple, red grape, sour cherry and pomegranate fruit juices and comparison of the phenolic contents of various juices. Major polyphenolic compounds of four different concentrated fruit juices from various industries were analyzed and characterized by liquid chromatography. RP-HPLC-DAD was used in our study as powerful and accurate method. The total and individual polyphenolic compounds differed significantly among the four selected red fruit juices. Among the tested juices, sour cherry and apple juices had the highest and the lowest contents of phenolic compounds, respectively.

Keywords: HPLC, polyphenolic, flavonoids, anthocyanin, fruit juice

INTRODUCTION

Phenolic compounds represent a large group of molecules with a variety of functions in plant growth, development, and defense. Polyphenolics include signaling molecules, pigments and flavors that can attract or repel, as well as compounds that can protect the plant against insects, fungi, bacteria, and viruses. Most phenolic compounds are present as esters or glycosides rather than as free compounds. Tannins and lignin are phenolic polymers **(Vermerris *et al*., 2006; Nakatani, 2000)**.
Tannins are used commercially as dyes and astringents, and lignin accounts for structural rigidity of cells and tissues and is essential to vascular development. From this brief overview it is apparent that phenolic compounds make up a large and fascinating family **(Khanbabaee *et al*., 2001; Lisperguer *et al*., 2009)**.
The term of phenolic cover a very large and diverse group of chemical compounds. These compounds can be classified in a number of ways. They can be in the one of these classes; simple phenolic, phenolic acids and aldehydes, acetophenones and phenyl acetic acids, cinnamic acids, coumarins, flavonoids, biflavonyls, benzophenones, xanthones and stilbenes, benzoquinones, anthraquinones and naphthaquinones, betacyanins, lignans, lignin, tannins and phlobaphenes (**Huang *et al*., 2010; Campo Dall-Orto *et al*., 2005)**.
There is a huge body of evidence that phenolic compounds have effects on human health. Perhaps the oldest medical application of phenolic compounds is the use of phenol as an antiseptic. Because of its negative side effects on living tissues, including blister formation, especially at higher concentrations, it is no longer used in this capacity. Another very common use of phenolic compounds is in sunscreens **(Boudet, 2007; Rauha *et al*. 2000)**. The presence of the aromatic ring results in the effective absorbance of the ultraviolet -B radiation (between 280 and 315 nm) mid-range ultraviolet from the sun and that is blocked by the ozone layer thus prevents sunburns **(Vioux-Chagnoleau *et al*., 2006)**.
A concern of the widespread use of phenolic compounds is the estrogenic activity these compounds may display, which impacts the hormone balance and may result in breast cancer in women **(Wang, 2012)**. Aside from medical applications, polyphenols, including the flavonoids and tannins, are an integral part of human and animal diets, because they represent one of the most numerous and ubiquitous groups of plant metabolites **(Scalbert *et al*., 2005; Habauzit *et al*., 2012)**.
Although traditionally regarded as anti-nutrients, because of their bad taste, unappealing color, or cause of browning of tissues, polyphenols and other food phenolics is the subject of increasing interest because of their possible beneficial effects on health **(Kondratyuk *et al*., 2004; Vauzour *et al*., 2010)**.
The major effects of polyphenoles on human health can be arised from these properties; antioxidant properties, disease prevention effect, activity against toxins **(Visioli *et al*., 2011; Habauzit *et al*., 2012)**. According to this fact that an industrial process decrease polyphenolic content of products, quality control is one of major and important step. By considering these negative effects we can suggest suitable preventive additives. For this purpose, simple, reliable, inexpensive and fast methods are critical for industrial quality control process. In this study, we compared the polyphenolic compounds profile of four different fruit concentrated juices ,using an HPLC method with a diode array detector as useful procedure for quality control of products.

MATERIAL AND METHODS

Materials

Chlorogenic acid, p-coumaric acid, catechin, epicatechin, quercetin, and sodium carbonate (Na_2CO_3) were purchased from Sigma Chemical Co. quercetin 3-galactoside, quercetin 3-glucoside, quercetin 3-rhamnoside, procyanidins B_1 and B_2, phloridzin, and cyanidin 3-galactoside were from Fluka. All of the solvents were of HPLC grade and were purchased from Caledon Ltd. The selected four red fruits under investigation were apple, red grape, sour cherry and pomegranate.

Sample Preparation

The concentrated fruit juices were collected from the local industries in the West Azerbaijan, Iran. All concentrated fruit juices were diluted to the concentrations that the Brix (define as the sugar content of an aqueous solution. One degree Brix is 1 gram of sucrose in 100 grams of solution and represents the strength of the solution as percentage by weight (% w/w)) of the diluted sample reach to the 11-13 ranges. 5 ml of the diluted sample was then transferred to a glass tube and 5 ml of the 70% aqueous methanol solution was added to the tube (at a 1:1 (w/v) ratio). The mixture was well mixed, homogenized and centrifuged at the 4500

rpm for 10 min at the 5˚C. The final solution was stored at -10 °C before being analyzed.

HPLC Conditions

The optimized extraction methods and HPLC procedure for study of phenolic contents of fruits were discussed completely in our previous study **(Hosseinzadeh *et al*., 2013)**. Briefly, HPLC system (Agilent Technology 1100 series, Palo Alto, CA) equipped with a quaternary pump, an inline degasser, a column oven, and a diode array detector (DAD) was used for the identification and quantification of various phenolic compounds in the samples. A Zorbox C18 analytical column (250 × 4.6 mm i.d.; particle size, 5 µm) was used for the separation.

The binary mobile phase consisted of a 6% acetic acid in 2 mM sodium acetate buffer (solvent A, pH 2.55, v/v) and acetonitrile (solvent B), and the gradient program was as follows: 0% B to 15% B in 45 min, 15% B to 30% B in 15 min, 30% B to 50% B in 5 min, and 50% B to 100% B in 5 min. There was a 10-min post-run going back to the starting conditions for reconditioning. The flow rate was 1.0 mL/min for a total run time of 70 min. The detector was set at 280, 320, 360, and 520 nm for simultaneous monitoring of the different groups of phenolic compounds. The amounts of total phenolics in the concentrated juices were calculated as the addition of all the different phenolic compounds quantified by means of the HPLC analysis.

RESULTS AND DISCUSSION

The phenolic compounds of the selected juice concentrates was evaluated by high performance liquid chromatography equipped with diode array detector (HPLC-DAD). The HPLC analyses allowed the quantification of different phenolic and polyphenolic compounds due to this fact that phenolic compounds have characteristic UV spectra and we can quantify them as different phenolic groups (Figures 1 and 2).

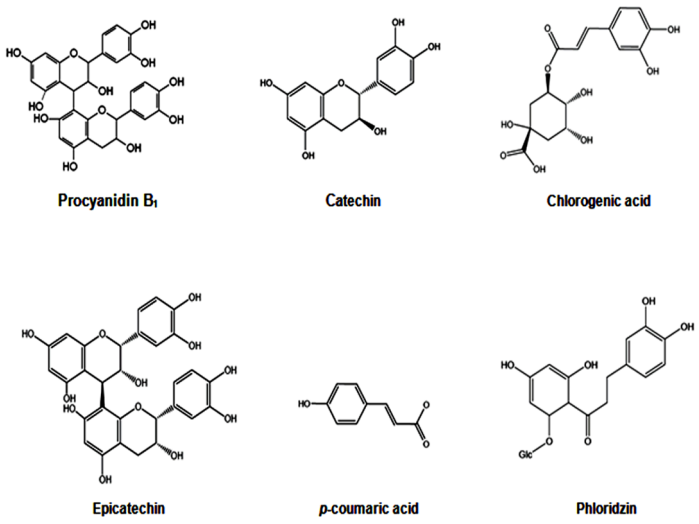

Figure 1 Chemical structures of some detected phenolic compounds

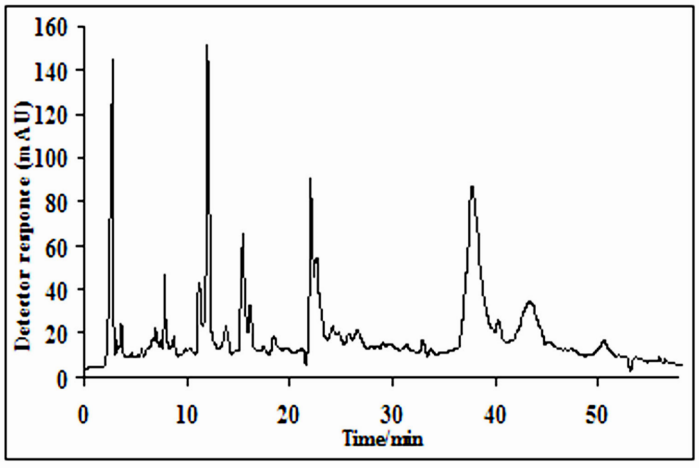

Figure 2 Sample chromatogram of polyphenolic compounds profile (retention times are given in Table 2).

Apples are rich in phenolic compounds. Five major polyphenolic groups are found in various apple varieties that the percentages of them in total phenolic compounds differed different apple varieties: hydroxycinnamic acids, flavan-3-ols/procyanidins, anthocyanins, flavonols, and dihydrochalcones **(Podsedek *et al*., 2000; Schieber *et al*., 2001)** .

In this study the apple juice concentrate contains, 1137.84 mg/kg of total phenolic compounds that in comparison with three other juices was low (see table 1). As it can be seen from table I the apple juice was reach in chlorogenic acid in comparison with other juices. Grape phenolic compounds can be divided into two groups: non-flavonoid (hydroxybenzoic and hydroxycinnamic acids and stilbenes) and flavonoid compounds anthocyanins, flavan-3-ols and flavonols). Anthocyanins are a family of polyphenols that are directly responsible for colour in grapes and young wines.

Table 1 Total polyphenolic contents of selected juices

Juice	Sour cherry	Apple	Pomegranate	Grape
Total phenolic content (mg/Kg)	4376.64	1137.84	2144.23	1308.11

Flavan-3-ols (monomeric cathechins and proanthocyanidins) are another large family of polyphenolic compounds in skin and seed of red grapes **(Waterhouse, 2002; Scola *et al*., 2010)**. The last group of flavonoids is flavonols (quercetin, myricetin, kaempferol, isorhamnetin and their glycosides) kaempferol, isorhamnetin and their glycosides, which display antioxidant activity **(Gomez-Alonso *et al*., 2007; Tutel'ian *et al*., 2013)** . The total polyphenolic content of the analyzed grape juice was 1308.11 mg/kg.

The quercetin 3-glucoside and phloridzin have the highest level in pomegranate juice comparison with other juices (Table 2). The main anthocyanins found in cherries (Prunus cerasus) are cyanidin (CY)-3-glucoside, cy-3-glucosylrutinoside, cy-3-sophoroside, and cy-3-rutinoside. Besides these anthocyanins others like cy-3-xylosylrutinoside, peonidin-3-glucoside, peonidin-3-rutinoside, and cy-3-gentiobioside can be found in low concentrations **(Simunic *et al*., 2005).**

Table 2 Contents (mg/Kg) and retention times of polyphenolic compounds in selected juices

Compounds	Retention time	Sour cherry juice	Apple juice	Pomegranate juice	Grape juice
procyanidin B_1	8.06	1213.62	461.84	1088.11	413.62
catechin	12.25	2428.84	426.24	250.64	445.12
procyanidin B_2	14.57	75.72	23.96	184.38	44.32
chlorogenic acid	16.04	55.88	135.38	50.34	17.83
cyanidin 3-galactoside	18.21	42.98	32.31	228.81	0
epicatechin	22.41	490.42	0	268.52	262.76
p-coumaric acid	29.24	17.02	17.62	28.83	24.82
quercetin 3-galactoside	42.94	25.36	16.49	0	13.12
quercetin 3-glucoside	44.74	0	2.78	0	43.58
quercetin 3-rhamnoside	51.72	12.94	12.34	30.08	24.88
phloridzin	55.52	13.86	8.88	14.52	18.06

Sour cherry juice was selected from this family and analyzed in here. Total phenolic compounds content of the sour cherry juice was 4376.64 mg/Kg of juice and had the highest content in comparison with other juices. Also the sour cherries had the high content of procyanidin B_1, Catechin, Epicatechin, and quercetin 3-galactoside in comparison with other analysed juices.

Polyphenols are relevant constituents regarding the organoleptic properties of pomegranate arils and juice as they are responsible for the distinctive red pigmentation and provide a mild astringency that is characteristic of pomegranate flavor **(Martínez, 2012)**. The husk and fruit membranes contain mainly ellagitannins that are water soluble (punicalagins), and small amounts of procyanidins (prodelphinidins and gallocatechin). Anthocyanins are also present in the skin, although the delphinidin derivatives are not generally observed and the cyanidin and pelargonidin derivatives coincide with those found in the juice **(Lila, 2004)**. During industrial processing, the technological treatment allows the release of water-soluble husk punicalagins into the juice, which has been related to the outstanding antioxidant activity observed in commercial pomegranate juice **(Seeram, *et al*., 2006)**.

The juice obtained from these arils contains anthocyanins (delphinidin, cyanidin, and pelargonidin 3-glucosides and 3, 5-diglucosides), ellagic acid glycosides (ellagic acid glucoside, arabinoside, and rhamnoside), free ellagic acid, ellagitannins (several punicalagin isomers, punicalin, and some punicalagin polymeric forms), and gallotannins.

According to the Table II, total polyphenolics in pomegranate was 2144.23 mg/Kg of juice that in comparison with grape and apple are rich from phenolics. Pomegranate juice in this study had the highest content of procyanidin B_2, cyaniding 3-galactoside, p-coumaric acid, and quercetin 3-rhamnoside in comparison with other juices.

CONCLUSION

A simple HPLC method using Diode-Array Detection (DAD) was developed for the determination of major polyphenolic contents of apple, pomegranate, Grape and Sour cherry.
Comparison between total polyphenolic contents was as:

Sour cherry > Pomegranate > Grape > Apple

According to the collected data in Table I, catechin was the major polyphenolic compound in the grape juice (34.03 % of total polyphenolic content) and sour cherry juice (55.50 % of total polyphenolic content). Procyanidin B1 was the major polyphenolic compound in apple juice (40.59 % of total polyphenolic content) and pomegranate juice (50.75 % of total polyphenolic content).
The industrial process and the processing conditions affect the polyphenolic content of juices. Temperature, additive, stabilizers, softeners and other conditions can be decreased these compound due to this fact that these compounds strongly depends on the condition stresses.

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