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The changes of bioactive ingredients and antioxidant properties in various berries during jam processing

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Abstract

Berries are a very rich source of bioactive compounds including vitamin C, E and phenolic compounds. In this study, chemical composition and the effects of jam processing were studied in various berries including *Fragaria ananassa, Rubus Fruticosus, Rubus idaeus, Morus rubra and Vaccinium corymbosum*. The samples were evaluated for ellagic acid (EA) content by high-performance liquid chromatography (HPLC), total polyphenol (TP) and total flavonoid (TF) contents as well as antioxidant activity (AA) and free radical scavenging activity (FRSA) using colorimetric methods. In fresh berries samples, the mean value concentrations of EA, TP, TF, AA and FRSA were detected 56.55 ± 47.66 µg/g, 2225.00 ± 934.00 µg/g, 4548.20 ± 1331.47 µg/g, 2.89 ± 0.94 mMol Fe+2 E/g and 45.93 ± 12.16 µg/ml respectively. After jam processing, the mean decrease percentage of EA, TP, TF, AA and FRSA in berries samples were 30.1% ± 9.1%, 37.7% ± 17.1%, 43.4% ± 17.2%, 38.8% ± 16.8% and 29.2% ± 21.6% respectively. Our findings support that berries and their jams are potentially useful sources of natural antioxidants and assessment of antioxidant compounds could be an important index for evaluation of processed fruits quality.

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Introduction

There is an inverse correlation between individuals who consume a rich diet in fruits and vegetables and their risk of developing cancer (Halliwell, 1994). These extortionary health-promoting effects in human could be related to high level of antioxidant capacity in these sources due to existing of natural phenolic compounds. Most of these antioxidant compounds possess antimutagenic, antibacterial, antiatherosclerotic, antiproliferative, antitumor, anti-inflammatory, anticarcinogenic or antiviral properties to a greater or lesser extent (Liu *et al*., 2002; Ratnam *et al*., 2006).

Berries are being used as fresh garden-fruits and also as constituents in foods. There are phenolic compounds, ascorbic acid and vitamin E as bioactive ingredients in various kinds of berries (Aaby *et al.,* 2005; sun *et al.,* 2002). Anthocyanines that are established as glycosides are known as one of the important phenolic compounds that can produce red, purple or blue colors in berries (Koponen *et al.,* 2007). Ellagic Acid (EA), a dimeric derivative of gallic acid (Vekiari *et al*., 2008), is the other dietary phenolic compound found in numerous fruits and vegetables including strawberries, raspberries, pomegranates, walnuts (Amakura *et al*., 2000) and blackberries (Rommel and Wrolstad, 1993). It seems that EA is preserved in the processed fruits such as jams and juices (Shahrzad and Bitsch, 1996). EA has an important role in cellular activities such as apoptosis and cell cycle arrest in a variety of cancer cells (Seeram *et al*., 2005). Previous researches demonstrated anticarcinogenic and antiproliferative effects (Daniel *et al*., 1989; Mandal *et al*., 1988) as well as antioxidant properties (Amakura *et al*., 2000) of EA consumption on human health.

There has been a growing attention in the past two decades about berries due to their notable antioxidant capacity. Since there has not been any widespread investigation about berries in Iran until now and the fresh fruit is not accessible all the year as well; the goal of this survey is defining whether the jams couId also signify a good source of bioactive compounds like antioxidants. While antioxidant content is becoming an essential factor considering the fruit quality concomitantly, it is of great interest to assess changes in antioxidant capacity during processing into jam. This research will be helpful for finding and quantifying new effective antioxidants from these fruits. For this purpose, we investigated EA, total polyphenol (TP) and total flavonoid (TF) contents as well as antioxidant activity (AA) and free radical scavenging activity (FRSA) in berries, which are available in local markets of Iran and their jams.

Materials and methods

Chemicals and Reagents

Methanol and acetonitrile (HPLC-grade), potassium dihydrogen phosphate (KH_2PO_4), ortho-phosphoric acid 85% (H_3PO_4), sodium hydroxide (NaOH), hydrochloric acid 37% (HCl), DPPH (1,1-diphenyl-2-picryl-hydrazil), gallic acid (GAE), Folin–Ciocalteu, $FeSO_4$, $FeCl_3$, TPTZ (tripyeridyl-s-triazine), $AlCl_3$ were purchased from Merck (Darmstadt, Germany). Ellagic acid (EA) standard (≥95.0% HPLC grade) was obtained from Sigma Aldrich (St. Louis, MO, USA).

Samples

Five kinds of berries including strawberry (Fragaria ananassa), blackberry (Rubus Fruticosus), raspberry (Rubus idaeus), mulberry (Morus rubra) and blueberry (*Vaccinium corymbosum*), grown in north and west regions of Iran, during the period May–July 2013, were harvested and delivered to the laboratory. One part of fresh berries stored at +4°C and analyzed within the earliest time, the other part was cooked to make jam.

Preparation of Jam

Jam samples were prepared in laboratory condition from fresh berries according to traditional method in Iran. Briefly, after washing the berries with water, 700 g of fresh berries were cooked with 700 g of sugar (sucrose) for 45 min. Then, the jams were allowed to cool at room temperature and stored at 20 °C until analysis.

Extraction

The extraction procedure was done as follows: At first 10 g of the fresh fruits or the prepared jams were weighed and then homogenized in 30 mL of methanol. The solutions were refluxed for 1 h and filtered. Distilled water (10 mL) and 100 µL of HCl solution (0.1 M) were added and the solvents were evaporated to dryness under reduced pressure below 40°C using a rotary evaporator. The residue was dissolved in 1 mL of distilled water. The contents of EA, total phenolic compounds, total flavonoid, antioxidant activity and free radical scavenging activity were then analyzed within the earliest time.

Determination of Ellagic Acid Contents by HPLC

Sample cleaning was carried out using a (Spe-ed) SPE Cartridge. The SPE Cartridge C18 was initially activated by passing methanol (10 mL) and distilled water (10 mL) in turn. The solution of sample was loaded to the SPE; the cartridge was washed with water (10 mL) and methanol (10 mL), respectively.

EA was eluted on the cartridge when 10 mL of methanol had been delivered to the SPE cartridge. At the end, the collected eluent was evaporated to dryness below 40°C under reduced pressure. The residue was dissolved in 5 mL methanol and filtered through a 0.22 µm filter (Whatman Pureadisc) prior to the injection into HPLC.

The HPLC applied system (Waters Chromatography Division, Milford, MA, USA) consisted of Waters 510 pump and solvent delivery system. The system was connected to Waters 486 UV Detector. Instrument control and data acquisition were performed with a personal computer running the software Autochro-2000 (Chromatograph Data System). For EA, separation was achieved using a column Intersil ODS-3V (5 µm, 250 x 4.6 mm, GL Sciences Inc, Japan) with guard column holder and a SPE column C18. The column was maintained at 30 °C and equilibrated for 40 min with mobile phase before sample injection. The isocratic mobile phase was employed including 5 mM potassium dihydrogen phosphate buffer solution (pH 2.5) and acetonitrile (82:18, v/v). The mobile phase was delivered at a flow rate of 1.4 mL/min, which was prepared freshly and degassed for 10 min by ultra-sonication. Absorption was set at 360 nm and volume injected was 20 µL. Quantitative determination was accomplished using calibration curve attained from stock standard solution of EA (1000 µg/mL) in methanol. Serial dilution and calibration curve were prepared in the concentration range (25-500 µg/mL) (Fig. 1). All stock solutions were sealed and stored at refrigerator.

The lower limit of quantification (LLOQ) of the method, defined as the minimum concentration that could be measured with a CV < 10% was found to be 25 µg/mL. The recovery of EA in samples ranged from 81% to 86%. A typical chromatogram of standard solution (500 µg/mL) is presented (Fig. 2). Identity and assessment of the desired peak were performed by comparing its retention time and AUCs in sample and standard chromatograms.

Determination of Total Polyphenol

The total polyphenol content of the extracted samples was measured by Folin–Ciocalteu colorimetric method (Singleton *et al.*, 1999). The absorbance was read in duplicates in a 96-well plate with a spectrophotometer (Biotec, Tecan US, Inc.) set at 725 nm against gallic acid (GAE) as standard. The results were expressed by GAE equivalents (µg/g).

Determination of Total Flavonoid

The total flavonoid content of the extracted samples was assayed using the colorimetric method (Meda *et al.*, 2005). The absorbance was read at 420 nm using spectrophotometer in a 96-well plate. Results were shown as mg quercetin equivalents in 1 g of the extract (µg QE/g extract).

Determination of Antioxidant Activity

The Ferric Reducing Ability of Plasma (FRAP) method was used to determine the antioxidant power of the extracted samples (Benzie *et al.*, 1996). The absorbance was read in duplicates in a 96-well plate with a spectrophotometer set at 593 nm. The antioxidant capacity of the extract was measured against standard curve of ferrous sulfate equivalent

(mMol Fe+2 E/g).

Determination of Free Radical Scavenging Activity

The free radical scavenging activity of the extracted samples was measured using DPPH according to the modified method (Brand-Williams *et al.*, 1995). The absorbance was read in duplicates in a 96-well plate with a spectrophotometer set at 517 nm. The inhibition of DPPH radicals by the samples were calculated according to the following equation: DPPH-scavenging activity (%) = [1 - absorbance of sample/absorbance of control] - 100.

Statistical Analysis

The results were expressed as Mean ± SD and compared using student T-test. The p-value < 0.05 was considered statistically significant.

Results

Our study showed that, in fresh berries samples, the mean values of EA, TP, TF, AA and FRSA were detected 56.55 ± 47.66 µg/g, 2225 ± 934 µg/g, 4548 ± 1331 µg/g, 2.89 ± 0.94 mMol Fe+2 E/g and 45.93 ± 12.16 µg/ml respectively. The highest level of EA, TP, TF, AA, and FRSA was found in strawberry samples 145.69 ± 14.56 µg/g, 3974 ± 188 µg/g, 5861 ± 512 µg/g, 4.21 ± 0.51 mMol Fe+2 E/g and 62.26 ± 7.94 µg/ml respectively. Also, the lowest content of EA, TP, TF, AA and FRSA were determined in blueberry samples 23.77 ± 2.38 µg/g, 1613 ± 112 µg/g, 3053 ± 415 µg/g,1.98±0.34 mMol Fe+2 E/g, 34.6 ± 4.1 µg/ml respectively.

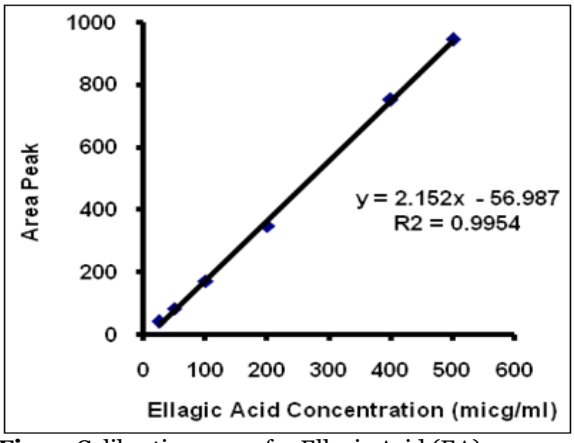

Fig. 1. Calibration curve for Ellagic Acid (EA).

After jam processing, the mean decrease percentage of EA, TP, TF, AA and FRSA in berries samples were calculated 30.12% ± 9.07% (ranged from 12.6% to 53.44%), 37.7% ± 17.1% (ranged from 20.12% to 69.4%), 43.4% ± 17.25% (ranged from 26.9% to 74.7%), 38.8% ± 16.8% (ranged from 14.8% to 66.4%) and 29.19% ± 21.58% (ranged from 3.82% to75.7%) respectively.

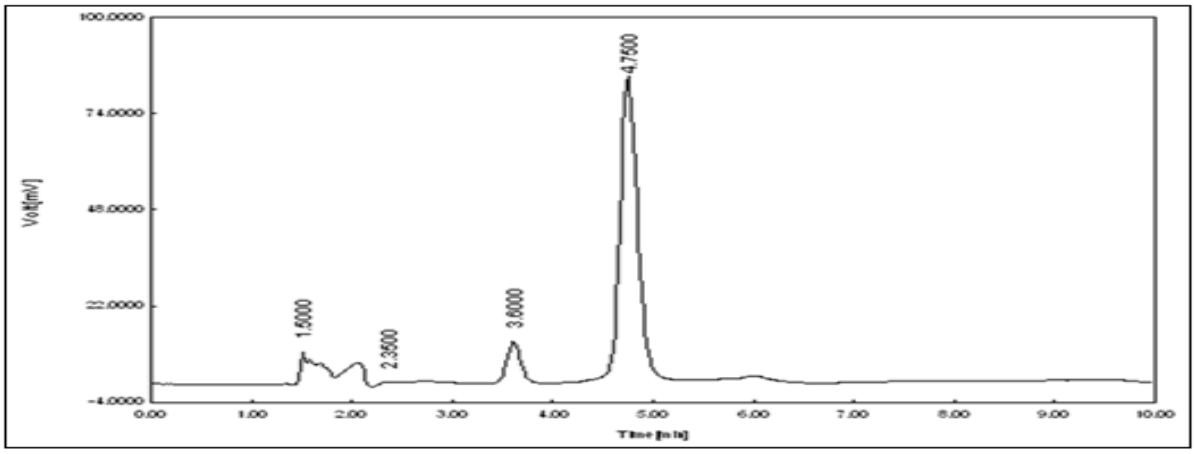

Fig. 2. Chromatogram of Ellagic Acid (EA) standard (500 µg/ml) concentration.

The level of EA content decreased significantly after jam processing in all samples except blueberry sample (Fig. 3). Also, the decrease of TP (Fig. 4) and TF contents (Fig. 5) were statistically significant in all of the jam samples compare to their fresh samples. As shown in Fig. 6 and 7, a significant decrease was observed in antioxidant and anti-radical properties in mulberry and blueberry samples in comparison with their related fresh samples.

Discussion

Based on present findings, it seems that strawberry is the main source for EA and the process of jam preparations in routine manner had no significant effect in decreasing concentration of EA. Today, it is well established that a diet, which is high in fruits and vegetables, is associated with reduced risk of oxidative stress mediated diseases such as cancer, cardiovascular and neurodegenerative diseases. However, it should be also noted that it is not possible to use some fresh fruits like berries, during the four seasons. Therefore, long-term storage like jam for food products can represent significant source of bioactive compounds in the diet with noticeable antioxidant capacity.

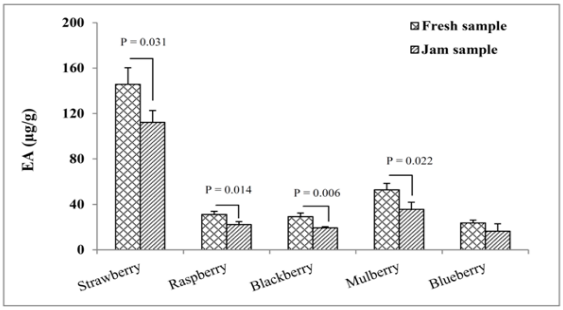

Fig. 3. Ellagic Acid (EA) content in fresh berries and their related jams. Values are expressed as mean ± SD.

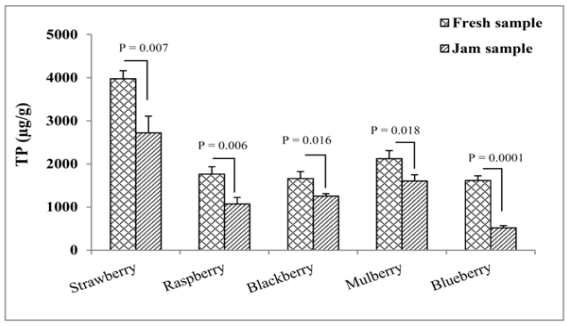

Fig. 4. Total Phenolic (TP) content in fresh berries and their related jams. Values are expressed as mean ± SD.

Berries are good sources of polyphenols such as flavonoids to which have been ascribed many beneficial effects like antioxidant and free radical scavenging activity (Savikin *et al.*, 2009). Therefore, it is important to determine the chemical characteristics of berries and influence of processing on their polyphenols contents which are health-promoting materials. In this research, we compare various berries from the viewpoint of antioxidant properties and the effects of traditional method in Iran for making jam on preservation of antioxidant storages. Although jam processing led to the loss of EA contents, total polyphenols, total flavonoids, free radical scavenging and antioxidant activity of five berries, jams are still outstanding resources of dietary substances with antioxidant potential. Our results are partially in keeping with the literature showing total phenols losses by 20 % and even more during jam processing (Amakura *et al.*, 2000; Hakkinen *et al.*, 2000). Current finding is consistent with Hakkinen *et al* and Levaj *et al* studies that proved, besides fresh strawberry fruit, the strawberry jams also possess prominent content of important bioactive compounds such as EA with considerable antioxidant capacity (Hakkinen *et al.*, 2000; Levaj *et al.*, 2012). According to the study, EA content in strawberry jam was 80% of that in unprocessed strawberries (Hakkinen *et al.*, 2000). In another study Wicklund *et al* prepared Jams from five strawberry cultivars and assessed the anthocyanin pigments and total antioxidant capacity of jams by FRAP-assay. Their results showed that the industry should consider storing the products at 4 ºC to achieve a good colored strawberry jam with high antioxidant capacity (Wicklund *et al.*, 2005). In a similar survey, Savikin *et al* also investigated the effects of domestic processing and storage in berries and found out that in spite of the losses in amount of total phenolics, total anthocyanins and radical scavenging activity, but frozen berries and related jams still could be considered as good sources of potentially antioxidant nutritional substances (Savikin *et al.*, 2009). Considering the values of the measured variables, some of the markers decreased significantly in fresh berries comparing to jam. After jam processing, the highest contents of TP and TF as well as anti-radical and antioxidant properties were detected in strawberry jam. Although, quantity of antioxidant compounds such as EA, TP and TF were significantly decreased, no remarkable difference was observed in anti-radical and antioxidant properties. This discrepancy may be explained at least in part by condition of jam processing and conversion of some

of the compounds to other antioxidants. For instance, release of hexahydroxydiphenic acid from ellagitannins because of the degradation of cell structures, which is transformed to EA or to an easier extractability of this compound from processed products during jam processing could compensate reduction of some unstable antioxidant agents (Savikin *et al.*, 2009). According to Turkmen *et al* suggestion, high temperatures in cooking process could inactivate peroxidases which are effective in pro-oxidant activity. Hence, thermal processing causes no reduction in antioxidant potential of vegetables and fruits and even augments it due to improvement of antioxidant properties of natural compounds or by formation of novel compounds which have antioxidant activity (Turkmen *et al.*, 2005).

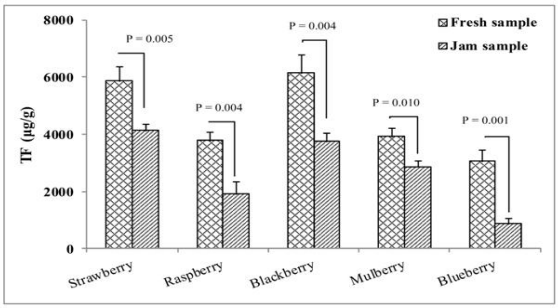

Fig. 5. Total flavonoid (TF) content in fresh berries and their related jams. Values are expressed as mean ± SD.

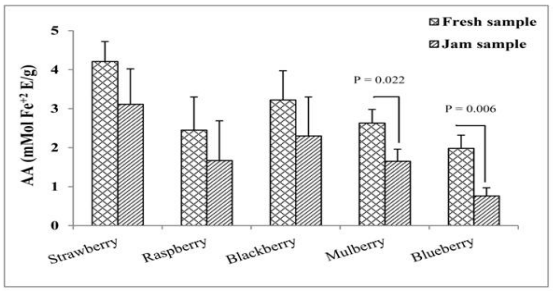

Fig. 6. Antioxidant activity (AA) in fresh berries and their related jams. Values are expressed as mean ± SD.

In the current study, we tried to investigate not only fresh strawberry (Fragaria ananassa) and its jam as a prominent and mostly used berry all over the world, but also four other kinds of berries including blackberry (Rubus Fruticosus), raspberry (Rubus idaeus), mulberry (Morus rubra) and blueberry (Vaccinium corymbosum) which can be considered as rich sources of antioxidants too.

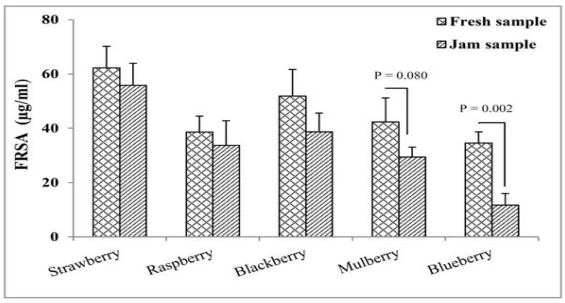

Fig. 7. Free radical scavenging activity (FRSA) in fresh berries and their related jams. Values are expressed as mean ± SD.

Taking together, we can conclude that berries are potentially as a useful source of natural antioxidants and determination of anti-radical properties and natural antioxidant values would be suitable indexes for evaluation of processed fruits quality.

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