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Hydrolytic Properties of Ellagic Acid in Commercial Pomegranate Juices

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***Abstract*—**Pomegranate and pomegranate juices (PJs) have taken great attention for their health benefits in the last years. As there is an increasing concern about potential health benefits of ellagic acid, it is of great interest to evaluate alterations in ellagic acid concentration of commercial PJs. The purpose of this study is to analyze total phenolic, free and total ellagic acid content of six commercial PJs sold in Turkish markets using HPLC.

The results showed that some commercial PJs had markedly high total phenolic and ellagic acid content. Total phenolic substances of commercial PJs range from 796.71 to 4608.91 mg GAE/l. Free amount of ellagic acid in commercial PJs range from 27.64 to 111.78 mg/l. Samples are hydrolyzed with concentrated HCl at 93^{o}C for 2 and 24 hour and influences of temperature and time parameters on hydrolization were investigated. Thermal processing for pasteurization increased ellagic acid via ellagitannins hydrolysis.

***Keywords*—**Ellagic acid, ellagitannin, pomegranate juice, total phenolic compounds

I. Introduction

IN the past few years, there has been an increasing interest in determining relevant dietary sources of antioxidant phenolics. Among fruits, pomegranate is an interesting rich source of anthocyanins and other phenolic compounds, with a demonstrated antioxidant activity [1]. All these activities may be related to diverse phenolic compounds present in pomegranate juice, including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin, pelargonidin 3-glucosides and 3,5-diglucosides) [2]. Ellagitannins (ETs) are water-soluble high molecular weight phenolic compounds in which hexahydroxydiphenic acid (HHDP) forms diesters with sugars [3]. Hydrolysis of ETs with acids or bases yields HHDP, which spontaneously lactonizes to ellagic acid (EA). This reaction has been utilized for the detection and quantification of ETs as EA equivalents after acid hydrolysis of food samples [4]. Also, in the human gastrointestinal tract, ingested dietary ETs are hydrolyzed to release EA. Both ETs and EA are largely metabolized by the colon microbiota of different mammals, including rats [5], pigs [6] and humans [7],[8]. Recent studies have indicated that ellagic acid possesses antimutagenic, antioxidant and anti-inflammatory activity in bacterial and mammalian systems [9],[10],[11]. Estrogenic/antiestrogenic activity as a selective ligand of estrogen receptor (ER) subtypes ERα and ERβ has been also proposed for ellagic acid [12].

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Some studies have reported that complex dietary ellagitannins from different sources are not absorbed in humans but hydrolyzed to yield ellagic acid, which is further metabolized by the human colonic microflora to yield bioavailable hydroxy-6*H*-dibenzo[*b*,*d*]pyran-6-one derivatives (mainly urolithins A and B) with estrogenic/ antiestrogenic activity [8],[9]. In Turkey, pomegranate is eaten as fresh as well as processed for jams, syrups and pomegranate juice products [13]. This fruit is one of the most important commercial fruits in Turkey and Turkey contributes 150,000 tons of the world annual production of one million tons [14].

As there is an increasing concern about potential health benefits of ellagic acid, it is of great interest to evaluate the alterations in ellagic acid contents of commercial PJs. Since no information previously existed on the ellagic acid content of commercial PJs and likewise, no information is available on ellagic acid conversion from its precursors associate with heating. Therefore, by evaluating hydrolysis time on the relationship of free ellagic acid from its precursors, the functional properties of these compounds can be evaluated as a result of processing. Accordingly, the objective of this research is to evaluate the changes in ellagic acid concentration of PJs.

II. Materials and Methods

A. Materials

PJs belonging to six different commercial trade marks were purchased from local markets. All commercial PJs labelled with 100% PJs and none of them contain extra added ingredients.

B. Acid Hydrolysis of Ellagic Acid

Hydrolysis was performed at concentrated hydrochloric acid in duplicate. The ellagic acid derivatives were hydrolyzed for 2 and 24 hrs at 93^{o}C.

C. Colorimetric Determination of Total Phenolics

The Folin-Ciocalteu assay was used for the determination of total phenol content in pomegranate juices [15]. Phenolics are expressed as gallic acid equivalents. Absorbance was measured at 760 nm with a spectrophotometer.

D. Analysis of Phenolics by HPLC

The identification of phenolics was performed by an Agilent 1100 HPLC system (Waldbronn, Germany) consisting of a binary pump, an autosampler, a diode array detector, and a temperature controlled column oven. The analytical separation was performed on an ACE 5 C18 column (250 ×4.6 mm, 5 μm). HP Chem-Station for LC software (rev.A08.01) was used for data processing. Samples were diluted (1:3) with methanol and filtered through 0.45 mm Millipore filter and injected onto HPLC. HPLC elution was carried out at room temperature.

The following solvent system and elution profiles were used: solvent A, the mixture of formic acid and water (5:95, v/v), and as solvent B, methanol. The elution profile was 15% solvent B isocratic for 5 min followed by a 15–30% linear gradient for 15 min and 30–50% linear gradient for 10 min with solvent B and holding with 50% solvent B for an additional 10 min, and finally followed by a 50–15% linear gradient with solvent B for 10 min [16]. The flow rate was 0.7 mL/min. Chromatograms were recorded at 254 nm with spectra (200-600 nm) taken continuously throughout the elution for confirmation.

E. Statistical Analysis

Data were assessed using analysis of variance (ANOVA) by the general linear model procedure of the SPSS 15.0 version for Windows statistical package programme. Data means were compared with the least significant difference test. All tests were considered to be statistically significant at $P < 0.05$.

III. Results and Discussion

Changes in total phenolics values in commercial PJs represent in Fig.1. Total phenolic contents (TPC) range from 1447.87 to 4608.91 mg GAE/l, except juice 4. This juice showed very low TPC level. Gil et al. [2] reported the TPC for a commercial PJ as 2566 ± 131 mg/l. TPC of six pomegranate arils from Mediterranean region of Turkey are reported between 1245 and 2076 mg/l [17], and of eight pomegranate arils widely grown in Turkey are between 2083–3436 mg/l [18].

Figure 3 represents the chromatograms of the ellagic acid concentration monitored at 254 nm. Ellagic acid has been identified in commercial pomegranate juice by comparing the retention times and spectral characteristics of the peaks against that of standard, as well as by spiking the samples with standard. Numerous polyphenolic compounds are present in commercial PJs. A total of 7 compounds were identified in all 100% juices. The retention times of gallic acid, protocatechuik acid, chlorogenic acid, caffeic acid, ferulic acid, *o*-coumaric acid and ellagic acid were 5.01, 7.60, 10.20, 16.45, 26.29, 23.32 and 35.53 min, respectively. Spike recovery percentages for ellagic acid was found to be %78.86. Concentrations of the ellagic acid found in the samples are listed in Fig. 2. As it can be seen from Fig. 3, sample 1 and 2 of the commercial pomegranate juices had relatively high concentrations of gallic acid and ellagic acid when compared to the others. Mousavinejad et al. [19] reported ellagic acid concentrations ranging from 7 to 160 mg/l for Iranian cultivars. For our sample set, we found that sample 6 had the highest ellagic acid content (111.78 mg/l).

The variation between the samples is likely due to differences in manufacturing methods or in the varieties used by the manufacturers.

During food processing ETs change to free EA and EA derivatives [20]. The amounts of EA in foods are determined as free EA and/or total EA after acid hydrolysis. The levels of ellagic acid derivatives were significantly influenced by increased hydrolysis time that accelerated conversion to free ellagic acid from ellagic acid precursors (Fig. 2).

Free ellagic acid hydrolysed with 2 hrs in commercial PJs (Fig. 2) changed by 14, 15, 9, 10, 10 and 9-fold with respect to unhydrolysed samples, respectively. These differences were due to the presence of ellagitannins. Ellagic acid aglycones showed strong correlation with total soluble phenolics, r=0.98.

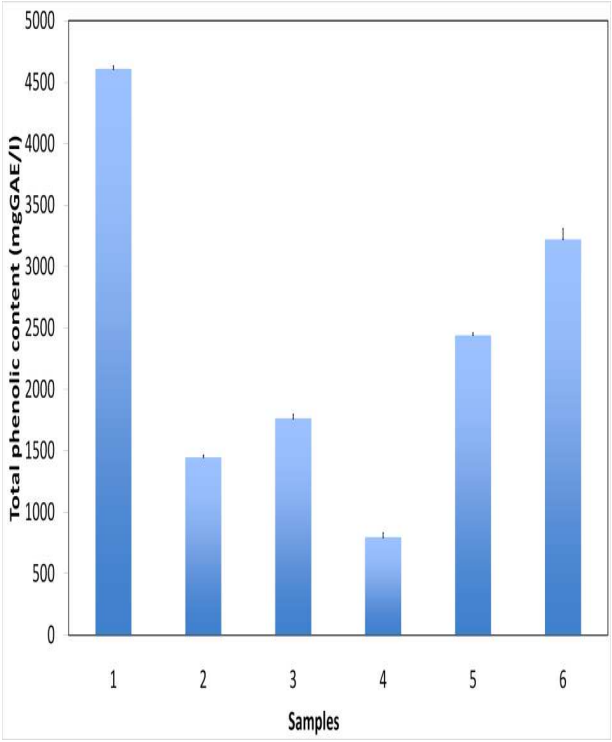

Fig. 1 Changes in total phenolics values in commercial PJs samples

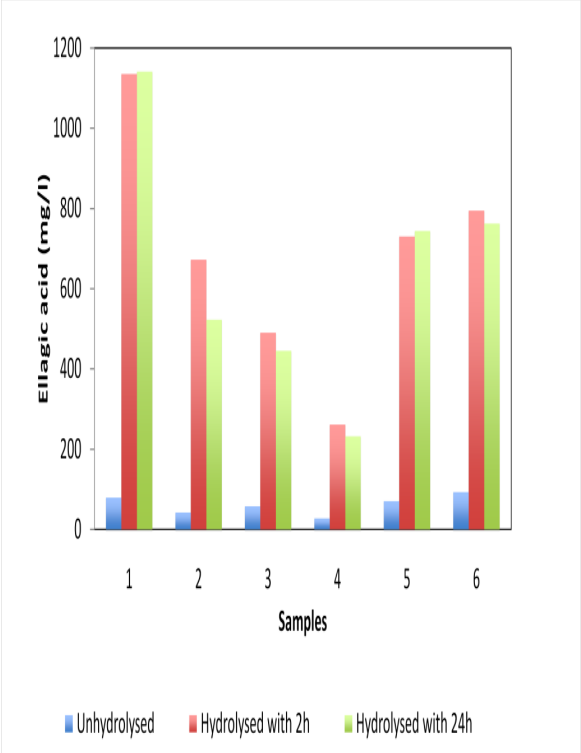

Fig. 2 Comparison of content of free and after hydrolysis ellagic acid in commercial PJs samples

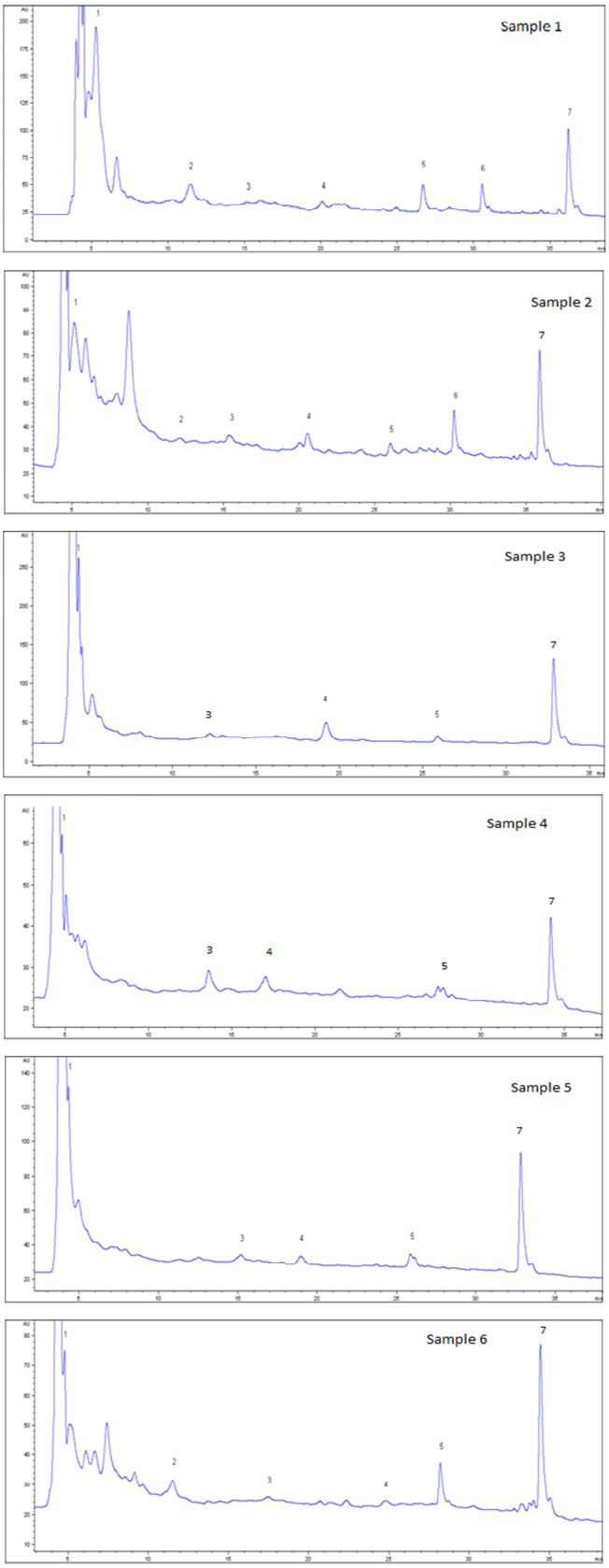

Fig. 3 Chromatograms of ellagic acid in commercial PJs monitored at 254 nm: Peaks: 1) gallic acid, 2) protocatechuik acid, 3) chlorogenic acid, 4) caffeic acid, 5) ferulic acid, 6) *o*-coumaric acid, 7) ellagic acid

IV. Conclusion

In the six commercial PJs studied in this work, in comparison to aril PJs and other fruit juices reported in the literature, much higher TPs were observed. Properties of ellagic acid derivatives were studied as affected by acid hydrolysis. Additional concentrated HCl completely hydrolyzed ellagitannins and ellagic acid glycosides in 2 and 24 hr. Results provided by these studies suggest beneficial reasons to consume pomegranate juices leading to improve the market value of this crop not only as juice but also as health promoting components in various types of processed food.

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