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Phenolic Contents and Antioxidant Activities of Persimmon and Red Beet Jams Produced by Sucrose Impregnation

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Abstract This study aims to bring different perspective to literature by defining changes in TPC and AA of sucrose impregnation applied jams produced from high antioxidant potential fruit and vegetables. Total phenolic contents were determined by the Folin-Ciocalteau method and total antioxidant activities were determined with ABTS and DPPH radical scavenging methods for both fresh fruits and the jams produced. Major phenolic compounds such as catechin, gallic acid, caffeic acid, hesperidin and betanin were identified by High performance liquid chromatography. In respect of results; jam processing by means of sucrose impregnation was found to result in insignificant decreases in total phenolic contents for persimmon (35.4%), however significant losses were observed for the red beet jam (49.9%). According to DPPH results, as a result of jam processing, insignificant decreases (about 16%) in antioxidant activity levels for persimmon was found whereas for sugar beet jam the reduction was found significant (43.4%). According to ABTS data, jam processing was found to have negative effect on antioxidant activity only for the red beet.

Keywords Persimmon, Red Beet, Antioxidant, Phenolic Content, Jam, Sucrose Impregnation

1. Introduction

Polyphenols are widely distributed in many fruits, vegetables, teas and beverages and they exert potential health-promoting effects as antioxidants besides their antitumor and anti-carcinogenic effects. There are many studies in literature showing the inverse relation between the consumption of “vegetable food” and cancer or heart diseases [1]. The attention paid to compounds showing high antioxidant activity (AA), such as carotenoids, anthocyanins and recently betalains has increased [2].

Persimmon (Diospyros kaki L.) is an important plant used for several purposes such as to stop bleeding, paralysis treatment, frosbite and burns [3]. There are some studies showing that it has medical effects on haemostasis, constipation, hypertension, apoplexy and atherosclerosis. It is a good source of antioxidants, vitamins, polyphenols and dietary fibers. Persimmon leaves have flavonoid oligomers, tannins, phenols, organic acids, chlorophyll, vitamin C and caffeine. There are also significant compounds such as catechin, epicatechin, gallic acid, kaempferol and quercetin exerting high AA [3-5]. It is an important bioactive fruit and has higher AA than apple, grape, tomato, blueberry and strawberry [6].

The hypolipidemic and antioxidant effects of two different diets in rats fed with cholesterol were investigated and compared as 7% of whole dry persimmon and 7% of phenol-free dry persimmon diets [7]. According to the researchers’ findings claimed that persimmon phenolics were positively influencing lipid levels The antioxidant effect of persimmon was primarily related with its phenolics [7].

Red beet (*Beta vulgaris* L.) includes specifically high amount of antioxidants. It contains betalains, which are significant natural food colorants, (composed of betacyanins and betaxanthins) and also many phenolic compounds such as ferulic acid conjugates, phenolic amides, and flavonoids [8, 9]. The most significant betalain compound is betanin, which is a glycoside composed of glucose and betanidin. Beetroot has a red and purple color due to betacyanin and betanin. Betacyanin can decrease LDL cholesterol oxidation and is effective on cardiovascular diseases [8, 9].

It is known that persimmon and red beet are rich in AA and many published *in vitro* studies showed the strong antiradical content and especially the AA of betalains from red beets [2]. However, their consumption is possible for only a few months of the year, since they are mostly consumed as a fresh fruit in Turkey. Persimmon is only marketable for 2-4 months by cold storage and red beet can be harvested in October and consumed for only 4 months.

These two fruits have only a very limited shelf-life. Therefore, similar to many fruits and vegetables as they are seasonally available processing into products such as juice, jam, dried fruit, ice cream, etc. which may be good solutions to benefit from their health effects throughout the whole year. Therefore, determination of changes in phenolic contents and AA upon processing is necessary for the products obtained from those two sources to evaluate their bioactive potential more accurately.

Effects of several processes such as; harvesting, preparation and handling of fruits and vegetables on antioxidant status have been reported by many researchers [10]. Numerous researchers have specifically studied the effect of traditional jam processing on phenolic content and AA of fruit and vegetables [11-13]. Rababah et al., (2011) studied the effect of jam processing on strawberry, cherry, apricot, fig and orange and reported significant losses in total phenolics (about 69-93% changing according to the type of the fruit), antioxidant activity (11-39%) and anthocyanins (60-99%) in jams, while they showed only moderate losses during storage for five months [11]. In contrast, Kim and Zakour, (2006) comprised different cultivars of cherries, plums and raspberries and their results indicated that more than 73% total phenolics and more than 65% antioxidant capacity were retained after processing fruits into jams, although anthocyanins were almost lost completely [12]. Contradictory results available in literature emerged the trials of different or modified applications for jam processing.

Impregnation may be defined as replacing the gas and liquid of a material through the internal pores by using an external liquid. Fruits are practically suitable for this application because of their homogeneous tissues accelerating mass transfer [14-15]. Use of carbohydrates, particularly sucrose might be a beneficial alternative for jam processing since it is able to diffuse through the cell wall and stay between the wall and membrane parts of fruits [16]. This ability was previously used for stabilizing colorful pigments found in foods. The working mechanism was related with either the reversible inclusion complexes formed between cavities in cyclodextrin moieties of carbohydrate sources and smaller molecules (often phenolic substances) or loss of water in fruits since anthocyanin color is known to increase upon the removal of water [17].

Although it is suggested for enriching foods with nutrients/additives and also for a better sensorial quality in terms of taste, appearance and smell, sucrose impregnation had only a limited use for jam processing and mainly applied to anthocyanin rich fruits such as strawberries [14].

The main objective of this study is to determine total phenolic content (TPC) and AA of red beet and persimmon both when they are both fresh and processed into jams by means of sucrose impregnation application. The losses in traditional jam processing from the literature were compared with that of sucrose impregnated ones in this study.

2. Materials and Methods

2.1. Materials

Persimmon and red beet were obtained from a local market in Istanbul, Turkey. Chemicals were used in the study such as methanol, formic acid, trifluoroacetic acid (TFA), sodium carbonate, potassium persulfate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate were obtained from Merck (Darmstadt, Germany). Gallic acid, Folin-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetonitrile were purchased from Sigma-Aldrich (Steinheim, Germany). 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and 2,2'-azinobis-3-ethylbenzo-thiazoline-6-sulphonic acid diammonium salt (ABTS) were obtained from Fluka (Buchs, Switzerland) and Applichem (Darmstadt, Germany), respectively. HPLC standards such as gallic acid, catechin, caffeic acid, hesperidin and betanin were obtained from Extrasynthese (Genay, France).

2.2. Methods

2.2.1. Preparation of Fruit Jams by Means of Impregnation

Fruits were washed with tap water to remove dirt from their peels. Red beet chopped into small pieces and persimmon was pureed with a hand blender for jam processing. 350 g red beet and 300 g persimmon were impregnated in 700 g (1:2 ratio, w/w) and 365 g (about 1:1 w/w) granulated sugar overnight, respectively, and boiled to about 88^{o}Bx which was checked with a pocket refractometer. Samples were cooled to room temperature and filled into a glass jar. Sampling was done into plastic bags and stored at -80^{o}C before extraction. Samples were ground to a fine powder in liquid nitrogen by using a precooled grinder (IKA-Werke GmbH & Co. KG, Staufen, Germany).

2.2.2. Moisture Analysis

To express the results in dry weight basis (DW), moisture content of samples was measured by using a vacuum oven method [18].

2.2.3. Extraction of Phenolics

One gram of each sample was weighed in test tubes under liquid nitrogen. 5 ml of 75% methanol: water (v/v) solution with 0.1% formic acid was added into each tube. Samples were sonicated for 15 min in an ultrasonic bath (Ultrasonic Cleaner-VWR, PA, USA) and centrifuged (Andreas Hettich GmbH&Co.KG, Tuttlingen, Germany) at 4000 rpm at 4^{o}C. This was repeated 4 times until 20 ml of solvent was used and extracts were stored at -20^{o}C until analysis [19].

2.2.4. Determination of Total Phenolic Content and Antioxidant Activity

TPC and AA analyses, by DPPH and ABTS radical

scavenging methods, were carried out by using UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). TPC was analysed by a modified Folin-Ciocalteu method [20]. According to the procedure, 100µl of extract or standard was mixed with 750 µl of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and then 750 µl sodium carbonate solution (60 g/l) was added. After incubating for 90 min, absorbance was measured at 725 nm. The calibration curve was prepared by using gallic acid as standard and the results were expressed in mg gallic acid equivalents (GAE) per 100 g of DW. AA analysis by DPPH radical scavenging method was performed [21]. 100 µl of extract or standard was added to 2 ml of DPPH in methanol solution (100 µM) in a test tube. After incubating for 30 minutes, the absorbance was measured at 517 nm. The calibration curve was prepared by using Trolox and the results were expressed in mg Trolox equivalents (TEAC) per 100 g of DW.

ABTS radical scavenging method was performed as described by Miller and Rice-Evans [22], 100 µl of extract or standard was added into 1 ml of prepared ABTS reagent mixture and mixed for 15 seconds. After waiting for 45 seconds, absorbance was measured at 734 nm. The calibration curve was prepared by using Trolox and the results were expressed in mg TEAC per 100 g of DW.

2.2.5. Phenolic Profile and Betalain Identification by HPLC

Major phenolic compounds and betalain were analyzed by using the HPLC-PDA method as described previously [19]. Standard calibration curves were prepared by using catechin, gallic acid, mallic acid, caffeic acid, hesperidin and betanin standards. Methanolic extracts were filtered through a 0.45-µm membrane filter and 1 ml of the filtered extract was placed into vials and analyzed in a Waters W600 HPLC system with PDA (Waters 996) detectors, for each sample. Luna C18 column (Phenomenex) was used as the stationary phase. The mobile phase was including solvent A, Milli-Q water with 0.1% (v/v) TFA and solvent B, acetonitrile with 0.1% (v/v) TFA. A linear gradient was used as follows: at 0 min, 95% solvent A and %5 solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min returns to initial conditions. The flow rate was 1 ml/min. Detections were done at 280, 312, 360 nm for phenolic compounds, and at 520 nm for betalain. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard curves. All analyses were performed in triplicate.

2.3. Statistical Analyses

All of the results were reported as mean value ± standard deviation. The results were analyzed statistically by using ANOVA and Duncan's New Multiple Range Test in SPSS 16.0 version at 0.05 significant level.

3. Results

Results of the spectrophometric assays were given in DW basis in Table 1. According to the results; TPC of the samples were changing between 276.70±0.08 and 1863.65±0.03 mg GA/100g DW. Among two fruits and their jams, fresh red beet showed the highest TPC (1863.65±0.03 mg GA/100g DW). Moreover, fresh red beet together with the red beet jam (929.40±0.03 mg GAE/100g DW) revealed significantly higher TPC ($p<0.05$) than fresh persimmon (428.62±0.12 mg GAE/100g DW) and persimmon jam (276.70±0.08 mg GAE/100g DW).

Among AA assays; results by DPPH radical were found to range between 61.12±0.02 and 217.60±0.09 mg TEAC/100g DW. Persimmon had the highest AA (217.60±0.09 mg TEAC/100g DW) among all samples followed by its jam (183.74± 0.05 mg TEAC/100g DW). Persimmon jam showed significantly higher ($p<0.05$) AA than red beet jam (61.12±0.02 mg TEAC/100g DW). Although fresh red beet possessed the highest TPC, it did not show the highest AA (108.30±0.03 mg TEAC/100g DW) according to DPPH radical scavenging method. ABTS radical scavenging activity results were found to be between 77.48±0.10 and 500.17±0.19 mg TEAC/100g DW. Fresh red beet was found to have the highest AA content (500.17±0.19 mg TEAC/100g DW) according to this method and therefore showed a similar trend with TPC results.

4. Discussion

TPC of all samples are given in Figure 1. Red beet had the highest level of phenolics. TPC decreased significantly (49.9%) when red beets were processed into red beet jam ($p<0.05$). In contrast, reduction in TPC (about 35.4%) when persimmon was processed into jam was found to be statistically insignificant ($p>0.05$).

Table 1. Total Phenolic Contents and Radical Scavenging Activities with DPPH and ABTS methods for fruits and jams

Sample	Total Phenolic Content (mg GA/ 100g DW)	DPPH Radical Scavenging Activity (mg TEAC /100g DW)	ABTS Radical Scavenging Activity (mg TEAC/ 100gDW)
Persimmon	428.62±0.12c	217.60±0.09a	364.85±0.14ab
Persimmon Jam	276.70±0.08c	183.74±0.05a	105.57±0.05ab
Red Beet	1863.65±0.03a	108.30±0.03b	500.17±0.19a
Red Beet Jam	929.40±0.03b	61.12±0.02c	77.48±0.10b

Previously other researchers have studied on the TPC of red beets and persimmon as fresh fruits or fruit juices. Fu et al. (2011) analyzed TPC of 62 fruits, including persimmon. They found persimmon as one of the fruits with strongest antioxidant activity among the tested fruits and determined its TPC as 112.09 ± 4.60 mg GAE/100 g FW [4]. In another study; analysis of TPC by the Folin-Ciocalteu method for 23 commercially vegetable juices such as carrot, tomato, beetroot, mixed vegetable, mixed fruit and vegetable juices

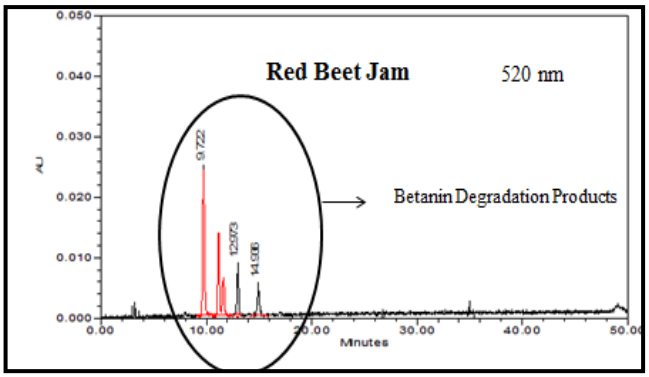

Figure 4b. Betalains of red beet jam

The outstanding component in red beet was betanin, which had high AA but low heat stability. In red beet other phytochemicals such as 4-hydroxy benzoic acid, cinnamic acid, vanillic, chlorogenic, trans-ferulic acid and caffeic acid were also determined by other researchers [29]. In red beet jam it was converted into other forms such as betanidin and its degradation products as shown in Fig. 4a and 4b**.** Herbach et al. (2004) studied thermal effect on red beet color and pigments. According to their study they also defined the major component as betanin [30]. After treatment at a high temperature (85 ˚C) for an hour, there were four degradation components (betanin, isobetanin, neobetanin, vulgaxanthin) as mentioned in our study.

5. Conclusions

In conclusion; two fruits in concern were affected differently from jam processing. This might be related with the different phytochemicals present in those fruits, their variable antioxidant activities and differences in those components' stability to heat and processing. Results revealed that persimmon may be a good raw material for jam processing with its potential and preserved phenolics and AA when compared with other jams available in the market. Sucrose impregnation application is conserving the phenolic contents and AA, so this application is recommended instead of boiling with sugar application that is traditionally applied during jam processing.

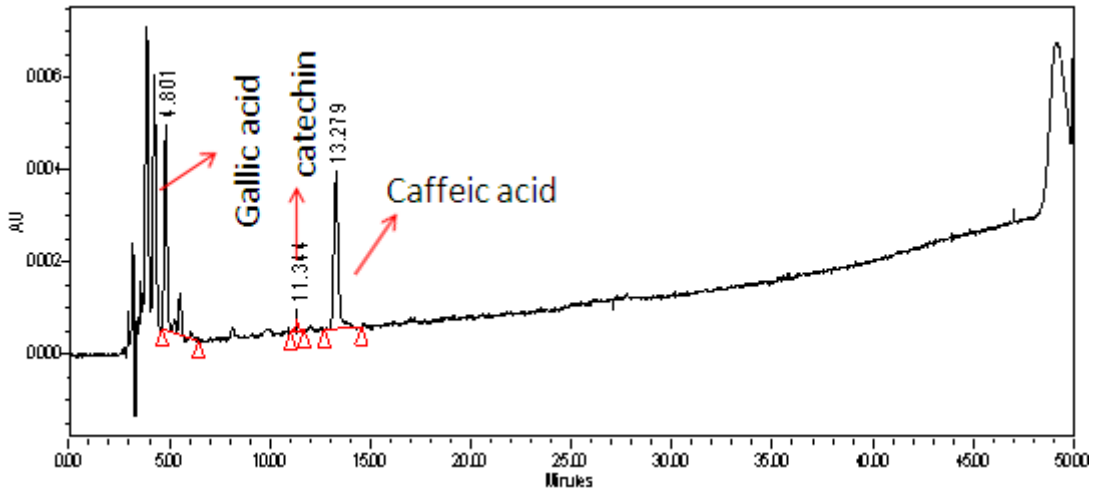

Figure 5a. Phenolic compounds of fresh persimmon

Figure 5b. Phenolic compounds of persimmon jam

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