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Total Phenolic Content, Antioxidant Activity, Some Physical and Chemical Properties of Pestil

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The objective of the present investigation is to determine total phenolic content, antioxidant activity, some physical and chemical properties of mulberry pestil (without additive, with glucose, with peanut, with walnut), plum pestil and cornelian cherry pestil. Total phenolic content of the water extracts of pestil samples was estimated as gallic acid equivalents (GAE) by Folin-Ciocalteu method spectrophotometrically. The antioxidant activity of water extracts prepared from pestil samples was measured by β-carotene bleaching method. Total phenolic content was found from 4.79 (mulberry pestil without additive) to 28.36 μg GAE/mg (plum pestil). The antioxidant activity of pestil samples varied from 40.05 % (mulberry pestil without additive) to 90.92 % (plum pestil). Dry matter, ash, protein, total sugar, reducing sugar, sucrose, pH, titratable acidity, colour (L, a, b), thickness of pestil samples were determined between 75.30-87.53 %, 0.18-3.42 %, 2.44-7.35 %, 30.91-54.60 %, 21.38-41.05 %, 0.83-27.54 %, 3.39-5.72 %, 0.06-6.25 % (malic acid); 27.35-41.35, (+1.25)-(+11.50), (+0.99)-(+18.22), 0.57-6.40 mm, respectively. The present study shows that pestil samples had high amount of phenolics content, antioxidant activity, carbohydrates and protein content.

Key Words: Antioxidant activity, Pestil, Mulberry, Plum, Cornelian cherry.

INTRODUCTION

Synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been popularly used in food and pharmaceutical formulations. It has been reported that these compounds have some side effects and are carcinogenic. Recently, natural antioxidants present in foods and other biological materials have attracted considerable interest due to their presumed safety and potential nutritional and therapeutic value and consumer concerns about the safety of synthetic antioxidants[1,2]. The increased interest in natural antioxidants has led to the antioxidant evaluation of many species of fruits, vegetables, herbs, spices and cereals. Special attention has been paid to fruits, as they are rich source of phenolic compounds[1].

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Fresh fruits and vegetables and their industrial by-products are rich in antioxidants such as vitamins C and E, carotenoids, tannins, lignans, catechin and polyphenols[3-6]. Polyphenols in fruits and vegetables include mainly phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid), flavonoids (flavonols, flavones, flavanones, isoflavones and anthocyanins)[3]. Antioxidants are substances that prevent or retard the oxidation of lipids, proteins and DNA and provide protection the compounds or tissues from damage caused by harmful free-radicals. Therefore, their health promoting effects reduce the risk of certain types of cancer, coronary heart disease (CHD), cardiovascular disease (CVD), stroke, atherosclerosis, aging, diabetes, obesity and other degenerative diseases associated with oxidative stress[3,5,7]. The antioxidant activity of these phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors. They can also play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Thus, natural antioxidants function as free-radical scavengers and chain breakers, complexing agents of prooxidant metal ions and quenchers of singlet-oxygen formation[5,8].

Pestil is a natural product of fruits. It is a well known and widely consumed traditional Turkish food. Fruits such as mulberry, grape, apricot, plum and cornelian cherry can be used in pestil production. The production steps of pestil differ according to the geographical regions and different ingredients like sugar, starch, or flour could be used in the production of pestil[9]. Pestil is prepared by removing moisture from a large flat-tray of wet puree until the desired cohesive 'leathery' composition is obtained. Pestil is light, pleasant to chew and for many fruits, quite tasty. Since it is light and low in moisture is stored for a long-term period and is economical to ship[10].

There is less of research about physical and chemical properties of mulberry, plum, apricot, grape pestil[9-13]. But, there is no information on the antioxidant activity and phenolic content in pestil. The aim of the present work is to determine the antioxidant activity, total phenolic content and some physical and chemical properties of six pestil samples commonly consumed and producted in Turkey.

EXPERIMENTAL

The commercial mulberry pestil (without additive, with glucose, with peanut, with walnut) plum pestil and cornelian cherry pestil samples were supplied from local supermarket and all reagents used were of analytical grade.

Preparation of pestil samples for antioxidant and phenolic analysis: The pestil samples were cut into small pieces before being dried in a hot air-blowing oven at 50 °C. All samples, after drying, had water contents below 10 %. They were ground to a fine powder in a mechanical blender and kept at room temperature prior to extraction. The dried pestil samples were used for the analysis of antioxidant activity and phenolic compounds. 10 mg ground sample was mixed with 10 mL water and stirred for 0.5 h on a magnetic stirrer. The suspension was filtered through

Whatman No. 1 filter paper. Final solutions were used as stock solution for the analysis of antioxidant activity and phenolic compounds.

Physical and chemical analyses: Total dry matter, protein, ash, pH and titratable acidity were determined according to standard AOAC method[14]; pH and titratable acidity were determined with a ATI ORION 420A model pH meter. Titratable acidity, expressed as percentage of malic acid, was determined with 0.1 N NaOH up to pH 8.1; protein content was determined by the Kjeldal method (N×6.25). Total sugar, reducing sugar and sucrose contents were analyzed by the Lane-Eynon method[15]. Reducing sugar concentration was measured before inversion whereas total sugar was determined after inversion. Sucrose was calculated by subtracting the reducing sugar concentration from the total invert sugar and multiplying the result by 0.95.

For colour analysis, the instrument was calibrated with a white reference before measurements. Colour of mulberry fruit was analyzed by measuring Hunter L (brightness; 100: white, 0: black), a (+: red; -: green) and b (+: yellow; -: blue) parameters with a colorimeter (Model CR 200, Chromometer, Minolta, Japan). Thickness of pestil was measured with a digital caliper compass (Mitutoyo, Absolute, CD-15CPX). Thickness of pestil was measured with a digital caliper compass (Mitutoyo, Absolute, CD-15CPX).

Determination of total phenolic content: The concentration of total phenolic compounds of pestil samples were determined by the Folin-Ciocalteau colorimetric method[16]. 1 mL of the solution (contains 1 mg sample) extract in water was pipetted into a flask. Then 46 mL of distilled water and 1 mL of Folin and Ciocalteu's reagent was added and mixed throughly. The mixture was left to stand for 3 min and then 3.0 mL of 2 % sodium carbonate were added. After 120 min incubation at ambient temperature with shaking, the resulting absorbance was measured at 760 nm. Measurements were carried out in dublicate, the calibration curve was performed with gallic acid and the results were expressed as μg of gallic acid equivalents per milligram of sample (μg GAE/ mg of sample).

Determination of antioxidant activity: The antioxidant activity of pestil water extracts was determined according to the β-carotene bleaching method described by Kaur and Kapoor[17] with some modifications. Briefly, 4 mL of β-carotene solution (0.1 mg in 1 mL chloroform), 40 mg of linoleic acid and 400 mg of Tween 40 were transferred to a round-bottom flask. The mixture was then evaporated at 50 °C by means of a rotary evaporator to remove chloroform. Then, 100 mL of oxygenated distilled water were added slowly to the residue and vigorously agitated to give a stable emulsion. Then, 800 μL of extracts were added to 3 mL aliquots of β-carotene/ linoleic acid emulsion. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The mixtures were incubated at 50 °C for 100 min. The measurement was carried out at 10 min intervals for 100 min. Water instead of pestil extract was used as control. A blank, devoid of β-carotene, was prepared for background subtraction. Butylated hydroxyanisole (BHA) was used as a standard. All samples were assayed in dublicate. Degradation

rate (DR) was calculated according to first order kinetics, using the following equation based on:

$$\ln(a/b)\times 1/t = DR_{sample} \text{ or } DR_{standard}$$

where, a is the initial absorbance (470 nm) at time 0, b is the absorbance (470 nm) at 100 min and t is time. Antioxidant activity (AA) was expressed as per cent of inhibition relative to the control, using the following formula:

$$AA = \left(\frac{DR_{control} - DR_{sample \text{ or } standard}}{DR_{control}} \right) \times 100$$

Statistical analysis: Analysis of variance was performed by ANOVA procedures (SPSS 9.0 for Windows). Significant differences between means were determined by Duncan's Multiple Range tests. P values < 0.05 were regarded as significant.

RESULTS AND DISCUSSION

Physical and chemical properties of pestil samples: The physical and chemical properties of pestil samples are given in Table-1. There were statistically differences among pestil samples in terms of total dry matter, ash, protein, total sugar, reducing sugar, sucrose, pH, titratable acidity, colour (L, a, b) and thickness of pestil samples ($p < 0.01$). There are variability in the contents of these analyzed physical and chemical properties between pestil samples. This might be explained by the difference in used fruit (mulberry, plum and cornelian cherry), additives (walnut, peanut and glucose) and process conditions. Mulberry pestil with glucose had the highest and plum pestil the lowest total dry matter content. The lowest sucrose (0.83 g/100 g) and total sugar content (30.91 g/100 g) was established in plum pestil. Reducing sugar content was determined the lowest amount in mulberry pestil with peanut. The highest protein content of samples was found in mulberry pestil with peanut (7.35 g/100 g). The ash content of cornelian cherry pestil (3.42 g/200 g) was higher than other pestil samples. In the studied pestil samples, pH and the titratable acidity varied from 3.39 (plum pestil) to 5.72 (mulberry pestil with peanut) and 0.07 % (mulberry pestil with walnut)-4.69 (plum pestil), respectively. The colour of the pestil samples were determined as L value 27.35 (plum pestil) and 41.35 (mulberry pestil without additive), a value 1.25 (plum pestil) and 11.50 (cornelian cherry pestil) and b values 0.99 (plum pestil) and 18.22 (mulberry pestil with glucose), respectively. The destruction of pigment components and occurence of Maillard reactions may ocur due to cooking pestil at high temperature. Thus, colour changes from cream, pink or purple to brown. The highest thickness was in the cornelian cherry pestil (6.40 mm), followed by the plum pestil (4.90 mm) and mulberry pestil with walnut (4.58 mm). Eksi and Artik[11] reported that mulberry pestil and plum pestil contained 14.3-19.5 % water, 85.7-80.5 % total dry matter, 83.4-79.0 % total sugar, 0.2-2.3 % total acidity, 2.0-2.0 % protein, 1.4-1.6 % ash, respectively. Thickness of this pestil samples was 0.5 mm (mulberry)-1.4 mm (plum). When present

results were compared with the results of above study on plum pestil and mulberry pestil[11], some differences were found in the contents of these analyzed compounds. These differences may be due to the different production of the pestil. Chemical and physical characteristics of pestil depend on the applied processes, ingredients which used in the production of pestil like sugar, starch or flour[9].

Total phenolics and antioxidant activity of pestil extracts: The total phenolics content was determined according to the colorimetric Folin-Ciocalteu method with gallic acid as a standard compound. Total phenolics content in studied pestil samples as shown in Table-2. One way ANOVA showed significant differences ($p < 0.01$) in total phenolics content among the six studied samples. Their total phenolics content ranged from 4.79 to 28.36 μgGAE/mg sample. Among the six pestil samples, plum demonstrated the highest phenolics content (28.36 μgGAE/mg sample), followed by mulberry with walnut (24.08 μgGAE/mg sample), cornelian cherry (20.15 μgGAE/mg sample), mulberry with peanut (18.36 μgGAE/mg sample), mulberry with glucose (11.57 μgGAE/mg sample) and mulberry without additive (4.79 μgGAE/mg sample). Plum fruit contain abundant amounts of natural phytonutrients such as flavonoids and phenolic acids, which may effective as natural antioxidants in our daily diet and have potential specific health benefits[18,19]. Phenolic compounds have antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[20]. Data on the total phenolics content and antioxidant activity of pestil are not in the literature.

The antioxidant activity of the extracts was determined based on the coupled oxidation of β-carotene and linoleic acid. Antioxidant activity is expressed as per cent inhibition relative to the control. In this assay, oxidation of linoleic acid produces hydroperoxide-derived free radicals which attack the chromophore of β-carotene, resulting in a bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant[21]. Table-2 illustrates the antioxidant activity of the extracts from pestil samples. This antioxidant activity is also compared to the BHA values in equivalent solutions. All extracts had an antioxidant activity. Antioxidant activity ranged from 40.05 to 90.92 %, with the following increasing order mulberry without additive (40.05 %) < with walnut (42.62 %) < with glucose (44.52 %) < with peanut (52.81 %) < cornelian cherry pestil (70.59 %) and plum pestil (90.92 %). Mulberry without additive had the lowest antioxidant activity which possessed the lowest phenolics content. It was demonstrated the higher antioxidant activity for the plum pestil. Wang *et al.*[22] showed that plum fruit had higher total antioxidant capacities than orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear and honeydew melon. Murcia *et al.*[23] reported that plum prove good scavenger activity against oxygen-derived free radicals such as hydroxyl and peroxyl radicals.

TABLE-1
PHYSICAL AND CHEMICAL PROPERTIES OF PESTIL SAMPLES*

Parameters	Pestil samples					
	Mulberry without additive	Mulberry with peanut	Mulberry with walnut	Mulberry with glucose	Plum pestil	Cornelian cherry pestil
Total dry matter g/100 g	86.91±0.17a	87.41±0.43a	85.57±0.07b	87.54±0.46a	75.30±0.15d	82.05±0.06c
Total sugar g/100 g	44.21±0.00c	36.58±0.00e	42.76±0.00d	54.59±0.00a	30.91±0.00f	45.59±0.00b
Sucrose g/100 g	3.16±0.00e	15.21±0.00c	16.90±0.00b	27.54±0.00a	0.83±0.00f	4.81±0.00d
Reducing sugar g/100 g	41.05±2.12a	21.38±0.71d	25.89±2.95c	27.06±0.55bc	30.07±0.00b	40.77±0.27a
Protein g/100 g	3.49±0.08d	7.35±0.00a	5.38±0.06c	6.43±0.18b	3.63±0.05a	2.44±0.18e
Ash g/100 g	0.18±0.03e	0.46±0.00d	0.30±0.07e	1.27±0.06c	2.86±0.02b	3.42±0.09a
pH	5.41± 0.04a	5.72±0.02a	5.59± 0.03a	3.73±0.21b	3.39±0.01b	3.66±0.35b
Titratable acidity g/100 g	0.41±0.01b	0.14±0.00c	0.07±0.00c	0.28±0.05b	4.69±0.40a	4.46±0.62a
L	41.35±0.40a	40.77±0.79a	39.37±4.29a	38.44±1.14a	27.35±0.38b	30.32±0.67b
a	7.61±0.52b	4.09±2.28c	7.63±0.05b	3.92±0.13c	1.25±0.05d	11.50±0.45a
b	15.56±1.85a	16.95±3.15a	16.34±2.09a	18.22±0.95a	0.99±0.06c	6.64±0.23b
Thickness	0.57±0.00d	3.41±0.72bc	4.58±1.31b	2.29±0.00c	4.90±0.00b	6.40±0.00a

*Means within the same line followed by same latter are not statistically significant ($p < 0.05$).

TABLE-2
TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF PESTIL SAMPLES*

Pestil samples	Total phenolic content, µg GAE/ mg of sample	Antioxidant activity, (%) of sample
Mulberry without additive	4.79±1.52d	40.05±4.28d
Mulberry with peanut	18.36±2.52b	52.81±6.39c
Mulberry with walnut,	24.08±4.55ab	42.62±4.89d
Mulberry with glucose	11.57±0.00c	44.52±2.47cd
Plum	28.36±3.54a	90.95±1.34a
Cornelian cherry	20.15±1.01b	70.59±1.99b
BHA(200 mg/L)	–	93.21±0.12a

*Means within the same lines followed by same latter are not statistically significant ($p < 0.05$).

In conclusion, large variability was found between all pestil samples in physical and chemical attributes, total phenolic content and antioxidant activity. These differences could be the results of used fruit (mulberry, plum and cornelian cherry), additives (walnut, peanut and glucose) and process conditions. The sucrose and total sugar were the lowest for plum pestil. The highest protein (7.35 g/100 g) were found for mulberry pestil with peanut. The results of this study show that the pestil samples have antioxidant activity and total phenolic content and can be considered as good sources of these compounds. Particularly, plum pestil (90.95 %) and cornelian cherry pestil (70.59 %) had a high antioxidant activity. Further studies will attempt to identify the individual phenolic compounds responsible for these properties.

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