P36: PEACH KERNEL: A POTENTIAL SOURCE FOR COSMECEUTICALS

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Abstract

Prunus persica (peach) is one of the most important deciduous fruit tree worldwide. The main waste of peach industrial processing is the kernel. In this study, kernels from four different peach cultivars from Imathia, Greece (kindly donated by the Members of Greek Canners Association) were examined, aiming to exploit kernels as high added value products. The total concentration of phenolic compounds, proteins and sugars was determined using the Folin-Ciocalteau, Bradford and DNS method, respectively. Fatty acids content was characterized by GC-MS analysis and the concentration of amygdalin, a cyanogenic glycoside which is toxic for humans, in samples was determined by LC-MS. The andioxidant activity of kernel extracts was estimated by $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays.

The results showed that peach kernels are rich in unsaturated fatty acids, mainly oleic and linoleic acid. Extracts from cultivar 1 presented the highest concentration of phenolic compounds (4.47 mg/g) and proteins (31.16 mg/g), while extracts from cultivar 2 presented the highest concentration of sugars. The highest concentration of amygdalin was detected in the methanolic extract of cultivar 4. The kernel extracts from all peach cultivars exhibited strong antioxidant activity, with cultivar 4 presenting the highest one. The results indicated that peach kernel is a promising source for unsaturated fatty acids and phenolic compounds possesing strong antioxidant activity and potential use as an active ingredient in cosmeceuticals and food supplements.

Keywords: *Prunus persica*, peach kernel, fatty acids, amygdalin

1. INTRODUCTION

Prunus persica is the third most cultivated deciduous tree worldwide [1]. Greece is the fourth largest peach producer in Europe and the eighth in the world. In Greece, peach is cultivated mainly in Macedonia and Thessalia, covering up to 80% of the domestic production.

A significant part of the harvested peaches used in industry after processing, results in a substantial amount of waste kernels, which leads to the necessity of its exploitation. Globally, several cosmetic products have been reported with peach kernel oil, such as soaps, shampoos, lotions, creams and massage oils. Peach kernel oil is used in cosmetic industry since it is a light, penetrating oil absorbed easily from the skin, offering hydration, without leaving a greasy feeling [1, 2]. Furthermore, peach kernel oil could be an appealing ingredient for the food industry due to its content in unsaturated fatty acids and antioxidant constituents. In Eastern countries, peach kernel oil is used as a cooking oil. Peach kernels are also used as biomass for energy production in Spain and Italy [3]. In Greece, kernels are partially used as biomass [4].

Previous studies of peach kernels from different origins (Spain, Canada, Brazil, Egypt, China), showed that they contain high concentration of fatty acids and phenolic compounds, exhibiting strong antioxidant activity [1]. The main fatty acids, which have been detected in peach kernels oil, were oleic and linoleic acid, which play an important role in the regulation of a variety of physical and biological functions [1, 5].

In this study, the content of peach kernels in phenolic compounds, proteins, sugars, free fatty acids and amygdalin was determined. Furthermore, the antioxidant activity of the sample was estimated, aiming to exploit kernels towards high added value products.

The amount of total phenolics can be determined by the Folin-Ciocalteau method. Phenols react with the Folin-Ciocalteau reagent to form a blue chromophore constituted by a complex which can be quantified by UV-Vis spectrometry [6]. The photometric method DNS was used for determining

the total concentration of sugars in samples. This method is based on the reductive properties of functional groups of sugars. The reduction of DNS gives a color to the mixture, and the reaction stops after adding water and sodium potassium tartrate [7]. Total concentration of proteins can be determined by the Bradford method, which is based on the change of the color of the colorant Coomassie Brilliant Blue G-250 when it creates complexes with proteins in acid solutions. This method is simple, quick and very sensitive [8].

There are many methods for the estimation of the antioxidant activity of samples, two of the most common are $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. Both of them are based on the capability of the samples to bind the free radicals of 1,1-diphenyl-2-picrylhydrazyl and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), respectively [9].

Amygdalin ($C_{20}H_{27}NO_{11}$) is a natural occurring sugar, which belong to the cyanoside glycosides family, because it has a characteristic cyanide group in its structure. It can be found in kernels of apricot, peach, nectarine, plums, cherries, bitter almonds, apples and pears [10]. Amygdalin can be metabolized to HCN in human body and it has been found that 1 g of amygdalin can produce 59.1 mg HCN. Hydrogen cyanide is extremely poisonous, because it binds irreversibly to the iron atom in hemoglobin, making it unavailable to transport the vital oxygen to the body's cells and tissues. There is a risk assessment of Hellenic Food Authority about the evaluation of the dangerous concentration of CN ions in bitter almonds and apricots and recommendations for consuming them by kids and adults [10]. Therefore, in the present study, amygdalin was quantified in samples of peach kernels.

2. EXPERIMENTAL

Four different peach kernel cultivars were tested in this study. Initially kernels were dried in oven at 30°C for 5 days in order to remove moisture. Dry kernels were extracted with different solvents, in ultrasound bath for 3 h, followed by centrifugation.

Phenolic compounds were extracted with a mixture of methanol: water (4:1) in an ultrasound bath for 3 h followed by centrifugation for 5 min in 4500 rpm. The supernatant was collected and the concentration of phenolic compounds was determined by the Folin-Ciocalteau method. Specifically, 0.05 mL of extract was mixed with 2.75 mL distilled water, 2 mL Na_2CO_3 (7% w/v), and 0.2 mL Folin-Ciocalteau reagent [8]. The samples were kept for 90 min, in dark. Following that, the absorbance was measured at 745 nm, in UV-Vis spectrophotometer (UV-1600 PC Spectrophotometer, VWR, USA). The total phenolic concentration was determined by using the calibration curve of gallic acid.

In addition, the extracts were studied with HPLC-DAD. Prior to chromatographic analysis, samples were filtered, in order to remove solids. The analysis carried out at a GraceSmart RP 18 (250 mm, ID 4.6mm, OD 5/16'', 5 μm) column (Grace Davison Discovery Sciences, USA). Injection volume was 20 μL and the flow rate at 1 mL/min. The elution system was (A) aqueous acetic acid 1% (v/v) and (B) acetonitrile and the elution gradient was t=0 min (A)=100%, t=4 min (A)=85% and (B)=15%, t=20 min (A)=60% and (B)=40%, t=40 min (A)=45% and (B)=55%. Detector set at 280 and 360 nm.

The total concentration of sugars was determined with the DNS method. Extracts were mixed with DNS and distilled water, followed by heating at 100 °C for 5 min, in order to react. Then, samples were left to cool at 25 °C and added a solution of sodium potassium tartrate (40% w/v). The absorbance was measured at 575 nm. The total concentration was determined by using the calibration curve of glucose.

The total concentration of proteins was estimated using the Bradford method. Proteins were extracted with water (1:2 w/v) in ultrasound bath for 3 h followed by centrifugation for 5 min in 4500 rpm. 1 mL of the extract was mixed with 1 mL of the Bradford reagent. After 5 min, the absorption was measured at 595 nm. The total phenolic concentration was determined by using calibration curve of albumin.

Fatty acids were extracted with hexane (1:3 w/v) for 3 h in an ultrasound bath followed by centrifugation at 4500 rpm for 5 min. The supernatant was collected and further diluted with hexane (1:10 v/v) and mixed with vortex (MS2 Minishaker, IKA, Germany) for 3 min. Chromatographic analysis was carried out in an Agilent Technologies 7890A GC system equipped with mass spectrometer (Agilent Technologies 5975C inertXL EI/CI MSD with triple-Axis Detector) on an HP-5MS (30 m × 250 μm) fused-silica capillary column (Agilent, CA, USA). The oven temperature was increased from 120 to 325 °C in a total run time of 31 min. The injector was set at splitless mode, at 200 °C.

The detection and quantification of amygdalin in samples was performed with LC-MS (LTQ-Orbitrap Discovery, Thermo Fisher Scientific, USA). Chromatographic separation was carried out on a Acquity UPLC HSS T3 1.8 µm, 2.1x100 mm column protected by aa pre-column Acquity UPLC HSS T3 1.8 µm, 2.1x5 mm (Advanced Chromatography Technologies, Aberdeen, UK). The elution gradient was: A [MeOH with 0.1% formic acid] and B [H_2O with 0.1% formic acid]; t=0 min A 15%, t=1 min A 15%, t=6 min A 40%, t=8 min A 55% and t=10 min MeOH 70%. The injection volume was 5 µL, the system operated at a flow rate of 500 µL/min and temperature of 40 °C. All measurements took place in positive ionization mode.

Antioxidants were extracted from samples with a mixture of methanol: water 4:1 in an ultrasound bath for 3 h followed by centrifugation at 4500 rpm for 5 min. The antioxidant activity of kernels was estimated using both the DPPH and ABTS assays. For the DPPH assay a mixture of DPPH – ethanol was prepared and then 1.9 mL of the solution was mixed with 0.1 mL of extract. The final mixture was measured by UV-Vis spectrophotometer after 5 min. Absorbance was measured at several time intervals.

For the ABTS method a mixture of ABTS- potassium persulfate was prepared and then was diluted with methanol, in order to have absorption equal to 1 at 734 nm. 3 mL of the diluted solution was mixed with 30 µL of kernel extract left to remain at room temperature for 6 min, and was measured in UV-Vis spectrophotometer. The concentration of the free radical was estimated using the calibration curve of trolox.

3. RESULTS AND DISCUSSION

The total concentration of phenolic compounds in samples was calculated by the calibration curve of gallic acid (Eq. 1).

$$Abs(745\,nm) = 82.9 * C_{sample} + 0.014 \quad (1)$$

The total concentration of phenolic compounds, in all four samples, was estimated to be around 3.4-4.5 mg/g_{kernel}.

Sugars in samples were quantified by equation 2.

$$Abs(575\,nm) - 3.757 * C_{sample} - 0.151 \quad (2)$$

The total amount of sugars in peach kernels ranged between 100-135 mg/g_{sample}.

Furthermore, the concentration of proteins was found to be at a range of 25-30 mg/g_{sample}. The estimation of total protein amount was calculated based on the calibration curve of (Eq. 3)

$$Abs(595\,nm) = 0.286 * C_{sample} + 0.104 \quad (3)$$

The determination of free fatty acids of the 4 different samples using gas chromatography showed that all samples were rich in fatty acids. Specifically, palmitic, oleic, linoleic and stearic acid were detected in all sample cultivars. Some other constituents, such as an aldehyde and a methylester, were also detected. Oleic and linoleic acid were co-eluted and their peak area percentage in all samples was at a range of 85-87%. Furthermore, the percentage of palmitic acid ranged between 6-8%, while the percentage of stearic acid between 3-5%.

The antioxidant activity of kernels was estimated with both DPPH and ABTS methods. In both assays, the samples showed strong ability to inhibit both free radicals tested. The estimation of the amount of the antioxidant activity was performed by the calibration curve of trolox for both DPPH and ABTS methods (Eq. 4 and Eq. 5, respectively)

$$Abs(517\,nm) = -17.18 * C_{sample} + 1.01 \quad (4)$$

As shown, after 24 h, 65-82% of the free radicals were inhibited, which indicate a strong antioxidant ability of the tested samples.

$$Abs(734\,nm) = -0.001 * C_{sample} + 0.947 \quad (5)$$

The results from the ABTS assay, showed that after 40 min, 94% of the free radicals were scavenged.

Finally, amygdalin was determined using LC-MS analysis. In all samples the amount of amygdalin was ranged between 5.1-18 µg/g_{sample}.

4. CONCLUSIONS

In this study, the content and the antioxidant activity of kernels of four different peach cultivars from Imathia were tested. The content of phenolic compounds and sugars in the peach kernels of the four different cultivars tested, was low in comparison with other byproducts of natural products. In these samples high concentration of proteins was measured compared with other natural products.

In peach kernels oleic and linoleic acid were determined, which have been also found in apricots' kernel.

Strong antioxidant activity was exhibited in all samples, by all assay tested.

Finally, the concentration of amygdalin was very low in all samples, which is an indication of low toxicity.

In conclusion, the combination of high concentration in free fatty acids, low toxicity and strong antioxidant activity, makes them a potent candidate as a cosmetic ingredient and food supplement.

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