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Evaluation of the total phenolic content and free radical scavenging capacity of almonds grown in Canakkale, Turkey

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ABSTRACT

Nuts comprise an important part of human diet due to their nutritional components in terms of lipids, carbohydrates, vitamins, minerals and bioactive compounds such as phenolics and flavonoids. Thus, nuts are widely grown throughout the world since they are mainly preferred for their nutritional, sensory and functional effects. Almond (*Prunus dulcis*) is naturally widely distributed throughout the world and has the highest production rate among nuts. A number of studies have shown that different almond cultivars exhibit different specific properties in terms of bioactive composition and antioxidant capacity depending on the climatic and regional conditions. Therefore, in the present study, the hexane and methanol extracts of Turkish almond cultivars Ak and Nurlu cultivated in Canakkale province, Turkey were investigated for their functional characteristics in terms of bioactive components and free radical scavenging capacity. The hexane extracts of both almond cultivars had higher total phenolic and flavonoid content when compared with the methanol extracts. On the other hand, the methanol extract of Nurlu almond was higher in anthocyanins than the Ak sample. Furthermore, the methanol extract of Nurlu almond exhibited better free radical scavenging capacity.

Keywords: Almond, Phenolics, Flavonoids, Anthocyanins, Free Radical Scavenging.

MATERIALS AND METHODS

Preparation of the almond extracts

The almonds were separated from the shells, without removing the brown skin. Then, the almond kernels were ground with a laboratory grinder until uniform flour was obtained. A sample (20 g) was weighed and extracted with hexane for 6 hours by using Soxhlet extraction device. From the oil-free sample obtained after hexane extraction, 5 g of sample were used for the extraction with methanol. The methanol extracts were obtained after 6 hours of extraction at the Soxhlet device. At the end of each extraction, the solvent (hexane or methanol) from the extracts was separated via rotary evaporator and the extract yields were recorded. The extraction procedure was repeated twice. All extracts were kept at 4oC until analysis.

Total phenolic content determination

The amount of total phenolics in the extracts of the almond samples was measured using the Folin-Ciocalteu reagent method of Djeridane et al. (2006). An appropriately diluted extract was taken in a test tube. 0.5 ml distilled water and 0.5 ml Folin–Ciocalteu reagent was added and the tubes were shaken thoroughly. After 1 min, 0.8 ml of sodium carbonate solution (7.5%) was added and the mixture was left for 30 min with intermittent shaking. The absorbance at 760 nm was measured by using a UV–VIS spectrophotometer (Thermo Aquamate). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram fresh almond sample.

Total flavonoids content determination

The total flavonoid content was evaluated according to Quettier-Deleu et al. (2000) using rutin as a standard. The total flavonoid content was expressed as rutin equivalents in milligram per gram fresh almond sample. Three replicates were used for the determination of the total flavonoids of the samples.

Total anthocyanin content determination

The total anthocyanin content of the methanol almond extracts was determined according to Gould et al. (2000). The total anthocyanin content was estimated as cyanidin-3-glucoside equivalents in milligram per gram fresh almond sample.

DPPH free radical-scavenging capacity determination

The effect of the oxidized extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated as described by Brand-Williams et al. (1995). Each extract was diluted appropriately. The DPPH solution was added to the diluted sample, thoroughly mixed, and left for 30 min at room temperature. After that, the absorbance was measured at 515 nm using a UV–VIS spectrophotometer (Thermo Aquamate). The absorbance of DPPH solution in methanol, without any antioxidant (control), was also measured. The percentage of DPPH radical scavenging activity was calculated by using the following equation: DPPH scavenging (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100$, where A_{sample} is the absorbance of the sample after the time necessary to reach the plateau (30 min) and $A_{control}$ is the absorbance of DPPH. Extract concentrations providing IC_{50} inhibition values (defined as the concentration of the compounds that was able to inhibit 50% of the total DPPH radicals) were calculated from graph plotting using nonlinear regression and expressed in microgram material equivalents per milliliter for sample extracts. Butylated hydroxytoluene (BHT) was used as positive control. Lower values of IC_{50} indicate higher antioxidant activity and vice versa.

Statistical analysis

The results were reported as mean ± standard deviation (SD). Oneway analysis of variance (ANOVA) was applied to determine the differences among means by using Statghaphics Centurion XV software. The values were considered to be significantly different at $P < 0.05$. The t-test was used to evaluate the differences between the anthocyanin content of the methanol extracts of the almond samples.

The bioactive component content and radical scavenging capacity of almond cultivars grown in Canakkale, Turkey.*

	BHT	Nurlu Almond**		Ak Almond	
		Hexane Extr.	Methanol Extr.	Hexane Extr.	Methanol Extr.
Total Phenolics (mg GAE / g fresh sample)		1.130±0.002b	1.762±0.006c	1.934±0.008d	0.445±0.001a
Total Flavonoids (mg rutin / g fresh sample)		6.612±0.080c	5.166±0.032a	5.946±0.052b	5.159±0.041a
Total Anthocyanins (mg cyanidin-3-glucoside / g fresh sample)			0.220±0.008b		0.194±0.010a
IC_{50} (µg/ml)	1.350a	8.460±0.063c	8.128±0.112b	12.215±0.235d	13.490±0.078e

*The values are given as mean ± standard deviation.

**Means with different letters within a row are significantly different at $P < 0.05$.

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