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# Antioxidant activity and properties of outer shell pistachios in different temperature of cooking

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# **ABSTRACT**

The method of cooking causes important changes in quality and safety of food. The purpose of this study was to survey the relation between some nutritional-qualitative factors of outer shell pistachios in different temperature of cooking. The outer shell pistachios were heated at 50°, 75°, and 100° C. The total carotenoids, flavonoids, chlorophylls Vitamin E and antioxidants capacity were measured. There were significant changes in total antioxidant in high temperature. This rate has increased at low temperatures (50° and 75° C). The carotenoids content also reduced with enhancing temperature. This study showed that significant differences may exist in some nutritional-qualitative factors such as vitamin E and carotenoids, and flavonoids levels between different temperatures of cooking. Enhancing antioxidant activity was shown with increasing temperature in our findings.

Keywords: vitamin E, carotenoids, flavonoids, total antioxidant, processing temperature

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# INTRODUCTION

The prevalence and incidence of diseases is expanding [1-7]. Therefore, methods of treatment using a functional and effective strategy. Pistachio nut (Pistacia vera L.) is one of the popular tree nuts. Several species of the genus Pistachio are referred to as pistachio, but only the fruits of P. vera attain a large enough size to be acceptable to consumers as edible nuts (8). Pistachio nut is grown mainly in Iran, USA, Syria, Turkey, Greece and Italy (9). Based on FAO statistics, Iran is the biggest producer of this commodity all around the world. Pistachios are a plentiful source of antioxidants, which fight damaging free radicals and may lower the risk for diseases associated with premature aging. Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as free radical scavengers. The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Examples of dietary antioxidants include betacarotene, lycopene, and vitamins A, C, and E (alpha-tocopherol) that found in plant sources. Today, interests in the application of these natural antioxidants, due to toxicological effects of synthetic antioxidants and consumer trend to natural products, have increased. The outer shell pistachio consumption is very high by some people. This product could be consumed in jams. The objective of this investigation was to study the antioxidant activity and properties of outer shell pistachio in different temperature of cooking.

#### MATERIALS AND METHODS

#### 2.1. Raw Material

The outer shell pistachios were collected. We used three temperatures for cooking. The outer shell pistachios was placed in water and boiled at 100C, 75, and 50 for 15 min. The shells were dried, and then turned into powder. The powers extracted with 100 mL of ethanol-water mixture at 70:30 (v/v). The mixture was stirred continuously for 24 h at 4 °C.

# 2.2. Determination of chlorophylls

The levels of chlorophylls were determined according to previous method (10). The extract absorbance was read on UV-260 spectrophotometer at 663 nm and 645 nm. The amount of Chlorophyll a and Chlorophyll b was calculated according to following formulas:

Chlorophyll a =  $(19.3 \times A663 - 0.86 \times A645) \text{ V}/100\text{W}$ Chlorophyll b =  $(19.3 \times A645 - 3.6 \times A663) \text{ V}/100\text{W}$ 

#### 2.3. Determination of carotenoids

The extract absorbance was read on spectrophotometer at 470 nm. Its carotenoids content were calculated on the basis of the standard curve of B carotene (11).

#### 2.4. Determination of vitamin E

Vitamin E content was measured according to a published method. Samples were exposed to Fe<sup>3</sup> solution, TPTZ and acetate buffer (pH 4). Then, the standard curve was prepared with appropriate vitamin E concentrations. The absorbance of samples was read at 595 nm wavelength (12).

# 2.5. Determination of total flavonoid compound

Total flavonoid content was assayed according to previous methods (13). Diluted extracts were mixed with reagent; ALCl3.6H2O 2% in methanol flavonoids could make complex with trivalent aluminum ion. Then, the samples were incubated in room condition for 10 minute. The absorbance of the samples was measured at 430 nm.

# 2.6. Determination of cupric ion reducing assay (cupric)

The cupric ion reducing capacity assay measures the cupric reducing capacity. The samples were mixed with solutions of CuCl<sub>2</sub>, neocuproine reagent in ammonium acetate buffer. The resulting absorbance at 450 nm is recorded either directly after incubation at 50 degrees C for 20 min (14).

# 2.7. Radical DPPH (1, 1- diphenyl 2-picrylhyorazyl) Scavenging Activity

The free radical scavenging activity of the extract based on the scavenging activity of DPPH (Upadhyay et al, 2014). A dose of 3.8 cc ethanol solution of DPPH (final concentration was 0.1 mM) and 0.2 cc extract (1% extract) were mixed (A sample). The respective extraction solvent was used as negative control (A control). The samples were shaken for 1 min and kept at room temperature in the dark for 30 min. Then, absorbance of them was read at 517 nm against ethanol blank. The percent of DPPH discolouration of the samples was calculated according to following formulas:

% discolouration =  $[1 - (Asample/Acontrol)] \times 100$ 

# **RESULTS**

The results are shown in Tables 1 and 2. The data values were expressed as mean  $\pm SD$ . The level of total Carotenoids and Chlorophyll a and b were measured (Table 1). The concentration of carotenoid pigments in the extracts was calculated using the standard curve obtained by a commercial  $\beta$ -carotene reagent. The formula used for the calculation was as follows:

y = 6.6201x - 0.0099;  $R^2 = 0.99$ 

In this study considerable content of total flavonoids was observed (Table 1).

Table 1. Level of total carotenoids, total flavonoids, Chlorophylls and Vitamin

	Chlorophylls(mg/g)		Vitamin E	Total flavonoids	Total carotenoids (mg/g)
	Chlorophylla	Chlorophyllb	(mg/g)	(mg/g)	Total carotellolds (flig/g)
Samples without any heating process	$0.45 \pm 0.005$	$0.35 \pm 0.001$	$0.48 \pm 1.15$	$26.9\pm 2.4$	$0.17 \pm 0.05$
Samples heated at (50°C)	$0.23 \pm 0.005$	$0.14\pm0.005$	$0.42\pm 3.4$	$18.5 \pm 0.27$	$0.11\pm0.01$
Samples heated at (75°C)	$0.13 \pm 0.005$	$0.13 \pm 0.001$	$0.42\pm 1.7$	$18.9 \pm 0.35$	$0.13\pm0.01$
Samples heated at (100°C)	$0.13 \pm 0.001$	$0.09 \pm 0.005$	$0.37\pm 3.1$	$17.16 \pm 0.16$	$0.11\pm0.04$

The levels of flavonoids contents were significantly different between groups. The result of vitamin E was significantly different between all groups except group 50° C comparing group 75° C. The changes in cholorophyllas were observed between control and 50°, 75°, and 100° C (P-value<0.05). Also there was significant different between groups in carotenoids content.

Table 2. Level of antioxidant activity

	DPPH	cupric assay
	% IP	(nm)
Samples without any heating process	$65.5 \pm 9.8$	$2.16 \pm 0.16$
Samples heated at (50°C)	$74 \pm 5.1$	$2.4\pm0.14$
Samples heated at (75°C)	$68 \pm 13.8$	$2.32\pm0.23$
Samples heated at (100°C)	61± 7.5	$2.4\pm0.09$

In our finding, total antioxidant capacity was investigated by two different methods: DPPH and cupric ion reducing capacity assay (Cupric assay). In DPPH assay, antioxidant activity as percentage of inhibition (%IP) was calculated (Table 2).

In Cupric assay the p value between control group with group heated at 100 was considerably significant (p=0.005). DPPH different also between the control and high group (P-value = 0.04).

#### DISCUSSION

In this study the total carotenoids, flavonoids, Chlorophylls, Vitamin E and antioxidant capacity of outer shell pistachios in three temperatures were measured and compared.

In present study, the amount of total flavonoids was less in cooked samples (50, 75, 100°C) in comparison with those heating without process. It has been demonstrated that flavonoids have C–glycoside bonds. Some processing such as heating or boiling hydrolyzes C–glycosides bonds. Therefore the level of flavonoids will be decreased (Sharma et al, 2015).

Our findings have demonstrated that the changes of carotenoids were not significant in different temperature processing, but these values was different with samples without any heating process. Because of their oxidation sensitivity, carotenoids must be protected from light, heat, and oxygen. In this study, carotenoids levels were significantly increased in heating (P<0.05) (Table 1).

The vitamin E level alters at  $100^{\circ}$ C. The results suggested that changes of vitamin E level depended to processing temperature. The amount of vitamin E will be destroying at high temperature. The degradation of vitamin E is low at  $50, 75^{\circ}$ C.

The total antioxidant activity was assessed with both DPPH and cupric assay. Significant DPPH activity was at 50  $^{\circ}$ C group. The DPPH radical scavenging increased with increasing temperature up to 50  $^{\circ}$ C and then decreased with further temperature.

Cupric assay is widely used to assay the activity of antioxidant agents. The results obtained from cupric assay suggest that 50  $^{\circ}$ C group possess high antioxidant activity. These results also confirmed the antioxidant capacity power of 50  $^{\circ}$ C. These results suggest that temperature more than 50  $^{\circ}$ C may destruct antioxidant components. The high antioxidant power in 50  $^{\circ}$ C may depend to the formation new components result of maillard reaction that has antioxidant activity (15).

The result of present study showed that the temperature processing could have an impact on health bioactive properties of outer shell pistachios. The increasing of temperature has a negative influence on the total flavonoids, carotenoids, chlorophylls, vitamin E and has a positive influence on antioxidant activity.

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