

## DETERMINATION OF THE CHLOROPHYLL CONTENT OF PISTACHIO (*Pistacia vera* L.) KERNELS USING VARIOUS EXTRACTION TECHNIQUES

### ODREĐIVANJE SADRŽAJA HLOROFILA U JEZGRU PISTAČIJA (PISTACIA VERA L.) KORIŠĆENJEM RAZLIČITIH TEHNIKA EKSTRAKCIJE

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#### ABSTRACT

The pistachio (*Pistacia vera* L.), a small nut tree belonging to the Anacardiaceae family, is extensively grown in Turkey, which is one of the leading world producers of pistachios. Kernel color is an important parameter for the evaluation of raw pistachios containing chlorophylls a and b as the major pigments. Moreover, kernel color is one of the most important quality attributes evaluated by consumers, producers and distributors. Chlorophylls a and b are the predominant pigments responsible for the green color of pistachios. Pigment concentrations are influenced by a number of factors such as geographic origin, ripeness degree, storage conditions and processing methods. Chlorophylls are sensitive to heat and can be degraded into pheophytins and pyropheophytins. Therefore, the purpose of this paper is to determine the chlorophyll content of the 'Uzun' pistachio cultivar (*Pistacia vera* L.) using the spectrophotometric methods and different solvents.

**Keywords:** pistachio, chlorophyll a, chlorophyll b, spectrophotometry

#### REZIME

Koštičavo voće *Pistacia vera* L. član je porodice Anacardiaceae, uzgaja u Turskoj, koja je jedan od vodećih svetskih proizvođača. Boja jezgre je važan parametar za procenu pistačija, a ona je definisana prisustvom hlorofila a i hlorofila b, kao najvažnijim pigmentima u sirovim pistačijama. Pored toga, boja je jedan od najvažnijih atributa kvaliteta koji ocenjuju potrošači, proizvođači i distributeri. Hlorofil a i b su pretežni pigmenti odgovorni za zelenu boju pistačija. Na koncentraciju pigmenta utiče niz faktora, uključujući geografsko poreklo, stepen zrelosti, uslovi skladištenja i metode prerade. Hlorofili su toplotno labilni i mogu se razgraditi da bi formirali feofite i pirofeofite. Zbog toga, cilj je bio da se utvrdi sadržaj hlorofila u pistačiju sorte Uzun (*Pistacia vera* L.) korišćenjem različitih rastvarača za spektrofotometrijske metode u ovoj studiji.

Prema dobijenim rezultatima, vrednosti hlorofila u različitim korišćenim rastvaračima imali su različite rezultate. Najveća vrednost postignuta rastvorom „acetona sa 1% HCl“ korišćenim za hlorofil a i hlorofil b, a za uzorke brašno jezgra i ulja od uzoraka pistačija. Sa druge strane, najviša vrednost hlorofila a i b dobijne su kada je brašno jezgre korišćeno kao ekstrakcioni materijal.

**Ključne reči:** pistači, hlorofil a, hlorofil b, spektrofotometrija.

#### INTRODUCTION

The pistachio (*Pistacia vera* L.) is one of the most popular nut crops worldwide. It is extensively cultivated in arid regions of the world owing to its ability to sustain hot and dry weather. Pistachios contain significant compounds beneficial to human health, which foster their global production (Ukhanova, et al., 2014). Iran, Turkey, USA, Syria, Italy, Tunisia and Greece are the main producers of pistachios in the world, growing various pistachio cultivars adapted to different environment conditions. Pistachio nuts have green or yellow kernels, characterized by the alternate bearing phenomena (Monastra et al., 1987), which ultimately determine their taste. Owing to its appreciable qualities such as shape, size, aroma and green color in particular (especially in hulled and peel-backed products), the pistachio is used as an ingredient in pastries, hams, salamis, ice creams, sweets and chocolates (Angelini, 1987). From a nutritional perspective, the energy value of pistachios is similar to almonds (2332 kJ/100 g). They are also rich in carbohydrates and minerals, mainly potassium (1025 mg/100 g) (USDA SR 18, 2005). The fat content of pistachios is about 50–70 % of the total nut weight relative to the variety. Carbohydrate formation occurs in chlorophyll facilitated by light energy in photosynthetic organisms. Chlorophylls play a vital factor in photosynthesis. The most active pigments involved in photosynthesis are chlorophylls, i.e. the green pigments of plants. Plants can be stressed by negative changes in chlorophyll contents. Therefore,

the chlorophyll content of the plant is very important for the accumulation of carbohydrates (Genty et al., 1989). Genotype, environmental conditions, early harvest date and selection of rootstocks have significant effects on kernel color (Kunter et al., 1995). Chlorophylls a and b are found in higher plants. Chlorophyll a has a blue-green color, whereas chlorophyll b features a yellow-green color. In general, chlorophylls a and b are present in equal proportions, but the a/b ratio can vary and is affected by sunlight. During maturation, the color of pistachio kernels turns from green to yellowish green (Anderson, 1986). However, there are many studies on the nutrient content of pistachios. For instance, Lutovska et al. (2014) researched the nutritive values of pistachios, almonds, soybeans and cereals. However, there are no studies on the variation in or the comparison between kernel and kernel oil chlorophyll contents of pistachio cultivars. The purpose of this study is to determine variations in the kernel chlorophyll content of the 'Uzun' pistachio cultivar using different extraction solvents.

#### MATERIAL AND METHOD

##### Material

Oil and kernel samples of the 'Uzun' pistachio cultivar were used as experimental materials in the present study. The 'Uzun' pistachio cultivar is a mid-season cultivar yielding pistachio fruits of medium quality. However, this cultivar produces higher yields than the 'Halebi' and "Kırmızı" cultivars. Notwithstanding its small size, 'Uzun' is globally known for its

taste. It matures earlier than other pistachio cultivars and produces higher yields. It is widely used in the food industry and is the most common cultivar in Turkey.

Chlorophyll analyses were performed using five different solvents: 90 % acetone, 90 % acetone with 1 % HCl, 100 % methanol, methanol with 1% HCl and 2:1 chloroform:methanol (v:v). The 'Uzun' cultivar kernels were randomly selected (a total of 100 nuts) and used for chlorophyll analysis. In addition, 100 g of randomly selected kernels were used in triplicate per sample for chlorophyll analysis with each solvent. The testa of the sample kernels was removed before they were ground by mortar and pestle. The powder thus obtained was used for oil extraction.

#### Methods

##### Oil Extraction

Dried and powdered pistachio samples were extracted for 1.5 h using the automatic Soxhlet equipment (Gerhardt Soxtherm) and hexane as a solvent. Triplicate analyses were performed for oil extraction. The residue was dried until the constant weight was obtained. Boron trifluoride/methanol was used for the preparation of fatty acid methyl esters (FAMES) (AOAC, 1990). The chlorophyll content of the 'Uzun' pistachio cultivar was measured using the spectrophotometric methods at wavelengths of 645 and 663 nm.

The chlorophyll a and b content calculations were done as follows:

$$\text{Chlorophyll a} = (12.7 \times 663 \text{ nm}) - (2.69 \times 645 \text{ nm})$$

$$\text{Chlorophyll b} = (22.9 \times 645 \text{ nm}) - (4.68 \times 663 \text{ nm}).$$

## RESULTS AND DISCUSSION

Chlorophylls are the essential photosynthetic pigments present in green plants and algae, enabling them to convert sunlight into organic compounds in the process of photosynthesis. Structurally, chlorophylls contain a central magnesium element and a long hydrocarbon (phytol) "tail". Chlorophyll is not a single molecule, but a family of related molecules designated chlorophylls a, b, c, and d. In most cases, acetone and methanol (each with varying percentages of water) are used cold or boiling for chlorophyll extraction. Extraction times vary from 10 minutes to 24 hours. The filters are sometimes ground and homogenized for extraction. After extraction, the extract is clarified by centrifugation. In photosynthesis, a blue/green substance called chlorophyll a and a yellow/green substance called chlorophyll b use light energy (normally sunlight but sometimes artificial light) to convert carbon dioxide and water into carbohydrates and oxygen in the green parts of the plant. Chlorophyll a is the primary photosynthetic pigment and chlorophyll b is the accessory pigment that collects energy and passes it onto chlorophyll a. Chlorophyll a absorbs energy from wavelengths of blue-violet and orange-red light, whereas chlorophyll b absorbs energy from wavelengths of green light. In green fruits, chlorophylls a and b are the main chloroplastic pigments, accounting for 21-47 % of the total pigment content of pistachios. The chlorophyll derivatives are colored intermediate compounds, which participate in the catabolic pathway of chlorophyll during the ripening and senescence of the green tissues (Rabadan et al., 2018). The extraction of the pistachio photosynthetic pigments was performed using a filter and a suitable volume of solvent. The chlorophylls a and b content of the 'Uzun' pistachio cultivar was determined using different extraction solvents and different samples (such as kernel powder and oils). The results obtained are presented in Table 1. The chlorophyll a content of the 'Uzun' pistachio cultivar ranged from 1.16 mg/l to 10.07 mg/l (Table 1).

Similar results were previously reported by Agar et al., (1994). The authors reported a chlorophyll a range of 0.55-5.81 mg/l. 'Momtaz' (2.89 mg/l) and 'Ravar No 2' (2.81 mg/l) are the only Iranian pistachio cultivars with chlorophyll a contents approximately as high as that of the Italian cultivar 'Ghiandolara' (2.89 mg/l). All the other Iranian pistachio cultivars were found to have significantly lower chlorophyll a contents.

Table 1. Chlorophyll a and b contents of the 'Uzun' pistachio cultivar using various solvent types and samples (mg/l)

Sample	Exp. N.	Solvent mixture	Sample Quantity	Chlorophyll a	Chlorophyll b
Oil	1	90 % Acetone	50 µ	1.16 ± 0.58	2.33 ± 0.16
	2	Acetone with 1 % HCl	50 µ	3.22 ± 0.64	7.47 ± 0.54
	3	100% Methanol	50 µ	2.10 ± 0.15	3.39 ± 0.30
	4	Methanol with 1 % HCl	50 µ	2.17 ± 0.26	3.70 ± 0.33
	5	Chloroform: Methanol (2:1)	50 µ	0.25 ± 0.02	0.40 ± 0.03
Kernel powder	6	90 % Acetone	50 mg	2.92 ± 0.98	4.15 ± 0.28
	7	Acetone with 1 % HCl	50 mg	10.07 ± 0.77	18.06 ± 0.78
	8	100 % Methanol	50 mg	2.56 ± 0.09	3.78 ± 0.42
	9	Methanol with 1 % HCl	50 mg	5.31 ± 0.96	8.09 ± 0.08
	10	Chloroform: Methanol (2:1)	50 mg	0.25 ± 0.01	0.64 ± 0.02

The chlorophyll b content of the 'Uzun' pistachio cultivar was found to vary significantly depending on the different solvents applied, ranging from 2.33 mg/l to 18.06 mg/l. The application of 100 % methanol and acetone with 1 % HCl resulted in the highest chlorophyll a and b values, respectively. In previous studies, the chlorophyll b content of the Iranian cultivars ranged from 0.62 mg/l to 3.11 mg/l, whereas the chlorophyll b content of the Italian cultivars varied between 1.26-2.98 mg/l. The chlorophyll b content of the Tunisian pistachio cultivars examined ranged between 1.87-2.34 mg/l, whereas the Greek pistachio cultivar 'Aeginea' and the Syrian pistachio cultivar 'Ashoury' had chlorophyll b contents of 1.11 mg/l and 0.62 mg/l, respectively (Agar ve ark., 1994).

## CONCLUSION

The chlorophyll a and b values of the 'Uzun' pistachio (*Pistacia vera L.*) cultivar were determined using different solvents and different samples such as oil and kernel powder. According to the results obtained, the chlorophyll content values of the 'Uzun' pistachio cultivar were greatly affected by different solvents applied. The highest values of chlorophyll a and b contents of both 'Uzun' pistachio kernel powder and oil samples were achieved using 1 % HCl. Moreover, the highest chlorophyll a and b contents were obtained when kernel powder was used as an extraction material.

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