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Original Article

Anthocyanins, chlorophylls and xanthophylls in pistachio nuts (*Pistacia vera*) of different geographic origin

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Abstract

The pistachio seed is particularly appreciated for its flavour and emerald-green colour. The aim of this paper was to study simultaneously the major coloured compounds in pistachio nuts collected from different geographic origins. Qualitative and quantitative analyses of pigments in commercial samples of pistachio nuts (coming from Greece, Iran, Italy and Turkey) were performed by HPLC. The results highlighted the presence of both of cyanidine-3-galactoside and cyanidine-3-glucoside, in the external skin of kernels, chlorophyll *a*, chlorophyll *b* and lutein in the inner hull. The degree of ripeness and the origin of the products influenced pigment concentration as well as colour parameters. Cyanidine-3-galactoside was absent or present in very low levels (21 mg/kg) in unripe products, with about 300–400 mg/kg in ripe samples. Chlorophylls (sum of $a+b$) were about 150 mg/kg in pistachios sold as "green" products, with a chlorophyll a/b ratio of about 3. The lowest chlorophyll value (25 mg/kg) was found in a ripe Turkish product. Lutein was the main carotenoid found in pistachio kernels, ranging from 18 to 52 mg/kg. The ripe Italian samples always had the highest pigments concentration.

Keywords: Pistachio; *Pistacia vera*; Anthocyanins; Cyanidine-3-Galactoside; Chlorophyll; Lutein; Colour parameters

1. Introduction

Pistachio (*Pistacia vera*) is a nut tree species which, because of its marked resistance to extreme environmental (pedoclimatic and hydrologic) conditions, is cultivated in Europe and Asia on soils that are unsuitable for other fruit crops. Iran, Turkey, USA, Syria, Italy, Tunisia and Greece are the main producers of cultivated pistachio; each cultivated area presents specific varieties (even though few in number in comparison with other fruit species) that produce pistachio nuts with green or yellow cotyledons and different percentages of empty fruits and alternate bearing (Monastra et al., 1987).

In Italy the pistachio is grown in Sicily in Bronte and Adrano (Catania). One variety (Bianca) is cultivated exclusively, representing 86% of cultivated surface in Italy (Fabbri and Valenti, 1998). Because only a few areas are cultivated entirely in pistachio, and as a result of pedoclimatic problems, onerous agronomic practices and alternate bearing, Italian production is very low in comparison to that of Asia and California; however it is compensated by very high quality (Di Marco, 1987). The pistachio, owing to its appreciable qualities such as shape, size, aroma and in particular its green colour (especially in hulled and peel-backed products) is used as an ingredient in cakes, pastries, ham, mortadella and ice-creams and in the confectionary industries (Angelini, 1987). Usually, Italian pistachio nuts, picked fully ripe, are sold as the green product, whereas in other countries they are sold at various degrees of ripeness: green (unripe), yellow/green (intermediate) or yellow (ripe) product.

From the nutritive point of view, the energy value of pistachios is similar to that of almonds (2332 kJ/100 g); they are high in carbohydrates and minerals, mainly potassium (1025 mg/100 g) (USDA SR 18, 2005). Fat content is about 50–70% of total nut weight according to variety. The fatty acids include about 25% essentially monounsaturated (with up to 50–70% of the total fatty acids in the form of oleic acid); the polyunsaturated fatty

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acid content is also high representing about 7% (linoleic acid generally up to 15–35% of the total fatty acids) (Garcia et al., 1992; Kafkas et al., 1995). As regards vitamin content, the presence of β-carotene 3.2 mg/kg, thiamin 8.7 mg/kg, vitamin E 23.0 mg/kg, riboflavin 1.6 mg/kg and 50.0 mg/kg of ascorbic acid have been reported (USDA SR 18, 2005).

In the past few years a number of studies have been carried out on the chemical composition of this nut (Aslan et al., 2002; Kucukoner and Yurt, 2003; Satil et al., 2003). Multivariate analysis has been applied in order to differentiate the geographic origin of pistachios (Anderson and Smith, 2005). Moreover, the antioxidant activity of pistachio hull extracts (Goli et al., 2005), as well as the effect of roasting on aflatoxins (Yazdanpanah et al., 2005) have been studied.

Although being an essential parameter in the pistachio trade, colour has been less studied. As concerns pistachios pigments, the first studies were those done by Giovannini and Condorelli (1958) who, in a paper concerning the metabolism of the chloroplastic pigments of this fruit, reported the presence chlorophyll *a*, chlorophyll *b*, β-carotene and lutein; moreover, they observed, with ripening, a first stage consisting in an accumulation of chlorophyll (August) followed by a degradation (September). The colour change from green to yellow-green with ripening has been also highlighted by Kunter et al. (1995). Other studies (Agar et al., 1998) report data on the variation of chlorophyll levels in pistachio varieties of different origin; they have highlighted the highest chlorophyll content in Italian samples.

As concerns the colour of the external skin, up to now only two studies have been carried out to identify the red pigments. The first one (Miniati, 1981) reported the presence of cyanidin-3-galactoside as the only anthocyanin in pistachios. Recently, presence of small amounts of cyanidin-3-glucoside has been found (Wu and Prior, 2005). Neither paper reports quantitative analyses. Studies concerning the simultaneous determination of the major pigments of pistachio kernels, as well as CIE parameters, have not been carried out. As consequence, no data are available in the literature about the correlation between pigments and ripening or origin.

In this paper, the quantitative levels of the major coloured compounds (anthocyanins, chlorophylls *a* and *b*, lutein) as well as colour parameters (CIE *L C h*), were determined in pistachios both of different origin and of different degrees of ripeness.

2. Materials and methods

Eight commercial pistachio samples of two independent lots were sampled, for a total of four samples each. They come from Greece (var. Kerman), Iran (var. Akbari), Italy (var. Bianca) and Turkey (var. Siirt). Turkish samples were supplied at three ripening stages: green (G), picked in the 3rd week of August; yellow/green (Y/G), picked at the end of August and beginning of September, which show a higher percentage of yellow product; yellow (Y), picked at ripeness (2nd–3rd week of September). Iranian sample was picked in the 3rd week of August (G); Greek sample was yellow/green (Y/G); Italian samples were green (G) products, but they were picked at ripeness, in September (2nd–3rd week) (Table 1). All samples refer to the crop agricultural year 2000/2001; the analyses were carried out 1 year later (2002). All chemical analyses were carried out in duplicate; data reported in tables are the average of eight HPLC injections.

Table 1
HPLC-DAD values of anthocyanin in mg/kg (dry matter); $n = 4$, analysed in duplicate

	Sample	Cyanidin-3-galactoside	Std. dev.
Unripe	1. Iran G	22.1	±3.73
	2. Turkey G	n.d.	—
Intermediate	3. Turkey Y/G 1	144.7	±5.08
	4. Turkey Y/G 2	106.8	±21.17
	5. Greece Y/G	138.2	±5.96
Ripe	6. Turkey Y	286.7	±23.5
	7. Italy (Bronte) G	281.0	±18.99
	8. Italy (Agrigento) G	426.4	±39.12

G = Green.
Y/G = Yellow/Green.
Y = Yellow.
n.d. = not detectable.
Std. dev. = standard deviation.

2.1. Sampling

As concerns the Italian samples (Agrigento, Bronte), each sample was representative of 1000–1500 kg of pistachio kernels, from which a 10 kg of intermediate sample was obtained, from which in turn 1 kg of pistachio for each sample was taken. The same procedure was used for the other samples, coming from commercial lots between 1000 and 40,000 kg, but, in this case it was not possible to have precise information about the initial amount.

2.2. Extraction and analysis of anthocyanins

Anthocyanin extraction was carried out as described by Gao et al. (1997). The external skins of pistachio kernels (300 mg), fine triturated, were extracted with 20 mL of aqueous formic acid (1%) as solvent for 1 h under magnetic stirring within an amber flask to prevent alteration of pigments. Two more extractions were carried out with 10 mL of methanol 0.1% HCl. Acidified methanol was used to take-up to the final volume. The sample obtained was filtered through 0.45 μm filter before HPLC analysis.

Anthocyanin identification and quantification was carried out by HPLC (VARIAN 9012 Q) equipped with PDA (Star 330 VARIAN); the separation was achieved on a