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Effect of harvesting time on nut quality of pistachio (*Pistacia vera* L.) cultivars

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ABSTRACT

The appropriate time of harvest is one of the most important factors affecting the quality of pistachio. Cultivars 'Ahmad-Aghaii', 'Kaleh-Ghoochi', 'Ohadi' and 'Badami-Zarand' were evaluated for qualitative indices of nut over a period of four years from 2001 to 2004. Nut samples were collected during eight successive weeks from 23 August to 11 October. Splitting, early splitting, hull cracking and hulling percentage continually increased toward the last harvest week while non-splitting, immaturity, number of nuts per ounce and moisture content decreased during the harvest weeks. The increment of splitting and the number of nuts per ounce was not significant from 20 September to the last week of harvest. Early splitting, hull cracking and aflatoxin contamination of kernel progressively increased from 13 September. Total crude fat and sugar contents showed a peak in the middle of September. The concentration of aflatoxins prior or at maturity stage of selected cultivars was lower than the critical level. In general, the appropriate time of harvest for the cultivars studied is the middle of September to avoid contamination of nuts to aflatoxin and to ensure the nut quality. This study emphasizes different quality indices for determining the optimum harvest time of pistachio crop.

1. Introduction

Pistachio (*Pistacia vera* L.) is an increasingly important nut crop, widely cultivated in hot dry areas of eastern Mediterranean (e.g. Iran, Turkey, Syria) and the US. Pistachio nut is a drupe characterized by a split in the shell (endocarp) along the suture at the calyx end of the nut and the hull (mesocarp) usually encloses the shell. There is a space between the hull interior and shell exterior, so the normal shell can split open without splitting the hull (Pearson et al., 1996; Doster and Michailides, 1995). Two types of hull rupture occur before harvest. In the first type, early splitting, the hull ruptures along the shell split. In the second type, hull cracking, the hull ruptures elsewhere (Doster and Michailides, 1999). The hull typically does not rupture before harvest and protect the kernel from fungal infection and insect infestation. Genetic characteristics of scion and rootstock, climatic conditions, unbalanced nutrition and irrigation and improper harvest date are responsible for production of early-split and cracked nuts (Hosseinifard and Panahi, 2006; Tajabadipour et al., 2006; Doster et al., 1999; Doster et al., 2001). Early-split nuts as well as blankness and cracking are among the most important physiological disorders affecting the quality and the yield of pistachio crop (Khezri et al., 2010).

The quality of a split pistachio nut is defined in particular by size and shapes of the nut, moisture content as well as biochemical compositions (e.g. carbohydrate, fat and protein) (Crane, 1978). These qualitative factors can be influenced by genetic characteristics of the scion and rootstock, ecological conditions, location and horticultural practices (Tajabadipour et al., 2006; Seferoglu et al., 2006).

Determining the optimal harvest date of pistachio nut is one of the most important horticultural practices to increase the quality of nut production. Both early and late harvesting time decrease the quality of pistachio nut. Early harvested nuts are mostly non-split and immature often including an undeveloped kernel. Pistachios harvested too late are clearly vulnerable to hull cracking, shell staining, hull and kernel deterioration, fruit shedding, mechanical injuries and insects and bird attacks (Ferguson et al., 2005). Of special concerns with late harvesting is kernel decay by *Aspergillus* spp. contaminated the early-split and hull cracked nuts. Pistachio nuts are a suitable target for aflatoxins accumulation and the aflatoxins contents in the tested samples is dependent on the cultivar's type (Bensassi et al., 2010). Species of the *Aspergillus* group produce secondary metabolites called aflatoxins which are harmful mycotoxins for humans and animals (Klich, 2009), therefore, regular sampling and testing of pistachios has been recommended to control the presence of aflatoxins for commercial trade (Fernane et al., 2010). Although the importance of early splitting and cracking for mold, aflatoxin and insect contamination is well established, very little is known concerning when early splits and cracking occur and become contaminated.

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As with other fruit crops, maturation in pistachio is uneven throughout the tree. Nuts on the upper southwest quadrant and those on the periphery mature sooner. Light crops also mature earlier than heavy crops (Ferguson et al., 2005). Some indices e.g. easy hull separating, the color turning of hull from green to rose, splitting percentage and the nut size have been reported as the most important common measures for assessing pistachio harvest date (Kader et al., 1982).

Although biochemical compositions e.g. carbohydrate, fat and protein are less attended by the growers, they affect the real taste of pistachio nut and ensure the nutritive and health value of nuts. Harvesting time must take into account these parameters, along with the quality characteristics essential for consumer acceptance. According to Labavitch et al. (1982), the maximum amount of carbohydrate, fat and protein are present at optimal harvesting time when the hull is fully matured, and thus inopportune harvest performance severely impresses the nut compositional characteristics and subsequently decreases the nut quality. However, there are not many reports which deal with the influence of harvesting dates on pistachio nut quality. The study reported here was carried out over a period of four years, 2001–2004, to determine the precise time at which different pistachio cultivars should be harvested to ensure the highest quality.

2. Material and methods

2.1. Plant material and experiment

Ten 15-year-old trees of 'Ahmad-Aghaii', 'Kaleh-Ghoochi', 'Ohadi' and 'Badami-Zarand' cultivars were selected as the sources of nut samples. These cultivars were grafted on *Pistacia vera* cv. Badami-Riz rootstock at Pistachio Research Institute, Rafsanjan, Iran. The experiment was arranged as randomized completely block design with three replicates, totally, 10 trees per cultivar. 'Kaleh-Ghoochi' and 'Ohadi' are among the popular Iranian cultivars also grown in the US and Turkey respectively. 'Ahmad-Aghaii' is a promising cultivar for new established orchards and 'Badami-Zarand' is widely cultivated as rootstock and somewhere as scion. Nut harvesting almost occur between the 6th of September and 6th of October. In this research, it was decided arbitrarily to harvest samples weekly during a two-month period from 23 August to 11 October. For each time of sampling, four fruit clusters were picked at random of each geographical direction of each tree, combined and hand sorted into split, non-split, blank and hull cracked nuts.

2.2. Compositional analysis

After removing and discarding all blank nuts, the samples were dehulled and the percentage of hulling was determined. 20 dehulled nuts of each geographical direction of each tree was weighted before drying, then dried to constant weight in an oven at 70 °C for 48 h and weighted for calculating the percentage of moisture. The dehulled dried split pistachios were used to determine the number of nuts per ounce (28.3 g).

Crude fat determination was performed by Soxhlet method (AOAC, 1990). 5 g dried ground kernels were weighted. The extraction procedure was done using automatic Soxtherm instrument (Gerhardt-Burteg, Germany) and pure hexane as the solvent. The extraction process was set at 180 °C for 3 h. Extracted samples were weighted and the percentage of crude fat was calculated.

Soluble sugars composition was determined by the phenol–sulphoric acid method according to the procedure of Nzima et al. (1997) with some modifications. The 10-mg fat-extracted kernel was added to 10 ml of 80% (v/v) methanol and homogenized for 40 s using a shaker (Gerhadt bonn-152, Germany). The extraction was repeated three times, each time using 10 ml of the methanol. Homogenates were centrifuged at 3000 × *g* for 5 min and decanted, and the three 10-ml extracts were combined. The methanol was evaporated to 3–5 ml using an oven at 50 °C, and then the volume was increased to 25 ml by adding deionized water. The methanol–water soluble fractions were deproteinated using 2% barium hydroxide and 2% zinc sulphate and then filtered through Whatman paper (No. 3). Extracts were diluted with 10 ml distilled water. 1 ml of phenol and 5 ml of sulphoric acid were then added to 2 ml of diluted extracts. The solutions were assayed by measuring absorbance at 485 nm using a spectrophotometer (Cecil CE-304, UK) and compared with glucose standards subjected to the same procedure.

The crude fat and soluble sugar composition were expressed as percentage on a dry weight basis.

2.3. Aflatoxin assay

Thin layer chromatography (TLC) is one of the most widely used separation techniques in aflatoxin analysis. Since 1990, TLC has been considered the AOAC official method and the method of choice to identify and quantitate aflatoxins at levels as low as 1 ng/g. In this research total aflatoxin (i.e. B1, B2, G1 and G2) and aflatoxin B1 were assessed by TLC according to the method of AOAC (1990) with some modifications. 4 g NaCl was mixed with 50 g of fat-extracted pistachio kernel. The extraction of aflatoxin was performed using 500 ml methanol (55%, v/v) and 200 ml pure hexane. After the extraction of aflatoxins, a clean-up step was performed. The dry extracts were dissolved in 200 μl acetonitrile–benzene (2:98, v/v) prior to the TLC analysis. The samples were separated by thin layer silica gel plates (20 cm × 20 cm, Merck, Germany). Plates were air-dried for 1 h and then activated at 105 °C for 2 h. The TLC plates were developed in first and second mobile phase consisting of diethyl ether and chloroform/acetone/methanol (86/10/4, v/v/v), respectively. Thereafter, the TLC plates were dried in the dark and exposed to long wave-ultra violet (365 nm) for visual estimation and comparison of sample spots to aflatoxin standard in terms of retention factors and intensity. Aflatoxin quantification was calculated as follows:

$$B_1\ (\mu g\ kg^{-1}\ \text{or ppb}) = \frac{C \times V_1 \times V_3}{m \times V_2}$$

where C = concentration of standard ($\mu g\ ml^{-1}$); V_1 = μl of final dilution of sample extract for TLC; V_3 = μl aflatoxin B1 standard matching unknown; V_2 = μl of sample extract matching C; m = weight of product represented by final extract for TLC.

Total aflatoxins measurement was subjected to the same procedure. Analysis was repeated at least 2–3 times with three replicate measurements each.

2.4. Statistical analysis

This experiment was conducted in four consecutive years from 2001 to 2004. Analyses of variance were performed using the General Linear Models procedure of SAS (SAS Institute Inc., Cary, NC, USA). Means were separated by Duncan's multiple range test ($P < 0.05$). Prior to statistical analysis, data were subjected to transformation, where necessary, for normalizing the frequency distribution.