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

Bahman Panahi a, Masood Khezri b, a

a Iran’s Pistachio Research Institute, Rafsanjan, Iran
b Horticultural Research Institute, Shahid Bahonar University of Kerman, Kerman, Iran

A R T I C L E   I N F O

Article history:
Received 19 February 2011
Received in revised form 28 April 2011
Accepted 30 May 2011

Keywords:
Early-split nut
Hull cracked nut
Immature nut
Blank nut
Aflatoxin contamination

A B S T R A C T

The appropriate time of harvest is one of the most important factors affecting the quality of pistachio. Cultivars ‘Ahmad-Aghai’, ‘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.

© 2011 Elsevier B.V. All rights reserved.

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., 1010). 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.

* Corresponding author.
E-mail addresses: khezri@mail.uk.ac.ir, masoodkhezri@gmail.com (M. Khezri).

0304-4283/ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2011.05.029
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. Lighter 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–sulphuric 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 (Gerhardt bonn-152, Germany). The extraction was repeated three times, each time using 10 ml of the methanol. Homogenates were centrifuged at 3000 x 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 deproteinized 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 quantify 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 x 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:

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

where C = concentration of standard (µg ml⁻¹); V₁ = µl of final dilution of sample extract for TLC; V₃ = µl aflatoxin B₁ standard matching unknown; V₂ = 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.
3. Results

3.1. Split, non-split and immature nuts

The percentage of split nuts significantly increased during the harvest weeks (e.g. ‘Ahmad-Aghaii’ from 49.7% on 23 August to 86.9% on 10 October). Among the cultivars, ‘Ahmad-Aghaii’ showed the highest (86.9%) and ‘Badami-Zandari’ had the lowest percentage of split nuts (73.3%) at the end of harvest date. The percentage of non-split and immature nuts gradually decreased toward 11 October. The lowest percentage of both non-split and immature nuts was found for ‘Ahmad-Aghaii’ (13.1% non-splitting and 6.4% immaturity) and the highest for ‘Badami-Zandari’ (26.8% non-splitting and 12.9% immaturity). For all the cultivars, there were no significant differences during 5th to 8th harvest weeks (20 September to 11 October) for the percentage of split, non-split and immature nuts (Table 1).

3.2. Early-split, cracked hulls and hulking

Early-splitting, cracking and hulking significantly increased during the harvest weeks for all the cultivars. At the last harvesting date, ‘Kaleh-Ghoochi’ was found to have the highest percentage of early-split (2%) and cracked nuts (31.3%) while ‘Ohadi’ had the lowest percentage of early-split (1.1%) and cracked nuts (10.3%). There was no significant difference among the cultivars from 13 September to 11 October for the percentage of hulking except of ‘Badami-Zandari’ which had the lowest in all harvesting dates (Table 1).

3.3. Number of nuts per ounce

The number of nuts per ounce is a marketing index, to show the size and weight of pistachio nuts, the lower the number of nuts per ounce, the larger the nut size. The number of nuts per ounce showed a constant decline for all the cultivars with progressive harvest dates but was not significantly different during 6th to 8th weeks of harvest (27 September to 11 October) and the differences was related to the type of cultivar. The lowest number of nuts per ounce was related to ‘Kaleh-Ghoochi’ while the highest was for ‘Badami-Zandari’ during all the harvest weeks. At the last harvesting date, the number of nuts per ounce was 22.6 and 28.1 for ‘Kaleh-Ghoochi’ and ‘Badami-Zandari’ respectively (Table 1).

3.4. Moisture, total soluble sugar and crude fat

For all the cultivars and harvest dates, there was a steady decrease in moisture content over time; however, the rate of this decrease differed according to the cultivars. The highest moisture content was related to ‘Ahmad-Aghaii’ declining steadily from 39.8% on 23 August to 35.7% on 11 October and the lowest was observed for ‘Kaleh-Ghoochi’ ranging from 34.2% at the first and
29.6% in 'Kaleh-Ghoochi' and 23.4% in 'Badami-Zarand' at the last date of harvesting. No clear trends were observed in the total sugar content of different cultivars. However, 'Kaleh-Ghoochi' showed the lowest sugar content (13.3%). Although total crude fat gradually increased with nut maturation, it was not significantly different. The percentage of crude fat was different among the cultivars and the highest crude fat content was found for 'Kaleh-Ghoochi' increasing from 56.7% on 23 August to 58.2% on 11 October (Fig. 1).

3.5. Total aflatoxin and aflatoxin B1

The onset of aflatoxin contamination was observed on 13 September (4th week of harvest) for 'Ahmad-Aghaii' and 'Kaleh-Ghoochi' while it was started on 27 September (6th week of harvest) for 'Ohadi' and 'Badmi-Zarand'. Aflatoxin contamination significantly increased toward the end of the harvest weeks. The highest amount of both total aflatoxin and aflatoxin B1 was found at the last harvest week for all the cultivars. 'Ahmad-Aghaii' and 'Kaleh-Ghoochi' significantly had higher concentrations of aflatoxins than 'Ohadi' and 'Badami-Zarand'. Depending on the cultivar, the concentration of total aflatoxin differed between 5.2 and 6.2 ppb while it was between 3.4 ppb and 4.2 ppb for aflatoxin B1 (Fig. 2).

4. Discussion

Pistachios have traditionally been harvested when the hull separates easily from the shell (Whitehouse, 1957). In the previous years, in addition to ease of hull separation, pistachio fruit color served as a common marker for maturation level, expressed as maturity index. It is not likely that color of hulls or shells can be used as an index of pistachio maturity because of the impact of seasonal variation on color of hull and shell (Labavitch et al., 1982). Hence, other indices are necessary for determining the optimum
harvest maturity of pistachio nuts. Means over four years and all harvest dates demonstrate fundamental differences among the cultivars with respect to harvest strategy. The degree of shell splitting is an important quality factor, as most pistachios are marketed in the shell. Although nut splitting continually increased even by 11 October, the percentage of early-split and cracked nuts as well as aflatoxin contamination also increased. Hulling percentage as a good index of fruit maturation continually increased toward the last week of harvest but improving this trait without hull cracking and shell staining is of great value for consumers. The shells of early split nuts frequently have a distinctive dark brown discoloration along the shell suture while this discoloration might be elsewhere on the shell for cracked nuts. The staining of pistachio shells can be extensive and is associated with poor quality nuts (Doster and Michailides, 1996). In the present study we clearly found that nuts from the delayed harvest weeks (27 September, 4 October and 11 October) were more likely to have shriveled hull and excessive shell staining than the earlier harvest weeks (23 August, 30 August and 6 September). It has been previously shown that early split nuts with shriveled hulls have higher incidences of moldy kernels and severely stained shells than early splits with smooth hulls (Doster and Michailides, 1998).

Early-split nuts have more moldy kernels than nuts with cracked hulls because early-splitting tend to occur earlier than nuts with cracked hulls (Doster and Michailides, 1994). Therefore, the percentage of early-split nuts and cracked hulls should be more considered for determining the optimum harvest time for pistachio nut.

Results of the present study showed that the incidence of aflatoxin contamination started from 13 September for all the cultivars (Fig. 2). Also, the percentage of early-splitting and cracking progressively increased from this week. Hence, it is better to do harvest around this week.

Occurrence of aflatoxin contamination in pistachio nuts before harvest has been reported previously (Thomson and Mehyd, 1978; Sommer et al., 1986). Currently, worldwide range of limits for aflatoxin B1 and total aflatoxin are <5 ppb and <15 ppb, respectively (Boutrif, 1998). We showed that the concentration of aflatoxin prior or at maturity stage is lower than the critical level which is in accordance with the report of Cheraghalizadeh and Yazdanpanah (2010). It seems that early-split and hull cracked nuts that are not infected in the orchard may become infected during transport and handling. High humidity and high temperature within bulk bins provide ideal conditions for the infection of early split and cracked nuts, which dramatically increases the incidence and level of aflatoxin contamination (Doster and Michailides, 1998). Therefore, our result emphasized that postharvest handling of pistachio nuts is as important as determining the optimum harvest management for preventing aflatoxin contamination.

Our study showed that the number of nuts per ounce was not significantly different from 13 September to the last harvest week (Table 1). This showed that this trait is not a limiting factor for the growers to harvest the pistachios earlier than 20 September.

The pistachio kernel is a rich source of fat which varies from 50% to 70% of total nut weight, depending on the cultivar (Agar et al., 1995). At optimum maturity, total crude fat and sugar contents of kernels reach a peak (Labavitch et al., 1982). Therefore it is better that the peak of crude fat and sugar contents coincides with nut harvesting. Our study showed that the approximate peak of total crude fat and sugar contents occurred around 13 September to 20 September depending on the cultivar.

In conclusion, all qualitative differences among cultivars were associated with the time of harvest. Although growers can harvest pistachios anytime during a several-week period, nut quality might suffer if harvest is delayed too late. Regarding to all quality factors over a period of four years research, we found that the optimum harvest time for ‘Ahmad-Aghaii’ and ‘Kaleh-Ghoochi’ occurred at the time between 4th and 5th week of harvest (13 September to 20 September), For ‘Oddi’ and ‘Badami-Riz’, the time between the 3rd and 4th week of harvest (6 September to 13 September) was the best. It is pertinent that criteria for correct decisions on harvest date consider other quality factors in addition to ease of hull separation for pistachio crop.

References