Investigating the Effect of Harvesting time on the Composition of Pistachio Nuts

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Abstract

Introduction: The main components of pistachios undergo significant changes during the ripening and development stages, making various uses of pistachios possible as fully grown product or green kernels Temporal Variations in Pistachio Nutrients. This research evaluated the main compounds and chlorophyll pigments of Ahmad Aghaei and Ohadi pistachios' varieties during ripening.

Materials and methods: Pistachios were harvested from the gardens of Rafsanjan city at four different times (August 10th and 25th and September 10th and 25th). After removing the soft skin, the pistachio and its kernel were weighed, and the moisture content was measured using incubating. Subsequently, they were dried at 70 degrees Celsius in the oven for 48 hours then the amount of protein, oil, carbohydrates, Fiber, chlorophyll, and ash were measured. The obtained data were statistically analyzed using Minitab version 17 software, and the averages were compared using Duncan's test at the 5% probability level.

Results: The amount of moisture in both cultivars decreased during ripening, and it was in the range of 46.5-28.8 and 49.5-31.8 percent in Ohadi and Ahmad Aghaei cultivars, respectively. Fat, protein, carbohydrates, and fiber increased during the ripening period. The amount of ash did not show a significant change, but the amount of chlorophyll decreased during the ripening period.

Conclusion: The composition of the pistachio kernel changes during ripening, and by considering these changes and according to the purpose of using pistachios, the appropriate harvesting time and pistachio quality can be determined.

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1. Introduction

According to taxonomic classification, pistachio (*Pistacia vera L.*) belongs to Anacardiaceae and contains 13 or more species [1]. Pistachio, a native of Western Asia and Asia Minor, can grow under saline and drought conditions. Iran, America, Turkey, China, and Syria are the main pistachio-producing countries in the world [2]. Iran is considered one of the largest producers and exporters of pistachios in the world, and this product is produced in different regions and has great economic importance [3]. It is known as green gold due to its high amount of chlorophyll, and it has other applications, including whole pistachios in roasted products and nuts and unripe kernels as green kernels in confectionery. The high nutritional value, especially the special green color, and pleasant aroma, is the main reason for its use in food products such as breakfast cereals, cakes, biscuits, chocolates, ice cream, sausages, pistachio butter [4, 5]. Pistachios are an important source of nutritious compounds such as protein, fiber, fat, minerals, and vitamins [6]. Several studies have been conducted on the components of pistachios. According to studies based on the constituents of different varieties of Iranian pistachios, the amount of these compounds in 100 grams of seeds includes 45-72% oil, 15-21.2% protein, 14.9-17.7% carbohydrates, 4 mg sodium, 1048-1142 mg potassium, 120-150 mg calcium, 5.8-11.4 mg iron, 1-1.4 mg copper, 157.5-165 mg magnesium and 494-514.5 mg phosphorus and the amount of pistachio energy is reported to be 567 kcal per 100 grams [7]. The composition of pistachio changes during ripening and in ,addition to changes in the main components (moisture, fat, protein, carbohydrates, and fiber) [8, 9], pistachio pigments and minor components Like phytochemicals, also experience changes [10, 11]. One of the most important pigment is chlorophyll, which decreases during ripening, so part of the pistachio product is harvested early and is called green pistachio, which is used in the confectionery industry [4, 5].

Previous studies have shown that the moisture content of pistachios decreases as they ripen [12]. Panahi and Khezri (2011) reported that the amount of protein is almost constant during the ripening period and decreases after pistachio ripening. But the amount of fat increases until ripening and reaches its highest value [8]. The results of another research have shown that the delay in harvesting of pistachios leads to an increase in the amount of sugar and a decrease in fat content [13]. Investigating the changes in pistachio constituents during ripening can help producers regarding the appropriate harvesting time of the product according to its practical purpose. In addition, it is effective in estimating thermal and thermodynamic properties and predicting them based on pistachio compounds for thermal processes such as drying. Therefore, in this research, the amounts of pistachio compounds, including moisture, protein, fat, fiber, carbohydrates, ash, and chlorophyll a, b pigments at two different varieties of pistachio nuts, Ahmad Aghaei and Ohadi, were investigated during four harvest times in the ripening stage (August 10th and 25th and September 10th and 25th).

2. Materials and methods

All chemicals used in this research, including n-hexane, methanol, barium hydroxide, zinc sulfate, sulfuric acid, acetone, acetic acid, nitric acid, and trichloroacetic acid, were obtained from Merck, Germany.

Sample preparation

Two varieties of pistachio, Ahmed Aghaei (Long) and Ohadi (Round), were randomly

taken from the pistachio orchards of Kashkouyeh region in 2022 during four different ripening times (August 10th and 25th and September 10th and 25th) in three replications. After harvesting the pistachio and separating the green hull and outer skin by hand, the pistachio kernel was dried at 70°C for 48 hours and subsequently its moisture was measured. Dried kernel was used to measure the constituent compounds [8].

Moisture measurement:

The moisture contents were determined according to the AOAC standard methods 930.15 [14].

Fat measurement

The percentage of fat was determined using the Soxhlet method using a auto-Soxhlet device (Buchi B- 811, Switzerland); hexane was used as a solvent [15].

Carbohydrate measurement

Soluble sugar was measured using the phenol-sulfuric acid method. 10 mg of fat-extracted kernel was added to 10 ml of 80% methanol and homogenized for 40 seconds using a shaker. The extraction was repeated three times and 10 ml of methanol was used each time. Then it was centrifuged for 5 minutes and three extracts (10 ml each) were combined. Methanol was evaporated to 3-5 ml using an vacuum distilled (Buchi R-114A29 B-480, Switzerland) at 50°C for 1 houre, and then its volume was increased to 25 ml by distilled water. Barium hydroxide and 2% zinc sulfate were applied to deproteinize methanol-water solution. subsequently, the solution was filtered using Whatman No. 3 paper. The resulting extract was diluted with 10 ml of distilled water. At the next stage,1 ml of phenol and 5 ml of sulfuric acid were added to 2 ml of diluted extract. The absorbance of the solution was read using a spectrophotometer (Cary 100, USA) at a wavelength of 485 and the results were finally compared with standard glucose subjected to the same procedure.

Ash measurement

First, 5 grams of pistachio powder was weighed with an accuracy of 1 mg in a bush, that had already reached a constant weight, and burned on a suitable flame until the end of smoking. Then, the crucible containing the sample was placed in a furnace (Nabertherm, Germany) at a temperature of 525 to 550°C until white ash was obtained. The crucible was cooled in a desiccator and weighed. The amount of ash was calculated using the following equation (1) [16];

$$\textbf{Weight percentage of total ash} = \left(\frac{M_2 - M}{M_1 - M}\right) \times 100 \quad (1)$$

where: M2 is weight the bush with ash (g); M is the empty crucible weight (g); M1 is weight the bush with test sample.

Protein measurement

To measure pistachio kernel protein, the Macro-Kjeldahl method was used, considering protein coefficient for pistachio kernel to be 6.25 [17].

Chlorophyll Measurement

To measure chlorophyll, 0.4 grams of pistachio nut powder was ground with five milliliters of 80% acetone in a mortar until homogenous state was achieved. In the next step, the samples were centrifuged (5000 rpm for 15 minutes). Then the Supernatant solution was separated and read by a spectrophotometer (Cary 100, Varian Australia) at wavelengths of 645 and 655 nm. The amount of Chlorophylls was calculated using the equation 2-4 [18].

$$C_a = 19.00A_{655} - 7.61A_{645} \quad (2)$$

$$C_b = 21.45A_{645} - 5.92A_{655} \quad (3)$$

$$C_{a+b} = 13.08A_{655} + 13.84A_{645} \quad (4)$$

Fiber measurement:

Amount of fiber was measured according to Iranian national standard No. 9636 [19]. Briefly, the amount of sample fiber was determined by boiling the sample in an acid mixture and separating and drying the undissolved material from the acid mixture, and afterward, reducing the weight of dried undissolved material ash from its weight.

First, approximately 100 ml of digesting liquid (900 ml of acetic acid, 60 ml of nitric acid and 24 g of trichloroacetic acid) was added to 5 grams of the sample in Erlenmeyer. Subsequently, it was was boiled for 30 minutes. The remaining raw fiber was transferred to the filter crucible and washed with 300-400 milliliters of distilled water at 70-80 °C. Then the flask was filled three times with acetone to remove the solvent without suction. Then the flask was filled twice with Ether and the solvent was passed through a filter and then the last traces of Ether were removed by suction. The crucible with the fiber remaining on it, was dried for 60 minutes in an oven that was previously dried to 120°C and cooled in a desiccator. Consequently, raw fiber were placed in the bushes in an electric furnace and burned for 30 minutes at a temperature of 550°C and weighed. The fiber content was calculated from the following equation (5)

$$R = \left(\frac{a-b}{\left(\frac{100-E}{100}\right)\times w}\right) * 100 \quad (5)$$

Where in, R: Crude fiber percentage based on dry body, a: The weight of the crucible with the fiber remaining on it after digestion and drying at 120 °C in (g), b: The weight of the filter crucible after burning it (g), W: test piece weight (g), E: moisture percentage of the sample.

statistical analysis:

All experiments were carried out in the form of completely randomized factorial designs with independent variables of harvest time in 4 levels and two varieties with three replications. The obtained data were statistically analyzed using SPSS software version 26, and the differences between means were compared using Duncan's test at the 5% probability level.

3. Results

Changes in pistachio constituents during ripening

As shown in Figure 1, moisture content decreased during ripening in both cultivars, and on 25 Sep, this value decreased from 46.5 and 49.5 in Ohadi and Ahmad Aghaei cultivars to 28.8 and 31.8 on wet basis, respectively. This amount in the Ahmad Aghaei variety was significantly ($p<0.05$) higher than the Ohadi variety in the ripening period.

The amount of fat in both cultivars increased during the ripening time, so that the highest amount of fat was observed on 25 Sep, which was about 37.9 on a wet basis. in general, the fat content on a dry basis in Ahmad Aghaei (52.9-56.7%) during ripening was significantly ($p<0.05$) higher than that of Ohadi (50.1-53.4%)

With the increase in the ripening time, the protein content in Ohadi and Ahmad Aghaei cultivars rose from 13.39 and 11.35% to 16.57 and 14.37% on wet basis, respectively. this value in the Ohadi cultivar was significantly higher ($p<0.05$) compared to Ahmad Aghaei. The protein content based on dry matter in pistachio shows that the highest amount of protein was determin ed on August 10th and in Ohadi and Ahmad Aghaei varieties as 25 and 22.46%,

respectively. On August 25th, this value decreased up to 23.4%. % and 21.1% and did not show any significant change until the end of the fourth stage of harvesting.

The amount of carbohydrates during the ripening period in Ohadi and Ahmad Aghaei varieties increased with a different rate. In Ahmad Aghaei variety, it gradually increased during ripening from 7.1 to 9.3% on wet basis, but in Ohadi variety from August 10th to August 25th, increased from 7.74 to 9.48% (w.b). and after that no significant change was observed.

The amount of fiber increased significantly during ripening ($p<0.05$). The lowest amount of fiber in both cultivars was on August 10th (Ohadi 5.56% and Ahmed Aghaei 5.3% on wet basis) and the highest on September 30th (Ohadi 6.91 and Ahmed Aghaei 6.59% w.b).

Regarding ash, during the ripening period no significant change was observed .it remained in the range of 2.97 to 3.66% in Ohadi cultivar and 3.1 to 3.47% in Ahmad Aghaei cultivar on wet basis.

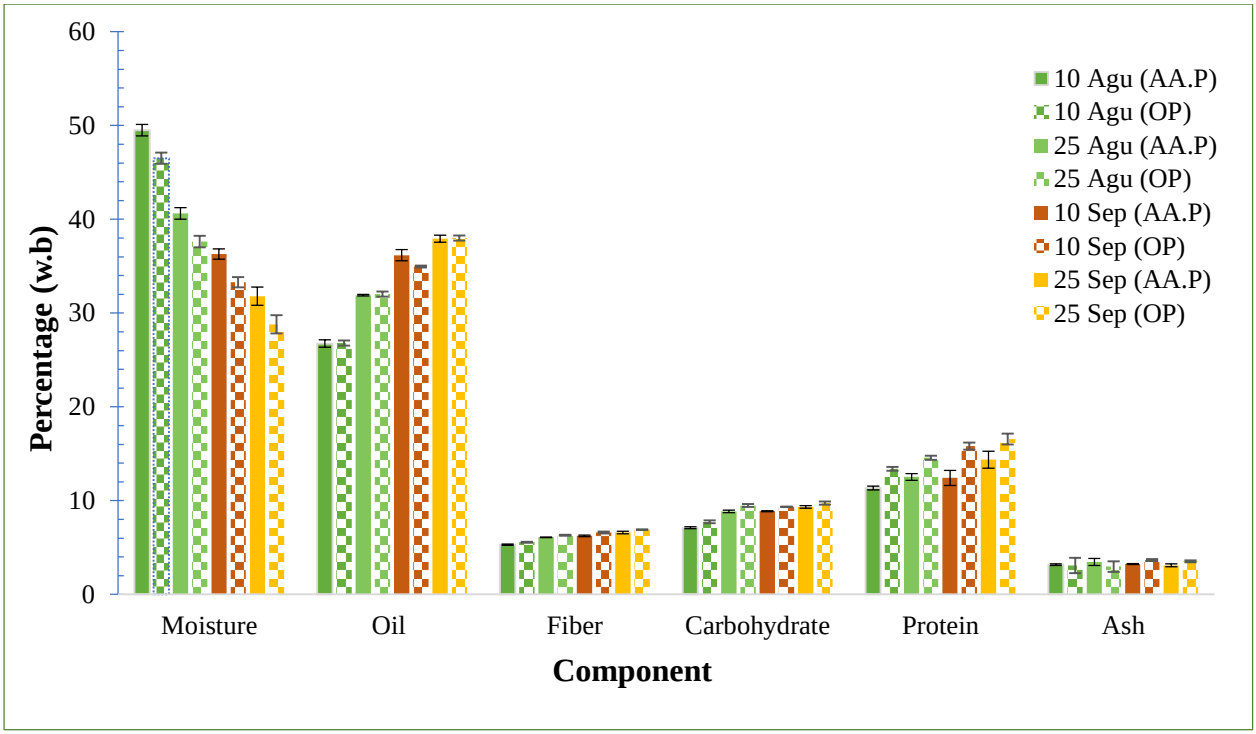

Fig 1. Effect of different harvesting dates on the percentage of kernel components

Chlorophyll (a, b) and total

As shown in Figure 2, The amount of chlorophyll a, b and total decreased significantly with ripening ($p<0.05$). This is the case for Ahmad Aghaei's figure from August 25th to September 25th, and no significant decrease was observed from August 15th to August 25th. The highest amount of chlorophyll a, b and total was observed in Ohadi variety (0.2, 0.136, 0.34 mg/g) on August 15th. The highest amount of chlorophyll a was observed in Ohadi cultivar on August 10th, and no significant difference was observed between the cultivars in the amount of this compound at other harvest times. However, regarding chlorophyll b, a significant difference was observed in the harvest times between the

two varieties, and its average was evaluated in Ohadi variety more than Ahmad Aghaei. In Ohadi cultivar, the amount of chlorophyll a, b, and total at the end of ripening time decreased by 90.7, 81.8, and 87.2% compared to August 10th, and in Ahmed Aghaei cultivar, the reduction percentage was 65.3, 63.5, and 64.4 %.

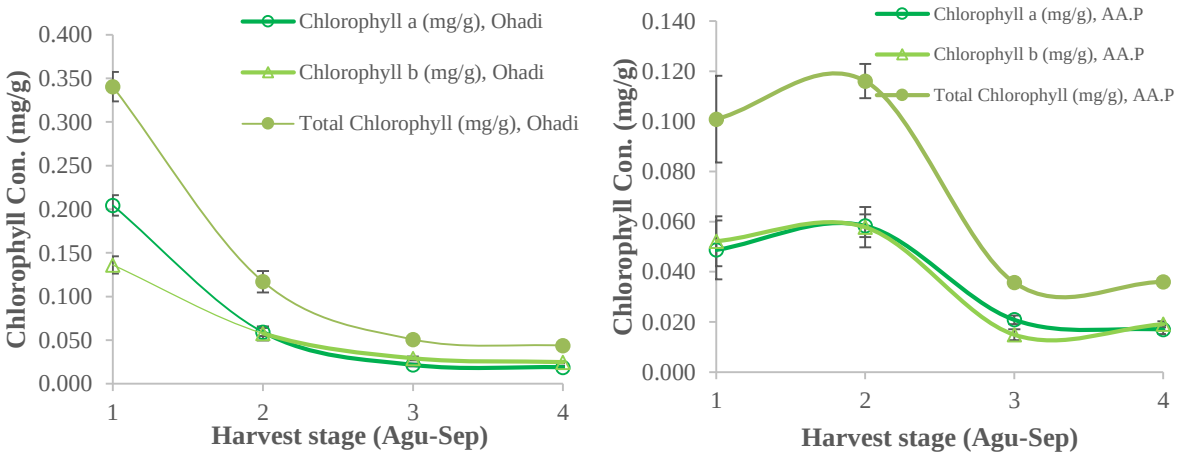

Fig 2. Effect of different harvesting dates on the Chlorophyll concentration of pistachio kernel. Sampling time was from 10 Agu (1), 25 Agu (2), 10 Sep (3), and 25 Sep (4).

4. Discussion

According to the results, the moisture content in both cultivars decreased with a constant rate during the arrival time until September 25th. According to the study carried out by Panahi and Khezri (2011), a steady decrease in pistachio moisture was observed during harvest [8], the rate of decrease was different based on the pistachio variety. The highest moisture content based on dry weight was related to Ahmad Aghaei cultivar, which decreased from 39.8% on August 23th to 35.7% on October 11th. According to the study of Chahed et al. (2006) on the evaluation of pistachio characteristics during ripening, the moisture content decreased with ripening. The maturation process of pistachio kernels was associated with the reduction of water and the accumulation of metabolites. the moisture content in the kernels reached 35% in the freshly harvested nuts at the maturity stage [12]. The moisture content of pistachio kernels is usually around 37-40% in fresh ones. In the present study, moisture was estimated to be about 40.4-46.6% on a dry basis in the pistachio kernel at the last stage of harvesting.

According to the results, the amount of fat in both cultivars increased during ripening. According to Afshari et al.'s study (2007) on the composition of pistachio kernel in three cultivars Ahmad Aghaei, Ohadi and Kale-ghochi, the highest percentage of fat was observed in Ahmed Aghaei cultivar. Panahi and Khezri (2011) reported that the amount of fat increases gradually during ripening. the results of the study by Chahed et al. (2006) and Karaca and Nizamoglu (1994) are also similar to the results of the present study [12, 20]. Nazoori et al. (2016) stated, the highest amount of fat (57.6%) was observed in the fourth stage of harvesting [21]. The results of Shekari's study (2009) on the biochemical changes of Ahmad Aghaei and Ohadi cultivars indicated an increase in fat and in these two cultivars, respectively, in 165 and

135 days after flowering and then it experienced a decrease in fat content [22]. According to the study of Karaca et al. (1994), the time of harvesting has no effect on the amount of fat, and this difference in the results can be explained by the measurement of fat on a dry basis. In the present study, the amount of fat on a dry basis during the harvesting time is in the range of 50-56 % which is in contrast to the increase in average fat during harvest, no significant difference was observed from August 25th onwards.

Pistachio kernel with a protein content of 21-25 (%d.b) is a good source of essential amino acids such as lysine and leucine that contain approximately 25% of its proteins [23]. According to the results, with the increase in the ripening time, the amount of protein on a wet basis increased significantly, but the amount of protein on a dry basis on August 10th was the highest in Ohadi and Ahmad Aghaei varieties (25% and 22.4%) and during the ripening period (August 25th) It decreased by 23.2% and 21% on dry basis, respectively, and no significant change was observed after that. According to the study of Esmaeilpour and Shakerardakani (2018), the highest protein content in pistachio variety Ohadi was observed on July 27th, 25.7% on dry basis, which gradually decreased during the ripening stage on September 6th to 20.6% on dry basis [24]. Nazouri et al. (2013) have reported that during maturity, the protein content of Ahmed Aghaei pistachio decreased from 20.5% d.b in the first stage harvest, last Agu , to 16.8% d.b in the third stage harvest , last Sep, [4]. The results of Esmaeilpour and Shakerardekani (2018) indicated that the amount of protein was different at different harvest times. At the first time of harvest (July 27), the amount of protein was the highest (27% d.b) and decreased to 22% d.b over time [24]. According to Labavitch et al. (1982), pistachio protein content was 30% d.b in the second week of August, but this value decreased over time [25]. The results of the mentioned studies are in accordance with the results of the present study. However, few researches such as the study by Nazouri et al. indicate an increase in the amount of protein up to the fourth stage of harvesting, last Agu, and then its decrease to 23% in the fifth stage of harvesting, mid Sep, which is different from the results of this research. variety, pests, rootstock and type of pollen, diseases, environmental conditions, and geographical location are examples of factors affecting the amount of protein [26]. The existence of such biotic and abiotic stresses can cause a decrease in protein levels and differences in the results presented in different studies. Therefore, Genetic control and environmental stress and their effect on protein expression can be mentioned as the most important reasons for protein reduction in the late period of pistachio kernel development [4].

The results concerning carbohydrates indicated their increase during the ripening time. Karaca and Nizamoglu (1994) reported that the highest amount of sugar was obtained at the time of ripening [20]. The results of Nazoori et al. (2016) indicated that the lowest content of carbohydrates was recorded in early August with the development of the kernel in September, the amount of carbohydrates (14.22%) increased [21]. The research of Saferoglu et al. (2006) confirmed that the average pistachio kernel sugar was the lowest in the first week of harvest (August) and gradually reached its maximum in the September [27]. According to the study of Panahi and Khezri (2011), no clear trend was obtained during harvest in the amount of soluble sugar of different cultivars [8].

The obtained results did not show a significant change in the amount of ash on a wet basis during the ripening stage. The amount of ash on a wet basis did not show a significant change during ripening and harvesting stages. The amount of pistachio ash was investigated 3-3.2 grams per 100 grams in previouse researches [28]. Esmaeilpour and Shakerardakani (2018) investigated the effect of early harvest on seed quality and physiological characteristics of pistachio during 5 harvest stages (July 27, August 6, August 16, August 27, and September 6). They observed the highest content of potassium, calcium, magnesium and nitrogen in the first stage of harvesting (July 27).

In general, the amount of chlorophyll a, chlorophyll b and total chlorophyll decreases with ripening. The results of Shekari's study (2009) indicate a regular and significant increase in chlorophyll up to 115 days after full flowering in Ahmed Aghaei and Ohadi and then a decrease in the value of this index was reported [22]. Kunter et al. (1994) investigated the effect of five harvest times with an interval of one week (starting from August 26) on Turkish pistachio cultivars. They reported that 3 is a ratio of chlorophyll a to b and found that there is a significant decrease in the amount of these chlorophylls in the delay in harvesting time, so that the green color of the kernel changes to yellowish green during ripening [29]. The results of Esmaeilpour and Shakerardekani (2018) indicated that the amount of chlorophyll was different at different harvest times. At the first time of harvest (July 27), the amount of chlorophyll a and chlorophyll b was the highest and declined over time [24]. Nazouri et al. (2013) reported that different stages of ripening have a significant effect on the amount of chlorophyll. at the first stage of harvesting, the highest amount of chlorophyll was observed, and then a significant decrease of this index was observed in the third stage of harvesting [4]. The gradual decrease of chlorophyll in ripe pistachios is the result of ripening changes and the activity of chlorophyll-decomposing enzymes (chlorophyllase and peroxidase), which significantly reduces the amount of chlorophyll after early August. Therefore, if pistachios are used to prepare green nuts for use in the confectionery industry, they should be harvested at this time.

Based on the results, the fiber content on a wet basis increased during the ripening time, so that the lowest amount was observed on the last 10th of August. In a research, the amount of fiber in ripe pistachios of Ohadi and Kerman cultivars was reported as 1.93 and 1.03%, respectively [30], but in another study, the amount of fiber in raw and roasted pistachios was 10.3 and 9.9 grams per 100 grams d.b, respectively. [31]. Various systems have been proposed for the classification of dietary fiber components. One of the accepted classifications for dietary fiber is based on their solubility in water and the ability to ferment in a laboratory system using an enzyme solution that represents the enzymes of the body's digestive system. Therefore, dietary fibers are divided into two categories: water-insoluble/less fermented fiber, such as cellulose, hemicellulose, lignin, and water-soluble fibers/well-fermented fiber, such as pectin, gum, and mucilage [32] . Pistachios are rich in insoluble fiber, so that 10% of pistachio weight is insoluble fiber and 0.3% is soluble fiber, and it is usually measured in pistachio insoluble fiber studies [31]

5. Conclusion

The results showed that the amount of moisture and chlorophyll a, b, and total in both varieties decreased during the ripening time until September 25th. Still, the amount of fat, protein, carbohydrate, and fiber increased in both varieties during the ripening time. So, the highest fat in both cultivars was observed in the last harvest. The amount of ash did not change significantly during ripening. The composition of the pistachio kernel changes during ripening, and considering these changes and according to the purpose of using pistachios, the appropriate harvesting time can be chosen. Regarding pistachio green kernels, early harvesting (early August) is recommended even though it has not yet reached the final weight due to their high economic value for use in the confectionery industry. Late September, due to the higher amounts of fat and carbohydrates in Pistachio, which improves the organoleptic properties, is the most appropriate harvest time for using pistachio for roasting, oil extraction, and pistachio butter. In general, determining changes in composition during ripening can be helpful in determining pistachio quality, developing its processing industry, and selecting superior pistachio genotypes for commercial cultivation.

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