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Changes of Contents of Polyphenols and Vitamin a of Organic and Conventional Fresh and Dried Apricot Cultivars *(Prunus armeniaca L.)*

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Abstract: Polyphenols are main functional components in apricots and can be separated into four groups as epicatechine, procyanidolic oligomers, chlorogenic acid and procyanidolic polymers which were found to be positive health effect. Conventional and organically grown fresh and dried apricot cultivars, Hacıhaliloğlu, Kabaaşı, Hasanbey and Zerdali type from the east part of Turkey, Malatya region, were analyzed in order to determine their phenolic and vitamin A contents. For the analyses, high performance liquid chromatography (HPLC) coupled with diode array detection was used. The concentrations of phenolic acid standards (ferrulic, *o*-coumaric, *p*-coumaric, caffeic, chlorogenic and ferrulic acids) and flavonoid standards ((+)-catechin, (-)-epicatechin, rutin besides vitamin A were used to determine characteristic differences among from the apricot cultivars. The polyphenol and vitamin A content of organically grown samples were found to be higher than the conventional cultivars in all samples ($p<0.05$). The most abundant phenolics in Hacihaliloğlu cultivar was quarcetin-3-rutinoside (rutine), whereas the less abundant phenolics was *p*-coumaric acid in Hasanbey cultivar. Though vitamin A content in different apricot cultivars varied the highest vitamin A level was found in Hacihaliloğlu cultivar. As a result, it was determined that types and concentrations of phenolics changed according to the different cultivars.

Key words: Apricot · Phenolics · Flavonoids · Conventional and organic agriculture · HPLC

INTRODUCTION

The Apricot (*Prunus armeniaca*, "Armenian plum" in Latin, syn. *Armeniaca vulgaris* Lam., Armenian: (**?????** "Tsiran") is a species of *Prunus*, classified with the plum in the subgenus *Prunus*. The native range is somewhat vague due to its widespread prehistoric farming, but most likely is India [1].

Although frequently thought of as a "subtropical" fruit, this is actually false-the Apricot is native to a [2], continental climate region with cold winters, although can grow in Mediterranean climates very well.

The tree is somewhat more cold-hardy than the peach, tolerating winter temperatures as cold as-30°C or lower if healthy. The limiting factor in apricot culture is spring frosts: They tend to flower very early, around the time of the vernal equinox even in the east locations spring frost often kills the flowers. Furthermore, the trees are sensitive to temperature changes during the winter season. In their native China, winters can be very cold, but temperatures tend to be more stable than in Turkey and especially the east Anatolia, where large temperature swings can occur in winter. The trees do need some winter cold (even if minimal) to bear and grow properly and do well in this location since spring frosts are less severe but there is some cool winter weather to tolerate a appropriate dormancy. The dry climate of these areas is best for good fruit production [3].

Apricot cultivars are most often grafted on plum or peach rootstocks. A cutting of an existing apricot plant provides the fruit characteristics such as flavor, size, etc. but the rootstock provides the growth characteristics of the plant. Apricots and plums can hybridize with each other and produce fruit that are variously called pluots, plumcots, or apriums.

There is an old adage that an apricot tree will not grow far from the mother tree. The implication is that apricots are particular about the soil conditions in which they are grown. They prefer a well-drained soil with a pH of 6.0 to 7.0. If fertilizer is needed, as indicated by

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yellow-green leaves, then 1/4 pound of 10-10-10 fertilizer should be applied in the second year. Granular fertilizer should be dotted underneath the branches of the tree. An additional 1/4 pound should be applied for every year of age of the tree in early spring, before growth starts. Apricots are self-compatible and do not require pollinizer trees, with the exception of the 'Moongold' and 'Sungold' cultivars, which can pollinate each other. Apricots are susceptible to numerous bacterial diseases including bacterial canker and blast, bacterial spot and crown gall. They are susceptible to an even longer list of fungal diseases including brown rot, Alternaria spot and fruit rot and powdery mildew. Other problems for apricots are nematodes and viral diseases, including graft-transmissible problems.

Turkey (Malatya region) is the leading apricot producer, followed by Iran. Seeds or kernels of the apricot grown in the east Anatolia and around Malatya are so sweet that they may be substituted for almonds. Cyanogenic glycosides (found in most stone fruit seeds, bark and leaves) are found in high concentration in apricot seeds. Laetrile, a purported alternative treatment for cancer, is extracted from apricot seeds. As early as the year 502, apricot seeds were used to treat tumors and in the 17th century, apricot oil was used in England against tumors and ulcers. However, in 1980 the National Cancer Institute in the USA described laetrile to be an ineffective cancer treatment [4].

In Europe, apricots were long considered an aphrodisiac and were used in this context in William Shakespeare's *A Midsummer Night's Dream* and as an inducer of childbirth, as depicted in John Webster's *The Duchess of Malfi*. Due to their high fiber to volume ratio, dried apricots are sometimes used to relieve constipation or induce diarrhea. Research shows that of any food, apricots possess the highest levels and widest variety of carotenoids. Carotenoids are antioxidants that help prevent heart disease, reduce "bad cholesterol" levels and protect against cancer. In conventional Chinese medicine, apricots are considered helpful in regenerating body fluids, detoxifying and quenching thirst. Some claim that the kernels also have healthy properties, including toning the respiratory system and alleviating a cough. However, the tip of the apricot holds a concentrated amount of the chemical laetrile, which can be upsetting to the system. The tips of the seeds should be removed and consumption should be limited to no more than five a day. Phenolic compounds are very suitable as chemotaxonomic markers and certain of them are characteristic to some species or varieties [5, 6], whereas quantitative differences may occur depending on fruit variety, stages of maturity, storage conditions [7-9] and the presence of the peel in fruit-based products [10]. For certain fruits characteristic phenolic compounds have been successfully used for the determination of adulteration of fruit nectars. Apricot varieties contain many phenolic compounds present in different concentrations. The hydroxycinnamic acid derivatives identified in apricot are caffeic, *p*-coumaric and ferrulic acids and their esters [11-13]. Other phenolic compounds determined in apricots are neochlorogenic acid (3-caffeoylquinic acid), (+)-catechin and (-)-epicatechin [14, 15], but chlorogenic acid (5-caffeoylquinic acid) is the dominant ester in apricots [16], Flavonols in apricot occur mostly as glucosides and rutinosides of quercetin and kaempferol and quercetin 3-rutinoside (rutin) is dominant [17]. Coumarins such as aesculetin and scopoletin have also been identified and quantified in small amounts and they were defined as chracteristic for apricot fruits [18-19]. In this study, the types and concentrations of the phenolic compounds in East Anatolia, Kabaaşı, Hasanbey, Hacıhaliloğlu apricot cultivars and Zerdali type, depends on conventional or organically grown conditions and also dried apricots of such varieties were investigated with high performance liquid chromatography (HPLC) technique in the determination of the apricot phenolic, Pressurized Liquid Extractions (PLE) was applied to extract phenolic from samples and analyzed by HPLC with diode array detection (DAD).

The major aim in our study was to characterization of similarities and differences, qualitative and quantitative levels of selected phenolic compounds of apricots obtained from the east of Anatolia, Malatya where the apricot harvesting is the major products and not only the over 90-95% of dried apricots exported all over the world from here, but also 50% of fresh apricots of Turkey’s production comes from Malatya; Hacıhaliloğlu, Kabaaşı, Hasanbey and Zerdali cultivars of conventionally or organically grown fresh and dried apricots were chosen as the main cultivars grown and their phenolic and vitamin A contents were evaluated.

MATERIALS AND METHODS

Plant Material: The apricots cultivar (*Prunus armeniaca*) used in all of the experiments were fresh or dried apricots harvested organically or conventionally. Fruits were harvested at deep-orange-1 (fully ripe), in Malatya Turkey. At each harvest date, we formed random lots,

each with 30 fruits. Immediately after picking, fruits were cut into small pieces, frozen in liquid nitrogen and stored at-80°C for subsequent analysis. Fruits used for polyphenol and vitamin isolation and characterization were harvested at the deep-orange-1 (fully ripe) stage. Immediately after picking, the unseeded cortex was cut into small pieces, frozen in liquid nitrogen, lyophilized and stored at-20°C until use. Dried samples were obtained fully ripped apricot samples

Chemicals and Standards: Chlorogenic, p-coumaric acid were obtained from Fluka (Neu-Ulm, Germany); (+)-catechin, (-)-epicatechin, quercetin-3-rutinoside, ferulic acid, vitamin A were obtained from Sigma (Deisenhofen, Germany); caffeic acid was obtained from Merck (Darmstadt, Germany). HPLC grade Ethanol, methanol, acetonitrile chloroform, acetone, hexane, acetic acid, 1, 2-dichloroethane, anhydrous sodium sulphate, *tert*butylhydroquinone (*t*BHQ).

Analysis of Polyphenols

Extraction: The polyphenols in examined samples were extracted using a procedure described by Dragovic-Uzelac based on the method of Bengoecha *et al.* [20] Each apricot fruit puree (50 g) was mixed with 50 mL methanol/HCl (100:1, v/v) which contained 2% *t*BHQ, in inert atmosphere (N_2) during 12 h at 35 °C in dark. The extract was then centrifuged at 4000 rpm min^{-1} and supernatant was evaporated to dryness under reduced pressure (35-40 °C). The residue was dissolved in 25 mL of water/ethanol (80:20, v/v) and extracted four times with 25 mL of ethyl acetate. The organic fractions were combined, dried for 30-40 min with anhydrous sodium sulphate, filtered through the Whatman No. 40 filter (Whatman International Ltd., Kent, England) and evaporated to dryness under vacuum (35-40 °C). The residue was dissolved in 1 mL of methanol/water (50:50, v/v) and filtered through 0.45 µm filter (Nylon Membranes, Supelco Inc., Bellefonte, PA, USA) before injected (20 µL) into the HPLC apparatus. Samples were extracted in triplicate.

Pressurized Liquid Extraction and Sample Preparation: Pressurized liquid extractions were performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA, USA) system. Percentages of methanol and water in the solvent, temperature, pressure and static extraction time were the parameters under study. The pre-set default conditions were as follows: pre-heating period, 5 min; solvent flush volume, 6 0% of the extraction cell volume; number of extraction cycles, 3; purge, 90 s using pressurized nitrogen (99.995% of purity, 150 p.s.i.); and collection, in 60 ml glass vials with teflon coated rubber caps (I-CHEM, New Castle, DE, USA). The solvent used was previously degassed in order to avoid the oxidation of the analytes under the operating conditions. Optimum extraction conditions were determined as 1500 psi pressure, 40 C° temperatures and an hour application time in PLE system. PEE (1g) was mixed in gradient conditions with (methanol/water/hydrochloric acid; 75:20:5) which contained 2% tBHQ in 11-or 22-ml stainless steel extraction cell. Then, each one was filtered through a 0.45 µm nylon membrane (Lida, Kenosha, WI, USA) and transferred to a 50 ml volumetric flask. Solvents was evaporated to dryness in a Turbovap LV Evaporator (Zymark, Hopkinton, MA, USA) provided with a nitrogen stream in a water bath at 40°C. The residue was reconstituted in (2 ml) methanol-water-aqueous (50:50, v/v) and which was brought up to its volume with methanol-water filtered through a 0.45 µm PTFE filter (Waters, Milford, CA, USA) prior to injection into the HPLC system.

HPLC Analysis: Polyphenol analysis were performed on a Agilent Series 1100 liquid chromatography, equipped with a vacuum degasser, a quaternary pump and a Agilent 1100, G 1315B DAD detector, connected to a HP ChemStation software. A reversed-phase ACE 5 C18 A11608 (250x4.6mm,4 µm) column were used. The content of solvents and used gradient elution conditions were previously described by Uzelac and others [21]. For gradient elution mobile phase a contained 3% acetic acid in water; solution B contained mixture of 3% acetic acid, 25% acetonitrile and 72% water. The following gradient was used: 0-40 min, from 100% A to 30% A, 70% B with flow rate 1 mL/ min; 40-45 min, from 30% A, 70% B to 20% A, 80% B with flow rate 1 mL/ min; 45-55 min, from 20% A, 80% B to 15% A, 85% B with flow rate 1.2 mL /min; 55-57 min, from 15% A, 85% B to 10% A, 90% B with flow rate 1.2 mL /min; and 57-75 min 10% A, 90% B with flow rate 1.2 mL/ min. Operating conditions were as follows: column temperature, 30 °C, injection volume, 20 µL, UV-VIS photo diode array detection at 280 nm. Detection was performed with UV-VIS photo diode array detector by scanning spectra from 210 to 360 nm. Stock standard solutions at a concentration of 1 mg ml^{-1} were prepared in methanol/water (1:1) and stored at 4°C in darkness. Different range calibration curves for different polyphenol components in apricots samples were used.

Table 1: HPLC Analysis Flow Chart

Time (Min.)	Solvent A	Solvent B	Elution Time (ml/min)	Temp. (°C)	Wavelength (nm)
1	100	0	1	30	280,290,355,310,329
40	30	70	1	30	280,290,355,310,329
40-45	20	80	1	30	280,290,355,310,329
45-55	15	85	1.2	30	280,290,355,310,329
55-57	10	90	1.2	30	280,290,355,310,329
57-75	10	90	1.2	30	280,290,355,310,329

Identification of phenolic compounds was carried out by comparing retention times and spectral data with those of authentic standards. Quantitative determinations were carried out using calibration curves of the standards. The compounds of propolis samples are identified and listed in Table 1. As mg/kg PEE. Data are reported as mean ± SD for at least three replicates.

HPLC Analysis of Vitamin A: Five mL supernatant was taken to 25 mL tubes with caps and 5% KOH solution was added. After it was vortexed, it was kept at 85°C for 15 min. The tubes were then taken and cooled to room temperature and 5 mL of pure water was added and mixed. Lypophilic molecules that didn't saponify were extracted with 2x5ml methanol/hexane. The phase was evaporated with nitrogen flow. It was dissolved in 1 mL (50+50% v/v) acetonitrile/methanol mixture and then analyzed with HPLC.

The mobile phase was acetonitirile/ methanol/ chloroform (47:42:11 v/v), the flow rate was determined to be 1 mL and a Agilent 1100, G 1315B DAD detector, connected to a HP ChemStation software. A reversed-phase ACE 5 C18 A11608 (250x4.6mm,4 µm) column were used

Detection was performed with a UV diode array detector by scanning from 210 to 360 nm. Identification of phenolic compounds was carried out by comparing retention times and spectral data with those of authentic standards. Identified phenolic compounds were quantified using the external standard method and quantification was based on peak area. Calibration curves of the standards were made by diluting stock standards in methanol to yield 2-30 mg L^{-1} (ferrulic acid), 5-50 mg L^{-1} (chlorogenic acid and catechins), 5-30 mg L^{-1} (caffeic and *p*-coumaric acid) and 2-20 mg L^{-1} rutin. Procyanidin B_2 and kaempferol 3-rutinoside were identified only by polarity and spectral data from the literature and they were not determined quantitatively except for kaempferol 3-rutinoside, which was quantified as quercetin 3-rutinoside. The samples were prepared and analyzed in triplicate. Data presented are mean ± standard deviation (SD).

Analytical Quality Control: Recoveries were measured by adding known amounts of each standard (2-25 mg L^{-1}) to apricot nectars or jams prior to extraction. In the calculation of final results, no correction for recovery was applied to the data. By analyzing dilution series of pure standard solutions ranging from 0.05 to 2 mg L^{-1}, minimum detectable quantities were determined for the phenolics.

RESULTS AND DISCUSSION

After extraction, recoveries of single phenolic compounds were tested. A known amount of each standard was added to apricot after which they were extracted as previously described under Materials and Methods. The recoveries were good or tolerable (71-94%) for most of the phenolic compounds studied in this work. All phenolic compounds showed a linear response within the range studied of 2-50 mg L^{-1} (r = 0.985-0.999). The following limits of detection were estimated using a signal-to-noise ratio of 4: 0.08 mg L^{-1} by *p*-coumaric acid, chlorogenic acid and (+)-catechin; 0.1 mg L^{-1} by caffeic acid and (-)-epicatechin; and 0.15 mg L^{-1} by ferrulic acid and rutin.

Phenolic extracts obtained from the raw and dried fruits grown conventionally or organically were HPLC analyzed using a UV photodiode array detector to record the UV spectra of the separated phenolic compounds. The phenolic compositions and vitamin A level of fresh, dried, organically and conventionally grown apricots are shown in Figure 1. Among all apricot cultivars (+)-catechin levels (Figure 1A) were found to be high in all fresh cultivars Kabaaşı, Hacıhaliloğlu, Hasanbey and Zerdali which were cultivated as organically ($p < 0.05$). When catechin levels of samples were investigated in terms of cultivation type, the catechin level decreased fresh > dried simultaneously in all samples. The most decrease in catechin level was in Zerdali cultivar, yet the highest level of catechin in all fresh samples was in Hacıhaliloğlu cultivar, 22.9 µg/dry sample, whereas the lowest level of catechin was found to be in Hasanbey cultivar, 5.2/ µg/dry sample. Either grown organically or conventionally the catechin level was varied, but in Hasanbey cultivar the level was the lowest.

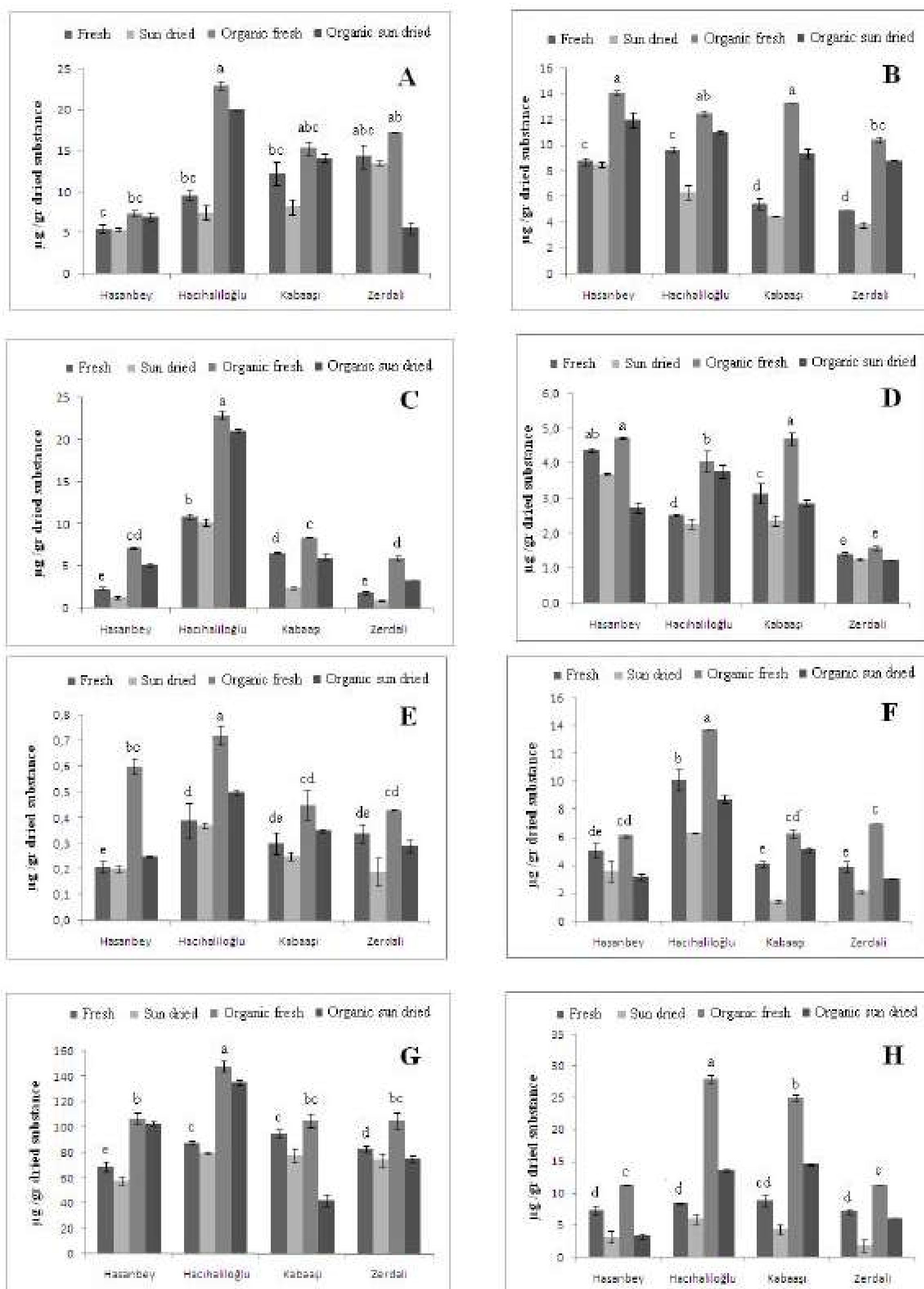

Fig. 1: (A) Catechin (B)Epicatechin (C)Ferrulic acid, (D) Caffeic acid, (E) p-coumaric acid, (F) chlorogenic acid, (G) Rutin, (H) Vitamin A level in Organic or Conventional Fresh and Sun Dried Apricots
Values are means ±SD (*n*=3), and they are given as µg/g of investigated samples

When epicatechin level was scrutinized in all samples (Figure 1B), in terms of harvesting type either grown organically or conventionally the difference in epicatechin level was relevant ($p < 0.05$) and when sundried samples in concern the level was decreased in all samples. Conventionally grown Hacıhaliloğlu samples showed over 30% decrease in epicatechin level the decrease in catechin level among other cultivar was the highest in Hacıhaliloğlu cultivar. In all fresh samples, Hasanbey showed the highest level of epicatechin 14.1 µg/dry sample, the lowest value was in the Zerdali 4.8 µg/dry sample.

In terms of ferrulic acid content (Figure 1C) in fresh samples as being Organic Hacıhaliloğlu> Conventional Hacıhaliloğlu> Organic Kabaaşı > Organic Hasanbey > Conventional Kabaaşı > Organic Zerdali > Conventional Zerdali and the difference in the ferrulic acid content was found to be relevant ($p < 0.05$). Organic Hacıhaliloğlu had the highest level of ferrulic acid as 22.9 µg/dry sample, whereas in Zerdali, 1.8 µg/dry samples, was the lowest. However, conventional Hacıhaliloğlu cultivar showed the ferrulic acid content of 10.9 µg/dry sample and the difference was rationalized statistically acceptable ($p< 0.05$). When the samples were sun dried, ferrulic acid level decreased in all, but the relevant difference was in Hasanbey and Zerdali which was about over 50%.

Figure 1D shows the caffeic acid level in all samples. The lowest level was in organic and conventional Zerdali, 1.4 µg/dry sample. The highest level was in organic Hasanbey and organic Kabaaşı cultivars, 4.7 µg/dry sample and the difference was relevant statistically ($p < 0.05$). When fresh samples were used the caffeic acid level as follows; Organic Hasanbey > Organic Kabaaşı > conventional Hasanbey> Organic Hacıhaliloğlu > Conventional Kabaaşı > Conventional Hacıhaliloğlu > Organic Zerdali >Conventional Zerdali. Sundried samples showed decrease in caffeic acid level in all samples, yet the highest loss was observed in Hasanbey nearly 50%.

p-coumaric acid (Figure 1E) content in all samples showed the following results; among the polyphenols the lowest value was *p*-coumaric acid in all samples. In fresh sample Hacıhaliloğlu variety showed 0.7 µg/dry sample, Hasanbey showed 0.2 µg/dry sample. When sundried all samples showed decrease in *p*-coumaric acid level, but the highest loss was in Organic Hasanbey 58%.

Chlorogenic acid level varied in all samples (Figure 1F), Organic Hacıhaliloğlu showed the highest value of 13.7 µg/dry samples, whereas the lowest value was in Zerdali which was about 03.8 µg/dry sample. Conventional Hacıhaliloğlu had the value of 10.0 µg/dry sample which was higher than Organic Kabaaşı, Hasanbey and Zerdali and the differences among them was statistically relevant ($p< 0.05$).

The rutin level, (Figure 1G), was the highest in all apricot samples and the difference in all samples was relevant ($p< 0.05$). Hacıhaliloğlu showed the highest value, 148.2 µg/dry samples, conventional Hasanbey showed 69.1 µg/dry samples. The highest loss among sun dried samples was in Organic Kabaaşı, which was about 44%.

Vitamin A level when investigated the results could be rationalized (Figure 1H); Organic Hacıhalioğlu cultivar, 27.9 µg/dry sample, conventional Zerdali, 7.1 µg/dry sample. It was noticeable that vitamin A level has been affected by the sun more than phenolics.

The products obtained by conventional cultivation techniques showed decrease in phenolics, these results were debatable among scientists. The quality of soil and fertilizer level has pronounced effect on phenolic content of the products. In this study, organically or conventionally grown Hasanbey, Hacıhaliloğlu, Kabaaşı and Zerdali cultivars were investigated in terms of phenolics. The concentrations of phenolic acid standards (ferulic, *o*-coumaric, *p*-coumaric, caffeic, chlorogenic and ferrulic acids) and flavonoid standards ((+)-catechin, (-)-epicatechin, rutin besides vitamin A were investigated. The level in terms of both phenolics was found to be varied in all samples and the difference among the cultivars was given. The highest level among polyphenols was rutin, whereas the lowest value was found to be p-coumaric acid. Polyphenol type and the contents varied from one cultivar to the other, these differences could be rationalized in terms of tanning and soil properties, ripeness and the region were the fruit was harvested. The vitamin a level was also was investigated and the results showed that the level was higher in fresh fruit than the sundried fruits in all samples. The decrease in polyphenol and vitamin A in all apricot samples of different cultivar was varied.

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