ELSEVIER

Food Chemistry 83 (2003) 205–212

Food Chemistry

www.elsevier.com/locate/foodchem

The vitamin and selenium contents of apricot fruit of different varieties cultivated in different geographical regions

Omer Munzuroglu[a,*], Fikret Karatas[b], Hikmet Geckil[c]

[a]*Department of Biology, Faculty of Science, Firat University, Elazig 23119, Turkey*
[b]*Department of Chemistry, Faculty of Science, Firat University, Elazig, Turkey*
[c]*Department of Biology, Inonu University, Turkey*

Received 21 August 2001; received in revised form 29 January 2003; accepted 29 January 2003

Abstract

High-pressure liquid chromatographic and fluorometric methods were utilized to determine the vitamin (A, C, E and β-carotene) and selenium levels in apricot fruit. The vitamins (A, C, and E), β-carotene and selenium levels of both fresh and sulphurized (sulphur-dried) apricot fruit from several common cultivars and wild type, grown in different regions, were analysed. The effects of post-harvest processing, regional difference and cultivar type on the levels of these nutrients were investigated. Cultivated varieties possessed significantly higher vitamin C ($P<0.01$), vitamin A and E ($P<0.05$) than the wild type. The vitamin C levels of fresh and sulphur-dried apricot from cultivated forms, for example, were 1.8–2.7- and 2.9–4.6-fold, respectively, higher than those of wild type ones. Among cultivated varieties, H cultivar was determined to have significantly highest vitamin A, E and β-carotene contents ($P<0.05$). In general, the vitamin, β-carotene and selenium levels were determined to be significantly different, both between varieties and between different regions within the same variety. Cultivars in region ES, a high altitude region, for example, had significantly ($P<0.05$) higher vitamin C content than the same cultivars grown in other regions. The selenium content, however, was significantly ($P<0.01$) higher only in fresh fruit from variety HB in region ES than the other two regions.

Keywords: Apricot; β-Carotene; HPLC; *Prunus armeniaca*; Selenium; Vitamins

1. Introduction

The anticarcinogenic and antioxidant properties of vitamins A, C, E, β-carotene and the essential element selenium are relatively newly known. Vitamins C, E, selenium and carotenoids may act independently or in combination as anti-cancer or cardioprotective agents by a variety of mechanisms. One such protective mechanism, attributed to vitamin C and E and the carotenoids, is antioxidant (radical-scavenging) activity (Rice-Evans, Miller, & Paganga, 1997). Vitamin C is known as the most important vitamin in fruits and vegetables for human nutrition (Lee & Kader, 2000). Selenium, functioning as part of glutathione peroxidase, has been recognized as a cellular antioxidant in addition to its protecting function against heavy metal toxicity (Al-Saleh & Al-Doush, 1997; Oster & Prellwitz, 1989). It has long been known that fruits constitue a rich source of vitamins and minerals. Among fruits, apricot is one of the least studied. Despite wide cultivation in many parts of the world, the favourable climatic and geographical factors in the Malatya-Elazig region, of eastern Turkey, is particularly important for apricot production and processing. According to recent statistics, more than half of the country's 500,000 t of fresh fruit and almost all sulphur-dried apricot production originates from this region. This figure is about 10% of world fresh apricot production. More importantly, about 80% of world dried apricot exports comes from this region (Asma, 2000; State Institute of Statistics 1998). As with many other fruits, the nutritional importance of this fruit was only recently realized and, since then, numerous investigations have been carried out on both fresh and dried forms, focussing mainly on their carbohydrate, amino acid and, to a lesser extent, mineral and vitamin contents (Belloso & Barriobero,

* Corresponding author. Tel.: +90-424-237-0000x6001; fax: +90-424-237-0062.
E-mail address: omunzuroglu@firat.edu.tr (O. Munzuroglu).

0308-8146/03/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/S0308-8146(03)00064-5

2001; Watt & Merrill, 1975). A study by Paunovic (1985) showed that 100 g of dried apricot contained 5 g of protein, 0.5 g fat, 66.5 g carbohydrate, 108 mg phosphorus, 979 mg potassium, and 12 mg vitamin C. Although, the chemical composition and nutritional value of apricot have been subject to many studies, and there are data on mineral and vitamin contents of apricot in food composition charts the levels in raw material and processed fruit have not been related (Belloso & Barriobero, 2001; Watt & Merrill, 1975). Furthermore, there has been no report, to our knowledge, on the vitamin E and selenium contents of this fruit. It has been determined that the levels of vitamins and especially minerals in fruits can vary greatly from region to region due to the content of elements in the soil (Al-Saleh & Al-Doush, 1997). The objective of this study was to expand the analysis by high-performance liquid chromatography (HPLC) of vitamins (A, C, and E) and β-carotene and fluorometry to include selenium contents of fresh and processed (sulphurized) apricot fruit. Both fruit types were from six common cultivars and wild-type grown in three geographical regions. Therefore, besides the determination of the levels of these compounds through utilization of two highly sensitive methods, other objectives of this study were to investigate how cultivar type, post-harvest processing, and regional difference were contributing factors to different levels of these compounds.

2. Materials and methods

2.1. Materials, chemicals and reagents

The apricot fruit from six common cultivars and wild type apricot (*Prunus armeniaca* L.) in three different regions was used in both fresh and sulphur-dried forms. Fresh apricot fruit, from similar ripening periods (ready to be sulphurized), were collected by random sampling, covered with aluminium foil, transported to laboratories and immediately subjected to analysis as it is known that maturity level is one of the major factors determining the compositional quality of fruits and vegetables (Lee & Kader, 2000). Sulphurized apricots were from local markets of each region. When not indicated otherwise, dry apricot, throughout, refers to sulphur-dried or sulphurized apricot. The apricot varieties selected for this study are known by their local names: Hacihaliloglu, Soganci, Hasanbey, Kabaasi, Cataloglu and Cologlu, abbreviated in this study as H, S, HB, KA, CAT, and COL, respectively, collected from regions, EK (Elazig-Kadikoy), ES (Elazig-Sahindere) and MT (Malatya-Tavsantepe).

Standards for vitamins, β-carotene and selenium were purchased from Sigma Chemical Co. Acetonitrile, *n*-hexane, methanol, chloroform, ethanol, nitric acid, perchloric acid were from Merck Chemical Co. All other chemicals and reagents used were of analytical grade. All glassware was acid-washed and rinsed with doubly distilled deionized water (ddH_2O). The recovery rates of standards were determined as 97.8% for vitamin A, 99.5% for vitamin E, 96.4% for β-carotene and 95.0% for selenium. Separation times, using a flow rate of 1 ml/min, were 1.9 min for retinoic acid, 3.1 min for retinol (sum of both given as vitamin A), 3.3 min for vitamin C, 5.5 min for vitamin E and 12.0 min for β-carotene.

2.2. Vitamin analysis

Both fresh and dried apricot samples (50 g) were mashed in a homogenizer and 2 g homogenate paste per sample were taken for extraction of vitamins A, E, β-carotene and 1 g per sample was taken for extraction of vitamin C. To the above homogenates, 4 ml of ethanol were added, vortexed and the mixture centrifuged (Mistral© 2000) at 2000 rpm for 3 min at 4 °C. The supernatant was also filtered through a Whatman No.1 paper, and to the filtrate 0.15 ml *n*-hexane was added and mixed. Vitamins A, E and β-carotene were extracted twice in the hexane phase and the collected extract was dried under a stream of liquid nitrogen. Dried extract was solubilized in 0.2 ml methanol for HPLC. Injections were made in duplicate for each sample. The quantification (Catignani, 1983; Miller, Lorr, & Yang, 1984) utilized absorption spectra of 326, 296 and 436 nm for vitamins A, E, and β-carotene, respectively. HPLC separations were accomplished at room temperature with a Perkin-Elmer liquid chromatograph system (Series 1100), consisting of a sample injection valve (Cotati 7125) with a 20 μl sample loop, an ultraviolet (UV) spectrophotometric detector (Cecil 68174), integrator (HP 3395) and a Techsphere ODS-2 packed (5 μm particle and 80 Å pore size) column (250×4.6 i.d.) with a methanol: acetonitrile: chloroform (47:42:11, v/v) mobile phase at 1 ml min^{-1} flow rate.

The extraction of vitamin C was according to the method of Cerhata, Bauerova, and Ginter (1994). To 1 g homogenized apricot paste, 1 ml of 0.5 M perchloric acid was added, vortexed and the volume adjusted to 5 ml by adding ddH_2O. The mixture was centrifuged at 5000 rpm for 8 min at 4 °C. The supernatant was filtered, as earlier, and the vitamin C level was determined using the method of Tavazzi, Lazzarino, Di-Pierro, and Giardina (1992) by HPLC, utilizing a column (250×3.9 i.d.) packed with Tecopak C18 reversed-phase material (10 μm particle size) with mobile phase (3.7 mM phosphate buffer, pH 4.0) at 1 ml min^{-1} flow rate.

2.3. Selenium analysis

The apricot samples for selenium determination were utilized as follows: to 2 g apricot samples (homogenate

paste), 5 ml nitric acid: perchloric acid (1:5, v/v) were added and the mixture was held in a Teflon bomb at 100 °C for 12 h for breaking the organic material (Breyer & Gilbert, 1987) and then cooled down to room temperature. Mixture was transferred into tubes and a 4.0 N HCl concentration was achieved by adding concentrated HCl (about 2 ml). The mixture was held at 90 °C for 15 min to reduce Se (VI) to Se (IV). To this mixture, 2 ml 2.5 M formic acid, 5 ml 0.1 M EDTA and 1.5 ml freshly prepared 3,3-diaminobenzidine (DAB) solution (1 mg/ml) were added and the pH was adjusted to 1.7 with 4 N NH_3. this was left to stand in the dark for 1 h for the formation of a metal–ligand complex. The mixture was made to 50 ml by adding ddH_2O and 5 ml toluene and mixed for 2 min. The mixture was transferred into a volumetric separation funnel and stood at room temperature for 2 min for phase separation. Selenium was separated in the toluene phase and its level was determined fluorometrically by a Perkin-Elmer 100 fluorescence spectrophotometer at 570 nm, using Standard Addition Method (Watkinson, 1960; Whetter & Ullrey, 1978). Minimum detectable level for selenium was 1 ng.

2.4. Statistical analysis

Data are the results from four separate analyses in duplicate. Statistical analysis was based on SPSS (version 10.0) program. In order to detect the significance of differences ($P<0.01$ or $P<0.05$) of variables a one-way ANOVA test was carried out. All values are expressed as mean ± SDEVs (σ_{n-1}).

3. Results

The levels of vitamins (A, C, and E), β-carotene and selenium in fresh and sulphur-dried apricot fruit from six common cultivars and wild-type (WT) grown in three different geographical regions are summarized in Figs. 1 and 2. The vitamin, β-carotene and selenium levels of both fresh and sulphur-dried apricot were determined to be dependent, both, on variety and region of cultivation. The mean vitamin levels per gramme of fresh apricot from all cultivars, including WT one, were 0.149 μg vitamin A (within the range of 0.109–0.192 μg), 62 μg vitamin C (47.9–71.4 μg, except WT one which showed 26 μg vitamin C level per g fresh apricot), 0.251 μg vitamin E (0.189–0.328 μg) and 16.6 μg β-carotene (14.6–20.2 μg). The mean selenium per gramme fresh apricot was 0.108 μg, within the range of 0.084–0.124 μg.

The levels of vitamins, β-carotene and selenium in sulphur-dried apricot from all varieties were (per gramme); 0.595 μg vitamin A (range: 0.409–0.820 μg), 192 μg vitamin C (range: 142–224 μg, except the WT which had a 48.7 μg vitamin C g^{-1} sulphur-dried apricot), 1.10 μg vitamin E (range: 0.884–1.34 μg) and 55.0 μg β-carotene (range: 45.4–65.5 μg). The selenium content was determined to be 0.312 μg g^{-1} sulphur-dried apricot in the range of 0.26–0.36 μg.

Fruit from WT, in general, was determined to have the lowest content of vitamins, β-carotene and selenium. Fruit (both fresh and sulphur-dried) from culture varieties showed significantly higher vitamin C ($P<0.01$), A and E ($P<0.05$) contents than fruit from WT (Figs. 1 and 2). Fruit from cultivars contained several-fold more vitamin C than fruit from WT (1.8–2.7-fold for fresh and 2.9–4.6-fold for sulphur-dried fruit). Among cultivars, both fresh and sulphur-dried fruit from variety H contained significantly more vitamin A, E, and β-carotene than other cultivars ($P<0.05$). When regional difference was considered, fresh fruit from apricot cultivars in region ES (Fig. 1a) had a significantly higher vitamin C ($P<0.05$) content than the same cultivars in the other two regions (Fig. 1b and c). Also, the vitamin C level of fresh apricots from regions ES and MT was significantly higher ($P<0.05$) than apricots from region EK. The level of this vitamin in CAT variety fruit from ES region was significantly higher than the same variety from the other two regions. Also, fresh fruit from varieties H and HB in region ES showed significantly ($P<0.05$) higher vitamin C content than the same varieties from region MT. The vitamin A content of CAT variety was also significantly higher in region ES cultivars than that of the same variety in region MT ($P<0.05$). The fruit from H variety in region ES had significantly higher vitamin E than this variety in both regions EK and MT ($P<0.05$). With the exception of significantly ($P<0.05$) higher selenium content of fresh fruit of variety HB grown in region ES (Fig. 1a), the level of this metal did not show any significant difference ($P>0.05$) between other varieties cultivated in different regions.

The sulphur-dried apricot from WT variety in region ES (Fig. 2a) and MT (Fig. 2c) was determined to have significantly higher ($P<0.01$) vitamin C content than the same variety in region EK (Fig. 2b). The vitamin C and β-carotene levels of HB and H varieties, respectively, from ES region were significantly ($P<0.05$) higher than the same varieties from regions EK and MT. The KA variety in region ES had a significantly higher β-carotene content than this variety in region EK ($P<0.05$). The variety S, in region ES, showed significantly higher vitamin A ($P<0.05$) and vitamin E ($P<0.01$) levels than the same variety in both other regions. The selenium level of KA from region ES was significantly higher than the selenium level of this variety from other regions ($P<0.05$).

Also, vitamins (A, C, and E), β-carotene and selenium levels of fresh and sulphur-dried apricot from six cultivars were compared to the wild-type one, which is still widely cultivated in these regions. Per cent differences of these compounds in cultivated culture varieties compared

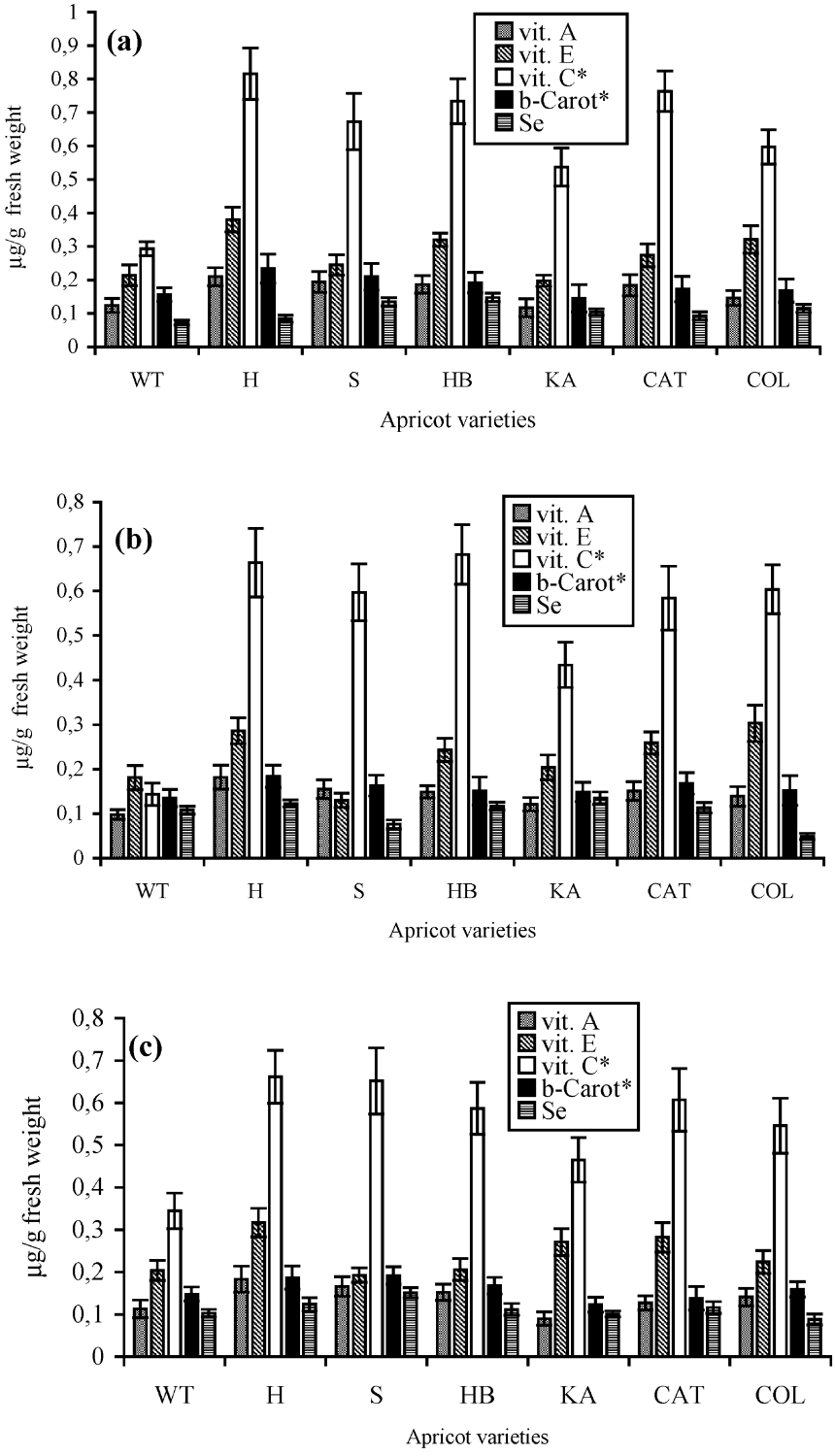

Fig. 1. The vitamin (A, E, C), β-carotene and selenium contents of fresh apricot fruit of commonly cultivated varieties and wild-type grown in region ES (a), EK (b), and MT (c). Error bars indicate standard deviations (σ_{n-1}) of $n=4$. *The vitamin C and β-carotene$\times 10^{-2}$.

with WT fruit are given in Figs. 3 and 4. In general, the cultivar types showed substantially higher level of vitamins, β-carotene and selenium than the WT. Among cultivars, variety H was determined to have the highest content of vitamins and β-carotene.

4. Discussion

The study carried out here compares the vitamin, β-carotene and selenium levels of apricot fruit of six commonly cultivated apricot varieties and the wild type

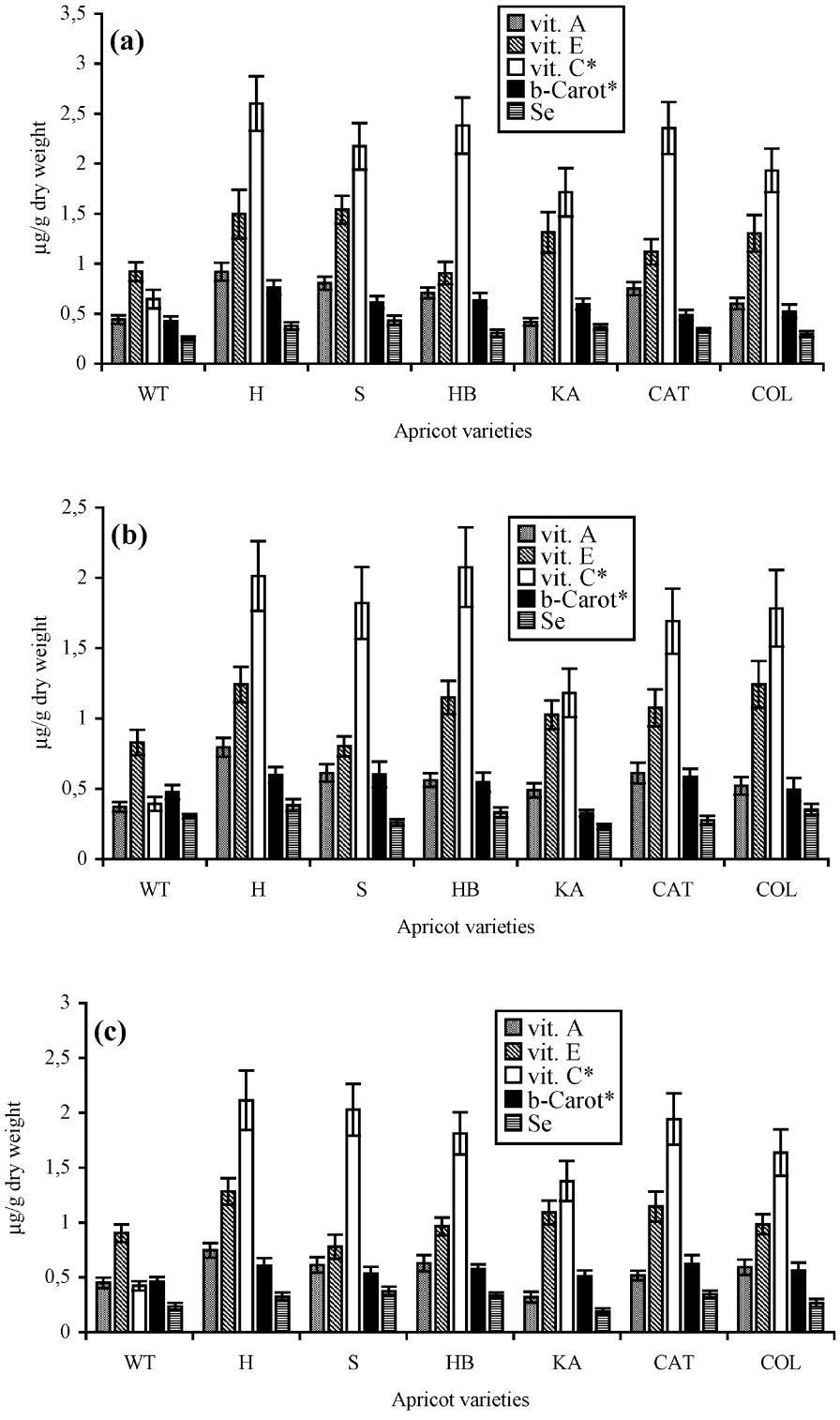

Fig. 2. The vitamin (A, E, C), β-carotene and selenium contents of sulphur-dried apricot fruit of cultivated varieties and wild-type grown in region ES (a), EK (b), and MT (c). Error bars indicate standard deviations (σ_{n-1}) of $n=4$. *The vitamin C and β-carotene$\times 10^{-2}$.

one grown in three different geographical regions. Also, sulphur-dried apricots were analysed for their vitamin, β-carotene, and selenium levels in order to determine how post-harvest processing affected their levels. In general, regional difference was a big factor determining the level of these compounds and selenium. The results in this study showed that post-harvest processing (sulphur-drying), however, did not cause an important loss in vitamin, β-carotene, or selenium levels. Although, the vitamin, and β-carotene and selenium levels (per

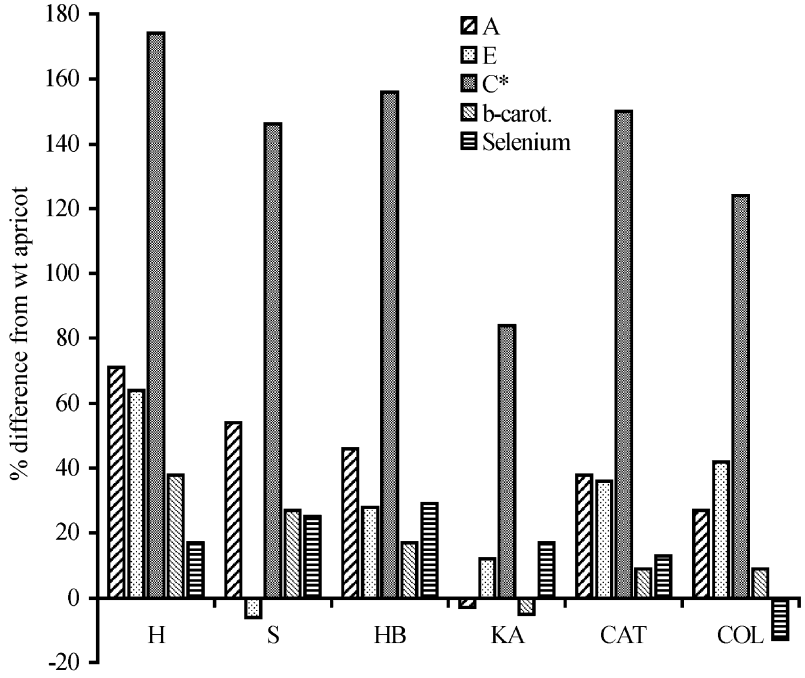

Fig. 3. The% differences of vitamin A, E, C, β-carotene and selenium levels in fresh apricot fruit of cultivated forms compared with wild-type fruit. *Vitamin C values were divided by a factor of 2.0 to bring the values into the *y*-axis scale.

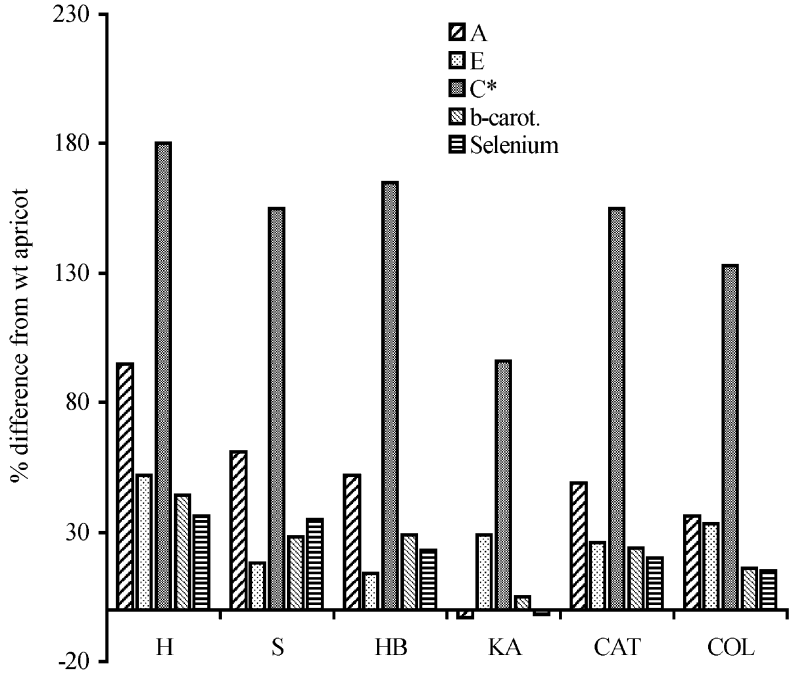

Fig. 4. The% differences of vitamins A, E, C, β-carotene and selenium levels in sulphurized apricot fruit of cultivated forms compared with wild-type fruit. *Vitamin C values were divided by a factor of 2.0 to bring the values into the *y*-axis scale.

gramme fruit weight) were 3–4-fold higher in dried fruit than in fresh fruit, the values found for fresh and dried fruit are comparable, since it is known that sulphur-drying causes an average of 3.75-fold weight reduction in fresh apricot (Asma, 2000) in varieties studied here. One notable difference was that, fruit (both fresh and sulphur-dried) of widely cultivated varieties showed significantly higher vitamin C ($P<0.01$), A and E ($P<0.05$) contents than fruit from WT. Fruit from commonly cultivated apricots contained 1.8–2.7-fold (for fresh fruit) and 2.9–4.6-fold (for dried fruit) more vitamin C than fruit from WT apricots. The substantial

reduction in vitamin C content of sulphur-dried fruit from the WT one compared with sulphur-dried fruit of cultivated varieties is due to excess liquid leakage during processing of WT fruit, causing considerable loss of this water-soluble vitamin. The pomological characteristics of fruit from WT might contribute to excess liquid loss during post-harvest processing. Compared with fruit from cultivated varieties studied here, fruit from WT is known to have a thinner skin, lower total soluble solids (TSS) and higher water content (Asma, 2000).

In general, selenium levels of apricot fruit of the same variety grown in different geographical regions were similar. With the exception of several similar vitamin values, vitamin and β-carotene contents of fruit of a specific variety grown in region ES were generally higher than the fruit from the same variety grown in the other two regions. However, this difference was significant ($P<0.05$) for vitamin C only in sulphur-dried fruit from H cultivar grown in this region compared with the same cultivar in regions MK and MT. Region ES has the highest altitude (1550 m from sea level) of three regions: regions MK and MT at 1020 and 1100 m, respectively. It is has been suggested that, the level of vitamins is increased in some vegetables with increasing altitude. In such regions, natural factors, such as radiation, temperature difference between day and night, limited water and mineral source and wind are only several of many conditions that plants have to resist. At the metabolic level, plants resist these changes by increasing the production of several vitamins and minerals with antioxidant properties (Munzuroglu, Karatas, & Gur, 2000). Also, pomological characteristics of this fruit (H variety) might favour post-harvest processing, since, both fresh and sulphur-dried fruit from this variety, from all three regions, showed higher vitamin levels than fruit from any other cultivar. Furthermore, Asma (2000) reported that fruit from variety H possessed a higher TSS/water ratio and lower acidity than fruit of other cultivated varieties and WT.

The mean vitamin A + β-carotene levels in apricot fruit of all varieties (including WT one) were determined as 28.1 (range: 24.7–34.2) in terms of I.U. (International Unit) equivalents (Sant'Ana, Stringheta, Brandao, & de Azeredo, 1998) for fresh apricot fruit and 93.4 I.U. (range: 77.5–112) for sulphur-dried apricot fruit. These values were found to be in agreement with values found for apricot fruit (Amotz & Fishler, 1998). Thus, the amount of vitamin A, plus its precursor β-carotene, in 200 g of fresh or 50 g of dried apricot fruit is more than enough to satisfy the dietary allowance of a male for vitamin A, established as 5000 I.U./day (USFNB, 1989).

The different levels of vitamins and minerals in apricot fruit of the same variety, or from different cultural forms of a variety, might be genetically as well as environmentally determined conditions. Furthermore, the collection of fruit, their ripening levels and differences in post-harvest processing are important factors for the observed differences in vitamin and mineral levels. Thus, differences found for vitamin A, C, E and β-carotene contents of fruit of varieties studied or of the same variety grown in different region can be attributed to these variables.

When the selenium values of fresh and sulphur-dried apricot fruit are compared with other fruits (or vegetables) (Hussein & Bruggeman, 1999; Kadrabova, Madaric, & Ginter, 1997), this fruit can be considered to be a rich source of selenium. The selenium content of this fruit (fresh) was determined to be considerably (up to five-fold) higher than values found for any vegetable or fruit to date. From a geographical point of view, there is a great variation in the selenium content of vegetables and fruits. This is due mostly to the content of this element in the soil. Also, it is known that, selenium is found in the protein fraction of foods, and plants with low protein contents are poor sources of this element (Al-Saleh & Al-Doush, 1997; Kadrabova et al., 1997). Vegetables and fruits contain trace amounts of selenium (1–33 μg kg^{-1}) compared with grains, meat and poultry. The richest sources of selenium are chicken (323 μg kg^{-1}), eggs (166 μg kg^{-1}) and fish (306 μg kg^{-1}) (Hussein & Bruggeman, 1999).

Results in this study showed that, apricot fruits of cultivated varieties are rich in vitamins A, C and β-carotene and selenium, at levels sufficient to meet RDAs of almost all healthy individuals with adequate servings (50–260 g sulphur-dried weight/day), but poor in vitamin E. The vitamin E levels were relatively low when compared with vegetables and fruits with high vitamin E values (Eckler & Fair, 1996). Although, the region of cultivation seems to be an important determinative factor, affecting the vitamin, β-carotene and selenium level of apricot fruit, selection of a cultivar with high vitamin, β-carotene and selenium levels and pomological characteristics that contribute to advantageous processing of the fruit are the major aspects to be considered by apricot growers.

References

Al-Saleh, I. A., & Al-Doush, I. (1997). Selenium levels in wheat grains grown in Saudi Arabia. *Bulletin of Environmental Contamination and Toxicology*, *59*, 590–594.

Amotz, B. A., & Fishler, R. (1998). Analysis of carotenoids with emphasis on 9-*cis* β-carotene in vegetables and fruits commonly consumed in Israel. *Food Chemistry*, *62*, 515–520.

Asma, B. M. (2000). *Apricot growing*. Malatya, Turkey: Evin Publishers (in Turkish).

Belloso, M. O., & Barriobero, L. E. (2001). Proximate composition, minerals and vitamins in selected canned vegetables. *European Food Research and Technology*, *212*, 182–187.

Breyer, P. H., & Gilbert, B. P. (1987). Determination of selenium (IV) differential pulse voltammeter of the 3,3′-diaminobenzidine piazselenol. *Analytica Chimica Acta*, *201*, 23–32.

Catignani, G. L. (1983). Simultaneous determination of retinol and α-tocopherol in serum or plasma by liquid chromatography. *Clinical Chemistry*, *2914*, 708–712.

Cerhata, D., Bauerova, A., & Ginter, E. (1994). Determination of ascorbic acid in blood serum using high performance liquid chromatography and its correlation with spectrophotometric. *Caska-Slov- Farm*, *43*(4), 166–168.

Eckler, J. A. L., & Fair, J. M. S. (1996). *Pharmacology essentials*. USA: W.B. Saunders Company.

Hussein, L., & Bruggeman, J. (1999). Selenium analysis of selected Egyptian foods and estimated daily intakes among a population group. *Food Chemistry*, *65*, 527–532.

Kadrabova, J., Madaric, A., & Ginter, E. (1997). The selenium content of selected food from the Slovak Republic. *Food Chemistry*, *58*, 29–32.

Lee, S. K., & Kader, A. A. (2000). Preharvest and postharvest factors influencing vitamin C content of horticultural crops. *Postharvest Biology and Technology*, *20*, 207–220.

Miller, K. W., Lorr, N. A., & Yang, C. S. (1984). Simultaneous determination of plasma retinol α-tocopherol, lycopene, α-carotene, and β-carotene by high performance liquid chromatography. *Analytical Biochemistry*, *138*, 340–345.

Munzuroglu, O., Karatas, F., & Gur, N. (2000). A study of the levels of vitamins A, E and C and selenium in rhubarb (*Rheum ribes* L.). *Turkish Journal of Biology*, *24*, 397–404 (in Turkish).

Oster, O., & Prellwitz, W. (1989). Are Germans selenium-deficient?. In *Selenium in biology and medicine* (pp. 229–233). Berlin Heidelberg, New York: Springer-Verlag.

Paunovic, S. A. (1985). Apricot culture and apricot science. *Acta Horticulturae*, *192*, 23–34.

Rice-Evans, C. A., Miller, N. J., & Paganga, G. (1997). Antioxidant properties of phenolic compounds. *Trends in Plant Science*, *2*, 152–159.

Sant'Ana, H. M. P., Stringheta, P. C., Brandao, S. C. C., & de Azeredo, R. M. C. (1998). Carotenoid retention and vitamin A value in carrot (*Daucus carota* L.) prepared by food service. *Food Chemistry*, *61*, 145–151.

State Institute of Statistics (SIS). (1998). *Agricultural structure and production*. Number of fruit trees and fruit production. Ankara, Turkey (in Turkish).

Tavazzi, B., Lazzarino, G., Di-Pierro, D., & Giardina, B. (1992). Malondialdehyde production and ascorbate decrease are associated to the eperfusion of the isolated postischemic rat heart. *Free Radical Biology Medicine*, *13*(1), 75–78.

USFNB. (1989). *Recommended dietary allowances* (10th ed). Washington, DC: National Academy of Sciences.

Watkinson, J. H. (1960). Fluorometric determination of traces of selenium. *Analytical Chemistry*, *32*(8), 981–983.

Watt, B. K., & Merrill, A. L. (1975). *Composition of foods*. Agriculture Handbook. No 8, Washington, DC.

Whetter, P. A., & Ullrey, D. E. (1978). Improved fluorometric method for determining selenium. *Journal Associate of Analytical Chemistry*, *61*(4), 927–931.