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Comparative analysis of tocopherol content in pulp and peel of eight apple cultivars

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ABSTRACT

Purpose: Tocopherols (vitamin E) are important bioactive components in some fruits, possessing potent antioxidant activity and exerting a significant influence on metabolic pathways, human nutrition, and health. However, the content of tocopherols in apple fruit peels and pulp has scarcely been investigated. **Research method:** Fruits of eight apple cultivars were evaluated for color, total soluble solids (TSS), and tocopherols from both the pulp and peels were extracted and analyzed. **Findings**: The content of α-tocopherol (0.073–0.656 and 0.01–0.02 mg/100 g fresh weight (FW) in the peel and pulp, respectively) was higher than that δ-tocopherol (0.002–0.01 and 0.0001–0.0014 mg 100 g FW in the peel and pulp, respectively), and both tocopherols were higher in the peels than in the pulp. The content of α-tocopherol followed the order: ‘Braeburn’ > ‘Golden Delicious’ > ‘Rome’ > ‘Red Delicious’ and ‘Royal Gala’ > ‘Jonagold’ > ‘Fuji’ > ‘Granny Smith’. Low levels of δ-tocopherol were detected in the peels. ‘Granny Smith’ apples had the highest δ-tocopherol content in the peel (0.01 mg 100 g FW), whereas ‘Rome Beauty’, ‘Royal Gala’ and ‘Fuji’ apples exhibited the lowest levels (0.002 mg 100 g FW). **Research limitations:** There were no limitations identified. **Originality/Value:** Our results indicate that tocopherols content in apple peels and pulp is relatively low compared to other types of fruits rich in vitamin E. However, regular consumption of whole apples may contribute to daily vitamin E intake and help prevent the oxidation of lipophilic biomolecules.

INTRODUCTION

Apples (*Malus domestica* Borkh) are among the most consumed fruit in the world and, therefore, they are an important source of bioactive compounds for human nutrition and health. Epidemiological studies have related the daily consumption of an apple (100 g/d) with a decrease in all mortality causes (Hodgson et al., 2016). Several bioactive compounds have widely been identified and quantified in apple fruit, including vitamin C, phenolic compounds, phytosterols, chlorophyll, and carotenoids (Bohn & Bouayed, 2020). However, other bioactive compounds in apple fruit such as tocopherols have received scarce attention (Akšic et al., 2021; Bohn & Bouayed, 2020; Górnaś et al., 2024; Fernández-Cancelo et al., 2022). In the past, the term Vitamin E included eight toco-chromanols naturally existing in plant materials (α-, β-,γ- and σ- tocopherol and the respective tocotrienols), which were highly recognized as quenchers of free radicals in lipid environments (Falk & Munné-Bosch, 2010). However, in recent years international health boards agreed that α-tocopherol is the only human bioactive form of vitamin E (Institute of Medicine, 2000; EFSA NDA Panel, 2024). The Daily Recommended Intake (DRI) of vitamin E is 15 mg/d of α-tocopherol, but its consumption usually is below this amount (IOM, 2001). In European dietary surveys, the consumption of vitamin E in adults varied from 9.7 to 16 mg/ day (EFSA, 2024). Vitamin E deficiency is associated with serious health problems related to neurological function, energy-sulfured amino acids, and single-carbon metabolism (Blaner et al., 2021). The bioavailability of vitamin E from enriched apples has been estimated to be nearly 10%, with this efficiency increased up to 20% and 33%, with 6% and 21% of dietary fat in meals, respectively (Bruno et al., 2006).

The intrinsic, but not relative, antioxidant activity of flavonoids as chain-breaking antioxidants was measured for few flavonoids and was found to be smaller than that of α-tocopherol (Pedrielli & Skribsted, 2002). Furthermore, there is an intimate relationship between polyphenolic compounds and vitamin E, which increase the importance of tocopherols in the apple matrix. For example, higher antioxidant effects of quercetin, epicatechin, and ferulic acid have been observed when they are in combination with α-tocopherol, due to a significant synergistic effect (Pedrielli & Skribsted, 2002; Trombino et al., 2004). In humans, a diet rich in vitamin E is correlated with the reduction of free radicals, and consequently protection against oxidation of some molecules like phospholipids in cellular membranes and low-density lipoproteins (Blaner et al., 2021). In addition, this vitamin seems to reduce the risk to suffer some types of cancer (prostate, liver, bladder, and pancreatic cancers) and a decrease in Alzheimer's disease incidence (Blaner et al., 2021; Yang et al., 2019).

In apples, like in some other fruits, the endogenous levels of tocopherols appear to have a great technological importance due to their protection of cellular membranes during low night temperatures in the pre-harvest stage and during cold storage in the post-harvest stage, which affects the commercial quality of the fruit (Fernández-Cancelo et al., 2022). Tocopherols also play important roles as antioxidants in chloroplasts and thylakoids, however, they are also involved in germination, phytohormonal balance, carbohydrate metabolism, growth, flowering, leaf senescence, and stress resistance in plants, fruits, and seeds (Falk & Munné-Bosch, 2010; Fernández-Cancelo et al., 2022; Sadiq et al., 2019). In Mexico, apples are extensively cultivated, where more than 800,000 tons being produced in 2022, placing the country within the top 20 producer countries in the world (FAOSTAT, 2022; SADER, 2023). However, several tons of apple pomace with significant quantities of bioactive compounds including tocopherols, are produced and wasted annually, and represent a significant source of environmental pollution. Although the content of tocopherols has been reported in the seeds of several apple cultivars, their content in apple skins and pulp has been scarcely reported (Fernández-Cancelo et al., 2022; Bianchi et al., 2020). Thus, the objective of this work was to investigate the tocopherol content in the peel and pulp of eight important apple cultivars.

MATERIALS AND METHODS

Chemicals and solvents

High-performance liquid chromatography (HPLC) grade methanol, reactive grade anhydrous granular sodium sulphate (Na_2SO_4), anhydrous ethanol, and petroleum ether were purchased from J.T. Baker (Baker Mallinckrodt, México). α- and σ-tocopherol standard compounds were purchased from Sigma (Sigma-Aldrich, St. Louis, MO). HPLC-grade water was prepared by a Milli-Qplus purification system (Millipore Corp., Bedford, MA, USA).

Plant material

Fruits from eight apple cultivars were obtained from local market in Querétaro, Mexico. Six cultivars ('Fuji', 'Rome Beauty', 'Jonagold', 'Red Delicious', 'Royal Gala' and 'Braeburn') were imported, while 'Granny Smith' and 'Golden Delicious' apples were produced in Mexico. Twenty apples of each cultivar were selected for uniform size and color, and freedom from defects.

Total soluble solids and color measurement

All apples were ripe and evaluated for total soluble solids content (% TSS) and color. TSS was measured in juice obtained from a representative portion of each fruit using a hand refractometer (ATAGO, Co. Ltd., Japan). The external color was evaluated by taking 2 readings in two opposite regions around of equatorial zone of each fruit, using a CM-2002 Minolta spectrophotometer (Minolta Co. Ltd., Japan), calibrated with a white pattern and zeroed on each occasion just before sampling.

Extraction and analysis of tocopherols

The apple peel was separated from the pulp using a potato peeler. Samples of one gram of peel or pulp tissue from each of five randomly selected fruit were ground in a mortar and Na_2SO4 was added until complete dehydration. Samples were extracted with 25 ml of ethanol for 15 min using an ultrasonic bath and then centrifuged at 5000 x *g* for 5 min at 2 °C. The pellet was eliminated, and 3.5 mL of petroleum ether was added to the apple extract, samples were then shaken, and 5 mL of water was added. The mixture was centrifuged again under the same conditions described above and the upper layer was separated and evaporated. The obtained residue was dissolved in 1.5 mL of methanol and filtered through a polyethylene membrane with a pore size of 0.45 μm (Millipore Corp., Bedford, MA) before injection of 50 μL into the High Performance Liquid Chromatographic (HPLC) system.

The HP 1100 series HPLC system (Hewlett-Packard GmbH, Waldbronn, Germany) was equipped with a fluorescence detector (FLD). The mobile phase was methanol/water (95:5 % v/v) and flow rate was at 1 mL/min through a Symmetry C18 analytical column (4.6 x 150 mm, 3.5 mm; Waters Co., Milford, MA) which was kept at 25°C. The tocopherols were monitored at λ_{ex}= 294 nm, λ_{em}= 326 nm. The identification and quantification of α- and σ-tocopherol were achieved by standard compounds.

Statistical analysis

All analyses were performed in triplicate, the collected data were analyzed by a one-way ANOVA and comparison of the means by the test of Tukey-Kramer, all analyses considered a $p < 0.05$ as statistical significance using JMP software (SAS Institute Inc., Cary, NC).

RESULTS

Color and total soluble solids content

The luminosity was higher in ‘Granny Smith’, ‘Golden Delicious’ and ‘Fuji’ apples, representing the lighter aspect of these fruits, whereas lower values were observed in ‘Rome’ and ‘Red Delicious’ apples, indicating their darker aspects (Table 1). The a* values representing the redder (positive values) to greener (negative values) coloration, were higher in ‘Red Gala’ apples which represent their intense coloration, followed by ‘Red Delicious’, ‘Jonagold’ and ‘Rome’ apples. The ‘Granny Smith’ apples had negative a* values, indicating the green color of the fruit of this cultivar. ‘Golden Delicious’ apples had the highest value of b* variable, indicating their yellow color. ‘Fuji’ and ‘Braeburn’ apples had a combination of lower redness and intermediate b* values, which describe their bicolor peels.

The TSS measured for the eight apple cultivars are shown in Table 1, and all cultivars were within the range of 11.7 to 15.4 °Brix. Color and TSS are important commercial indicators of fruit quality and ripeness. Apple color is an important fruit quality attribute that influences the consumer’s preference. The ranges of the skin color are related to both the absolute and relative concentrations of at least three pigments. Chlorophylls are the most abundant in the epidermal and hypodermal cell layers of unripe green apples, while the color of ripe red apples is due to the content of anthocyanins, and carotenoids are the most abundant pigment in ripe yellow cultivars (Fernández-Cancelo et al., 2022). The purpose of the color evaluation in this study was to characterize and indicate the physiological stage of the fruit at the moment of evaluation. The color variables were similar to fruits of the same cultivars cultivated in Greece (‘Granny Smith’, ‘Golden Delicious’ and ‘Fuji’ apples), Belgium (‘Jonagold’ apples), Argentina (‘Granny Smith’ and ‘Red Delicious’), and India (‘Golden Delicious’, ‘Granny Smith’, ‘Red Gala’, and ‘Red Delicious’) (Drogoudi et al., 2008; Gwanpua et al., 2014; Kumar et al., 2018; Piagentini & Pirovani, 2017).

TSS (°Brix) is a good indicator of fruit sugar content and sweetness. For many apple cultivars, like in ‘Golden Delicious’, the TSS needs to be around 12%, while others, like ‘Royal Gala’, it needs to be above 12% (Hoehn et al., 2003). In others cultivars such as ‘Granny Smith’, a proper TSS is around 11%. All eight cultivars in our study had adequate values of color and TSS for consumption and good quality (Table 1).

Table 1. Fruit maturity parameters of the eight apple cultivars analyzed.

Cultivar	TSS	Color variables L*	 a*	 b*
‘Granny Smith’	11.7 ± 0.2 c	69.4 ± 0.3 a	-5.6 ± 0.2 g	37.1 ± 0.3 b
‘Red Gala’	13.4 ± 0.3 b	53.6 ± 0.8 c	27.7 ± 0.7 a	19.0 ± 0.6 e
‘Golden Delicious’	15.4 ± 0.2 a	74.1 ± 0.4 a	4.2 ± 0.4 f	42.0 ± 0.3 a
‘Red Delicious’	13.5 ± 0.3 b	40.9 ± 0.3 d	20.9 ± 0.4 c	5.2 ± 0.3 f
‘Jonagold’	14.6 ± 0.3 a	52.1 ± 1.0 c	23.5 ± 0.9 b	21.0 ± 0.9 de
‘Rome’	12.9 ± 0.2 b	38.0 ± 0.3 e	23.1 ± 0.4 b	5.3 ± 0.3 f
‘Fuji’	13.1 ± 0.3 b	61.8 ± 1.1 b	7.5 ± 1.2 e	25.3 ± 0.9 c
‘Braeburn’	12.7 ± 0.2 b	55.6 ± 0.9 c	14.8 ± 1.0 d	23.1 ± 1.0 cd

TSS: Total soluble solids, the color variables include: Lightness (L*), green to red (a*), and blue to yellow (b*). Different letters within the same column indicate significant differences between apple varieties.

Content of tocopherols

The content of α-tocopherol (0.073 – 0.656 and 0.01 – 0.02 mg 100 g^{-1} FW in peel and pulp, respectively) was higher than δ-tocopherol (0.002 – 0.01 and 0.0001 – 0.0014 mg 100 g^{-1} FW in peel and pulp, respectively) in fruit of all apple cultivars (Fig. 1 and 2). The α-tocopherol content (Fig. 1) was considerably lower in the pulp than in the peel, and was 0.01-0.02 mg 100 g^{-1} of fresh pulp of 'Braeburn', which had the highest content. In both pulp and peel tissues, the levels of δ-tocopherol were low (Fig. 2). The peel of 'Granny Smith' and 'Braeburn' apples had the highest amount of δ-tocopherol (0.01 and 0.007 mg 100 g^{-1} FW, respectively). In the pulp, the content of δ-tocopherol was lower than 0.0015 mg 100 g^{-1} of fresh pulp in all of the cultivars, being 'Granny Smith' apples with the highest content. This vitamer was not detected in 'Red Delicious' apples. In the chromatograms (Fig. 3), it was possible to observe several peaks (at least 3 of them) between the δ- and α-tocopherol peaks that exhibited similar fluorescent characteristics to tocopherols. Therefore, it is suspected that these apples contain at least another tocopherol, β or γ, tocotrienols or both.

The content of α-tocopherol in the tested apple cultivars was higher than that reported in yellow peels of 'Golden Reinders' apples cultivated in the mountain and valley regions of Spain (0.0105 – 0.012 mg 100 g^{-1}) (Fernández-Cancelo et al., 2022). The peel of 'Granny Smith' and 'Fuji' apples had the lowest amount of α-tocopherol (Fig. 1) possibly because these cultivars do not show the process of the chlorophyll degradation during ripening. Chlorophyll biodegradation produces phytol, which is a limiting factor in the synthesis of tocopherols (Fernández-Cancelo et al., 2022; Sadiq et al., 2019). The rest of the cultivars, especially 'Golden Delicious' and 'Braeburn', had a high amount of α-tocopherol in the peel (Fig. 1), and exhibited chlorophyll degradation and biosynthesis of carotenoids. Interestingly, the content of α-tocopherol was significantly lower in the apple pulp than reported previously for 24 different cultivars from Italy (0.13 – 0.33 mg 100 g^{-1} FW) (Bianchi et al., 2020).

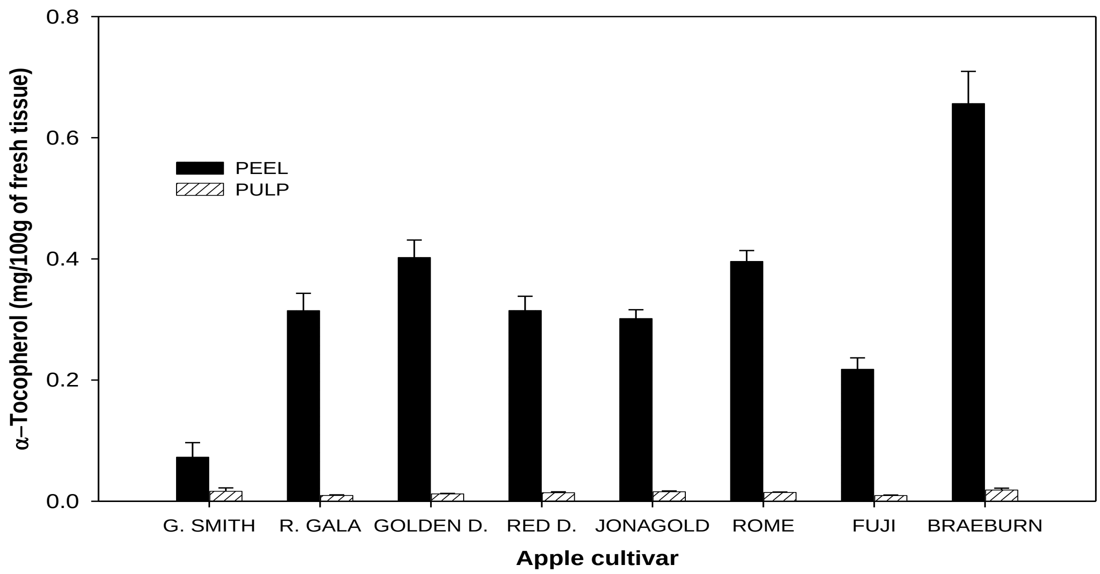

Fig. 1. α-Tocopherol content in the peel and pulp of eight apple cultivars of apples. Upper bars are the standard error of the mean. Different letters in peel and pulp bars indicate significant differences between apple cultivars. The asterisk indicates significant differences between the peel and the pulp (p<0.05).

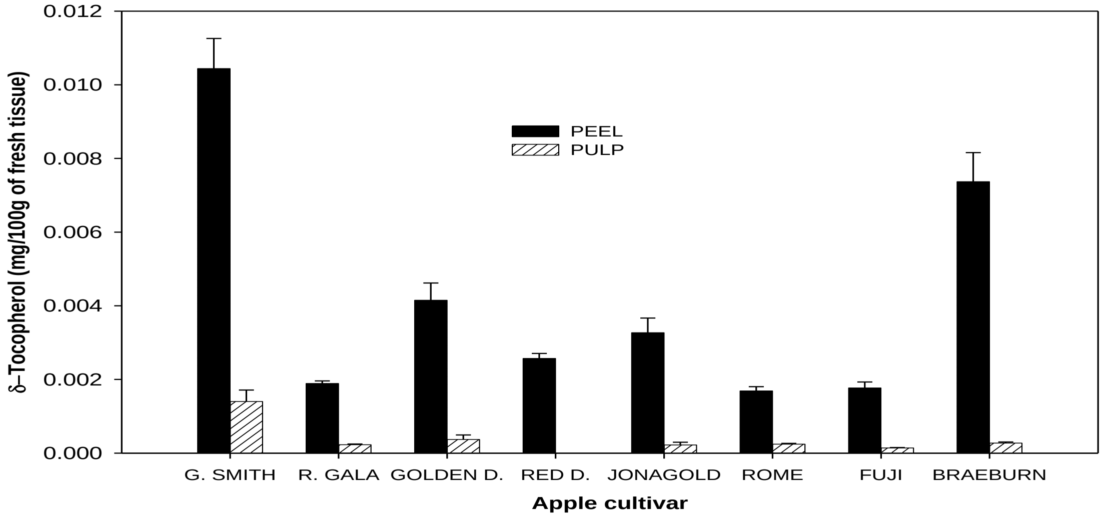

Fig. 2. δ-Tocopherol content in the peel and pulp of eight apple cultivars. Upper bars are the standard error of the mean. Different letters in peel and pulp bars indicate significant differences between apple cultivars. The asterisk indicates significant differences between the peel and the pulp ($p<0.05$).

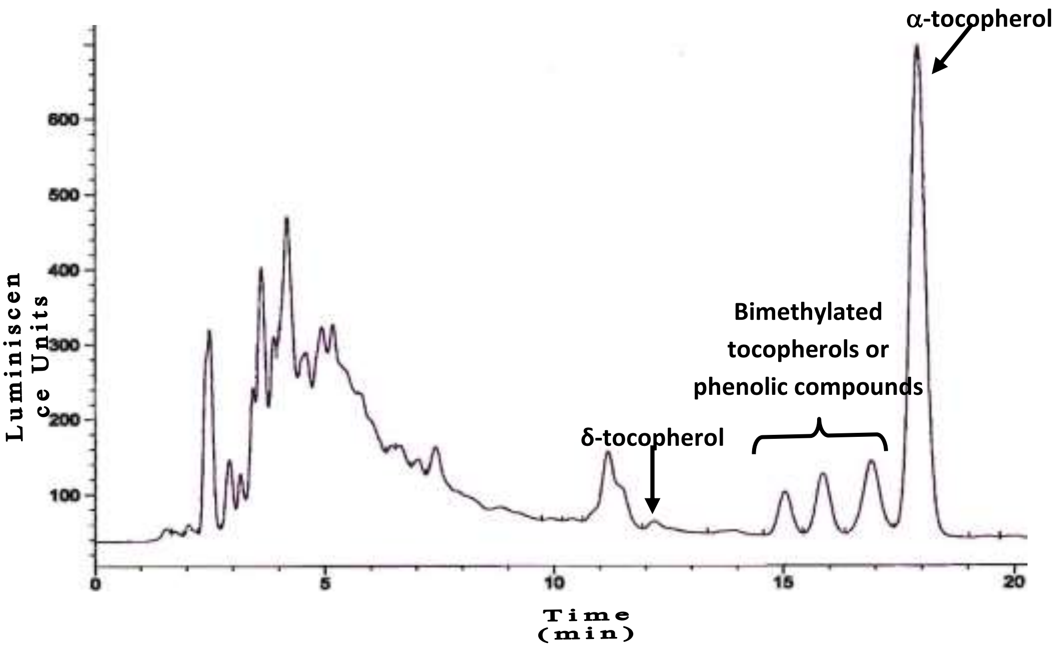

Fig. 3. Typical High Performance Liquid Chromatography (HPLC) chromatogram of tocopherols contained in extracts of apple peel.

Fernández-Cancelo et al. (2022) found γ-tocopherol in yellow peels of 'Golden Reinders' apples, however, at lower values than that of δ-tocopherol in our study (0.001 – 0.004 mg 100 g^{-1}). The variation in the content of tocopherols among the different cultivars might be explained by genetic determinants (Akšic et al., 2021), but no significant relationship was found between the apple origin and the content of tocopherols (Górnaś et al., 2023). Fernández-Cancelo et al. (2022) found up to 10-fold higher content of γ-tocopherols in peels of apples from mountain regions than in fruits from valleys in Spain, however, α-tocopherol was not affected.

The tentative identification of other tocopherols must be taken with discretion since most of the authors point out that it is not possible to separate β or γ-tocopherol using reversed phase (Górnaś et al., 2024), despite the fact that there are reports that indicate the opposite (Akšic et al., 2021), and some peaks could be a mixture of bimethylated tocopherols. On the other hand, these peaks could originate from phenolic compounds such as catechin or epicatechin, which are excited at similarλ, and also fluoresce.

CONCLUSION

Our results indicate that fruits of the apple cultivars analyzed contain low levels of α-tocopherol (vitamin E). However, these low levels represent an important loss of phytochemicals and antioxidant compounds when the peel of the fruit is removed, as is the custom of some consumers due to safety or other reasons, not only because of the loss of tocopherols, but also because of the synergistic and regenerative effects of these with other antioxidant compounds.

Conflict of interest

The authors declare no conflict of interest.

Author statement

EMY was responsible of the investigation, conceptualization, and funding acquisition, reviewing, editing supervision, writing the original draft. JJOP was responsible for methodology, data analysis, data curation, writing the original draft, reviewing and editing. CIVC was responsible for data curation, writing the original draft, reviewing and editing.

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