

# Antioxidant Properties, Oxidative Stability, and Fatty Acid Profile of Pitaya Fruit (*Hylocereus polyrhizus* and *Hylocereus undatus*) Seeds Cultivated in Turkey

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Pitaya is a tropical fruit from a newly cultivated plant in Turkey that has increasing economic value. In this study, its seed properties were investigated. Pitaya fruit samples used in the research were obtained from local producers in the Gazipaşa/Antalya region. The dry matter, protein, oil, and ash content of *H. polyrhizus* and *H. undatus* pitaya seeds were 89.7% to 89.1%, 19.8% to 17.5%, 22.8% to 24.0%, and 2.8% to 4.09%, respectively. The oil and protein contents of the seeds were very high. The total phenolic content,  $\alpha$ -tocopherol content,  $\gamma$ -tocopherol content, free radical scavenging activity, and induction time of *H. polyrhizus* and *H. undatus* pitaya seeds were 12.8 to 11.9 mg GAE/g dry sample, 3.67 to 2.75 g/kg oil, 1.29, 1.64 g/kg oil, 46.9% to 51.5%, and 5.37 to 5.07 h, respectively. Seeds contained significant amounts of phenolic compounds and tocopherols, which play an important role in increasing oxidative stability. The percent inhibition of DPPH indicated that pitaya seeds may be evaluated as an antioxidant source. Unsaturated fatty acids were high in seed oils of both pitaya species. Linoleic acid, a polyunsaturated fatty acid, was dominant in both pitaya species. The chemical properties of the seeds were similar to those of species grown in tropical countries. Future studies should investigate other pitaya species grown in Turkey.

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## INTRODUCTION

Pitaya fruit is a climbing ivy cactus species first grown as an ornamental plant and later as a fruit product (Gunasena *et al.* 2007; Perween *et al.* 2018; Shen *et al.* 2020). *Hylocereus* spp., which is known as pitaya fruit, is an exotic fruit that belongs to the Cactaceae family, and is indigenous to tropical regions of Mexico and Central as well as South America (Dembitsky *et al.* 2011; Jiang *et al.* 2021; Younis *et al.* 2023). Currently, pitaya is widely cultivated in Asia, including Vietnam, Latin America, Colombia, Malaysia, and South China (Britton and Rose 1963; Liu *et al.* 2019; Le 2021). Pitaya seeds are black sesame-like kernels embedded in the flesh of a pitaya; the number of seeds in a pitaya is approximately 5000 to 15,000, and the fruit seeds account for 1.3 to 1.5% of the fresh fruit weight (Liu *et al.* 2022). *Hylocereus undatus* and *Hylocereus polyrhizus* are two varieties of commonly called pitaya fruits (Dembitsky *et al.* 2011). As one of the most important fruit crops, it is widely cultivated in worldwide tropical or sub-tropical areas, and the taste of its juicy and succulent fruit is delicious. Generally, pitaya could be

classified according to the appearance of flesh and peel, namely, red flesh/red peel, white flesh/red peel, and white flesh/yellow peel pitaya (Jiang *et al.* 2021).

The pitaya fruit consists of 36% to 37% non-renewable shell, 47% to 49% edible flesh (pulp), and 14% seeds (Lim *et al.* 2010). The mass of seeds in 100 g of fruit ranges between 4.6 g and 8.01g (Esquivel *et al.* 2007). Carbohydrates in pitaya seeds are generally composed of nutritional fibers. They also contain pectin, protein, fat, and ash (Villalobos-Gutiérrez *et al.* 2012; Le *et al.* 2021). Ibrahim *et al.* (2018) performed an extensive bibliographic review, describing 15 sterols and triterpenes, 30 fatty acids, and 11 miscellaneous compounds located mainly in the seeds of different types of pitaya.

Several parts of the pitaya plant (*e.g.*, leaf, flower, and fruit) have healthy functions (Zhang 2002; Chien *et al.* 2007; Liaotrakoon 2013; Le *et al.* 2021). Pitaya seeds and peels were found to have higher total polyphenols and betacyanins compared to pulps. These bioactive components exhibit regulative influences on the human gut microbiota, glycemic response, and lipid accumulation (Huang *et al.* 2021; Jiang *et al.* 2021). Pitaya fruit is known for its peculiar flavour and appearance (Joshi and Prabhakar 2020; Al-Radadi 2022), and its seeds are a good source of dietary fibre, vitamin C, minerals, carotenoids, phenolic acids, organic acids, protein, flavonoid compounds, phosphorus, iron, and phytoalbumins (Utpott *et al.* 2020; de Araujo *et al.* 2021). In addition to aiding digestion, the peel and seeds of the pitaya fruit reduce cholesterol levels and protect against diabetes and colon cancer by neutralising the harmful effects of heavy metals and other environmental toxins (Utpott *et al.* 2020; Al-Radadi 2022). Red pitaya has health benefits, including cancer prevention, anti-inflammatory effects, antidiabetic effects, and cardiovascular risk-reducing properties (Stintzing *et al.* 2002; Cos *et al.* 2004; Herbach *et al.* 2006).

The pitaya seeds can be used in the extraction of functional lipids, as a new source of essential oil, with high content of saturated (myristic, palmitic, and stearic acids) and unsaturated (oleic, linoleic and linolenic acids) fatty acids (de Araujo *et al.* 2021). The oils of pitaya seeds are rich in unsaturated fatty acid (UFA). Most of the UFA is linoleic acid, an essential fatty acid that cannot be synthesized in the body and must be part of the diet (Pomeranz 2003). Essential fatty acids, namely, linoleic acid and linolenic acid, form a significant percentage of the unsaturated fatty acids of the seed oil extract. The two pitaya varieties contain about 50% essential fatty acids (Dembitsky *et al.* 2011). The high linoleic acid content is favorable for medicinal and nutritional applications because it is responsible for cardio-protective, antidiabetic, and antimicrobial activities (Das 2000; Szentmihályi *et al.* 2002). Tocopherols ( $\alpha$ -tocopherol and  $\gamma$ -tocopherol) and phytosterols (campesterol, stigmasterol,  $\beta$ -sitosterol and  $\gamma$ -sitosterol) were also found in the seed oil of *H. undatus* and *H. polyrhizus*. These compounds have medicinal properties (including antioxidant, anti-cancer and anti-tumour activities) and can contribute to health maintenance (de Araujo *et al.* 2021).

Akram and Mushtaq (2019) reported that the seeds of pitaya fruit are rich in essential fatty acids, principally *omega-3* and *omega-6* fatty acids as well as tocopherols. The levels of linoleic acid attributed to pitaya seed oil (500 g/kg of oil) are comparable with flaxseed, canola, and sesame oils. The seed oil of pitaya fruit may become a viable source of good quality oleonutrients for food and cosmetic industries.

There is little information on the anti-nutritional factor studies related to pitaya fruit. Toxicological studies on pitaya are scarce, and further research is very much needed (Jeronimo *et al.* 2017). Jeronimo *et al.* (2017) in the study on acute toxicity with rats, stated neither observed mortality nor signs of acute or subchronic toxicity, nor significant difference in body weight, organ weight, or hematologic parameters in subchronic study.

The oral administered extract of pitaya fruits is relatively safe. İbrahim *et al.* (2018) have reported that acute and subchronic toxicity studies of pitaya fruits did not show any mortality and adverse effects to female and male rats of the MeOH extract of pitaya fruits. Researchers have reported that the NOAEL (no-observed-adverse-effect level) *via* oral administration for both pulp and peel extracts in mice were more than 5 g/kg, and also intake of exaggerated amounts of fruit resulted in pseudo-hematuria, which is a harmless reddish discoloration of the feces and urine. Besides the medicinal and nutritional value of pitaya fruits, there is a potential allergenicity risk due to the protein content of seeds. Investigations have reported the allergenic potential of pitaya fruits and indicated that they are capable of inducing immune reactions in sensitive individuals (Hao *et al.* 2022; Liu *et al.* 2022). Hao *et al.* (2022) have reported cases of suspected allergy to white- and/or red-fleshed pitaya, and the allergic symptoms after the consumption of this fruit included vomiting, urticaria, *etc.*

Fruits may also have a spatial and temporal effect on human health, as their production is controlled by environmental factors of the area and thus can differ in the phytochemical content (Jincy Rose and Jayadev 2018). Jincy Rose and Jayadev (2018) have reported mango has highest saponin values (162.1 µg/g) and pitaya fruit has the lowest (130.5 µg/g).

Three types of *H. undatus* (white pitaya), *H. polyrhizus* (red pitaya), and *H. megalanthus* (yellow pitaya) are the most preferred species for consumption by consumers in Vietnam, Colombia, Costa Rica, Israel, Malaysia, Thailand, and Florida (Zahid 2014; Chia and Chong 2015; Rahmati *et al.* 2015; Truong and Dang 2016). Some of the leading farmers on the south coast of Turkey have started to grow pitaya, especially in Antalya and Mersin, and the product has taken its place in markets. Since pitaya fruit is a newly emerging fruit crop, there is currently no United States export, import, or per capita consumption data available reported through the U.S. Department of Agriculture's Economic Research Service or Foreign Agricultural Service ("Fruit and Tree Nut Data" - ERS, 2021) (Anonymous 2022).

Pitaya (*Hylocereus* spp.) is a tropical fruit that has just started to be grown in the Mediterranean climate zone in Turkey. Pitaya fruit contains essential nutrients such as vitamins, minerals, complex carbohydrates, dietary fibers and antioxidants that are beneficial for human health. There is no published study on the composition of pitaya seeds grown in Turkey. Therefore, this study focuses on some chemical properties of seeds and oxidative stability of seed oils of *Hylocereus polyrhizus* and *Hylocereus undatus* which are tropical fruits, harvested on the south coast of Turkey.

## EXPERIMENTAL

### Plant Material

Researchers have reported that pitaya species generally contain between 82% to 90% moisture when harvested (Liaotrakoon 2013). In the present study, pitaya samples were collected when the fruits reached maturity. The research was carried out with two species of pitaya (*H. polyrhizus* and *H. undatus*) obtained from local producers in Antalya/Turkey. Both species had a red outer shell, but the pulp color of *H. undatus* and *H. polyrhizus* had white and red colors, respectively. Following the harvest, the samples were transferred to the laboratory and stored in deep freeze until the analysis. Pitaya seeds were separated by filtration after optimum enzymatic liquefaction of the pulp fraction

(Villalobos-Gutiérrez *et al.* 2012). Seeds were washed with water, air-dried, and stored at room temperature. Pitaya species and their seeds used in this study are shown in Fig. 1.

### Physical Analysis in Seeds

The total oil (AOAC 2019), moisture (AOAC 2000), ash (AOAC 2005), and protein contents (Yetim 2002) were determined according to methods described in the literature. Protein content was determined by NDA 701 Dumas Nitrogen Analyzer (VELP Scientifica Srl, Usmate/Italy).

### Total Phenol Analysis in Seeds

Oil was first extracted from seeds. 2 g air-dried seed samples were extracted twice with petroleum ether to obtain oil for fatty acid composition. Total phenol analysis was done on oil-free residue. The oil-free ground seeds were extracted by mixing with methanol 1/10 (g sample/ml methanol) for total phenol analysis. The obtained extracts were powdered after being freeze-dried. The total phenolic determination of these powders was done according to the Folin-Ciocalteu spectrophotometric method (Singleton *et al.* 1999). A total of 100 mL of the extract was transferred into a test tube, and 500 mL of Folin-Ciocalteu reagent, 1.5 mL of saturated sodium carbonate were added and then completed to a volume of 10 mL with pure water. After standing in the dark at room temperature for 2 h, the mixture was measured at 760 nm against solvent using a UV-Vis spectrophotometer. The standard calibration (5 to 2000 mg/L) curve was plotted using gallic acid. The content of pitaya seed phenolics in extracts was expressed in gallic acid equivalent (mg of GA/g of extract) (Babbar *et al.* 2011).

### DPPH Free Radical Scavenging Activity Determination of Total Antioxidant Activity

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method, adopted with suitable modifications (Stankovic 2011). A total of 3.9 mL of 0.1 mM DPPH prepared in methanol was added to the 100 mL extract in a reaction tube. The reaction mixture tubes were incubated at ambient temperature for 30 min in the dark. The DPPH absorption values at 515 nm were read relative to methanol. Total antioxidant activity (TAC) was calculated as percent inhibition using the following equation (Singh *et al.* 2002; Babbar *et al.* 2011):

$$\% \text{ DPPH} = [(\text{Methanol Absorbance} - \text{Sample absorbance}) / \text{Methanol Absorbance}] \times 100$$

### Induction Period of Seed Oils

The induction period is a value in which a certain breaking point is determined in parallel with the increase of volatile components formed due to the oxidation of oils at a certain temperature and airflow. The induction period was determined by the change of conductivity of water as a result of the transfer of fragmentation products to distilled water. A longer induction period indicates higher oxidative stability of the oil. The samples were analyzed with a Ransimat 892 device (Metrohm AG, Herisau, Switzerland) using a flux of 20 L/ hour at 110 °C and according to AOCS Cd 12b-92 (AOCS 1992). The induction period results were given in h.

### Determination of Fatty Acid Composition

2 g air-dried seed samples were extracted twice with petroleum ether to obtain oil for fatty acid composition. First, the oil samples (5 to 100 mg) were converted to their fatty acid methyl esters (FAME) (AOAC 1984). A standard fatty acid methyl ester mixture (Sigma-Aldrich, St. Louis, MO, USA) was used to identify sample peaks. Next, the fatty acids (1 ml) were analyzed in gas chromatography (Agilent 7890A, Santa Clara, CA, USA) equipped with a flame ionizing detector (FID), autosampler (Agilent 7683 Injector and sample tray), with a fused silica capillary column (HP-88, 100 m x 0.25 mm x 0.2  $\mu$ m). It was operated under the following conditions: GC inlet was 260 °C, the split ratio was 30:1, oven temperature prog was from 140 °C (5 min) to 240 °C (15 min) with a rate of 4°C/min, injection volume was 1  $\mu$ L, carrier gas was helium, the constant flow was 20 cm/s, the detector was mass selection detector (5975C MSD), solvent peak exit time was 10.5 min, and detection mode was scan (40 to 400 amu).

### Determination of Tocopherols of Seeds Oil

Tocopherol analysis was carried out according to IUPAC (1992) (no: 2.432). A total of 1.5 g of oil and 10 mL of hexane were dissolved and injected into the HPLC system. HPLC conditions were as follows: column was LiChroCART Si 60 column (25 cm x 4 mm x 5  $\mu$ m) (Merck, Darmstadt, Germany), the detector was SPD-M20A Prominence diode-array detector which was fixed at a wavelength of 295 nm, and the mobile phase was 0.5% isopropanol in n-hexane. The total run time was 40 min, and the injection volume was 20  $\mu$ L. Tocopherols were quantified by a standard external method;  $\alpha$ -,  $\beta$ -,  $\gamma$  and  $\delta$ -tocopherol standards were obtained from Sigma-Aldrich.

### Statistical Analysis

Statistical analysis of the data was performed using the prog Minitab V.16 (Minitab LLC, State College, PA, USA). Differences between the groups were determined by the Tukey Multiple Comparison Test.

## RESULTS AND DISCUSSION

Pitaya fruit cultivation has increased because of reports highlighting promising medicinal uses of these plants (Hao *et al.* 2022). The fruits consist of plenty of grainy seeds. Like other fruit seeds, pitaya fruit seeds contain oil mainly polyunsaturated fatty acids. It is known to be a good source of ascorbic acid, retinol, and trace amounts of thiamine and riboflavin. Because of its high lipid content and functional properties, pitaya fruit oil extracted is viewed as a high value product (Boyapati *et al.* 2022).

As shown in Table 1, the difference between the dry matter, protein, and oil ratios of the seeds of the two pitaya species were statistically insignificant. These values were similar to each other, while ash values of *H. undatus* species (2.78%) were higher than *H. polyrhizus* species (4.09%) ( $P < 0.05$ ). The protein content of both species ranged from 17.5% to 19.8%, and the oil yield was from 22.8% to 24.0%. The oil, protein, and ash content of pitaya seeds have been reported in the ranges of 22% to 32%, 20% to 25%, and 3% to 6%, respectively (Yu *et al.* 2007; Ariffin *et al.* 2009; Chemah *et al.* 2010; Lim *et al.* 2010; Villalobos-Gutiérrez *et al.* 2012). Pitaya seeds (18.8% to 27.5%) have a similar oil yield to apple seeds (27.7%) (Yu *et al.* 2007). *H. undatus* and *H. polyrhizus* harvested from the south coast of Turkey showed similar findings to related references concerning oil and

ash content, the amount of protein detected in the pitaya samples was lower. The differences in the composition of pitaya seeds may be due to soil properties, climate, the origin of the plant, growing, and harvest conditions (Liaotrakoon 2013).

**Table 1.** Some Physical Properties of Pitaya Seeds

Species	n	Total Dry Matter (%)	Ash (%)	Protein (%)	Oil (%)
<i>H. polyrhizus</i>	3	89.68±0.65 <sup>a**</sup>	2.78±0.62 <sup>b</sup>	19.79±1.54 <sup>a</sup>	22.78±0.85 <sup>a*</sup>
<i>H. undatus</i>	3	89.13±0.32 <sup>a</sup>	4.09±0.10 <sup>a</sup>	17.54±1.21 <sup>a</sup>	23.97±0.17 <sup>a</sup>

\*Mean (three replicates) ± standard deviation of each parameter

\*\*Significant (P < 0.01) differences within a column are denoted by different superscript letters

The percentage of edible fruit was approximately  $39.75 \pm 3.45$  %, the portion of the fruit that is non-edible is around  $51.92 \pm 4.73$  %, and the percentage of seeds is around  $7.89 \pm 1.21$  % (Boyapati *et al.* 2022). The protein content of the red pitaya seeds from Central America was reported to be around  $206 \pm 6$  mg/kg seed (FW basis) (Villalobos-Gutiérrez *et al.* 2012, Boyapati *et al.* 2022). The present results showed that both pitaya seeds yielded more oil (22.8% to 24.0%) than the oil yield reported by Andasuryani *et al.* (2020) for Passion fruit seed, which was in the range of 6 to 12%.

Pitaya seeds contained up to 32.0% fat, 22.1% protein, 21.0% starch, 1.75% total sugar, 10.4% crude fiber, and 3.18% ash. The red pitaya seeds contained 6.4% moisture, 24.8% crude protein, 31.8% crude fat, 13.4% crude fiber, 2.58 g/100 g ash, and 20.4 g/100 g carbohydrate, in addition to  $\alpha$ -tocopherol and  $\gamma$ -tocopherol, with 5.78 mg/100 g and 10.5 mg/100 g, respectively (Liu *et al.* 2022).

Pitaya fruit seed samples' ash content ranged from  $2.78 \pm 0.62$  % to  $4.09 \pm 0.10$  % (Table 1), which is found to be higher when compared with the ash content reported as  $0.25 \pm 0.22$  g/100 g seed by Boyapati *et al.* (2022). Moisture level results were found higher for pitaya fruit seeds compared to the reported moisture content of date palm seeds (1.66% to 2.33%) by Boyapati *et al.* (2022). The carbohydrate content ranged from 79.3 to 83.0% among different varieties of date seeds (Dehdivan and Panahi 2017), while Villalobos-Gutiérrez *et al.* (2012) reported  $352 \pm 15$  mg/kg in red pitaya seeds of Central American origin. Pitaya fruit has more minerals in content than mangosteen and other tropical fruits such as mango and pineapple, including potassium, phosphorus, sodium, and magnesium. Pitaya fruit oil can be considered a high-value product due to its high lipid content and functional properties (Boyapati *et al.* 2022). It may be recommended to add pitaya seed oil to dietary supplements or functional foods (Liu *et al.* 2022).

Pitaya fruits contain natural antioxidant compounds in the shell, pulp, and seeds (Wee *et al.* 2014; Zahid 2014; Barcelon *et al.* 2015; Obenland *et al.* 2016; Ferreres *et al.* 2017). The seeds of pitaya fruits are rich in the natural antioxidant sources including phenolic compounds,  $\alpha$ -tocopherol, and  $\gamma$ -tocopherol (Lim *et al.* 2010; Villalobos-Gutiérrez *et al.* 2012). As shown in Table 2, the difference in the total amount of phenolics between the two species was statistically significant (P<0.05). Adnan *et al.* (2011) determined the total phenolic substance in pitaya kernels as 13.56 mg GAE / g dry matter. They reported that the phenolic components are catechin, quercetin, myricetin, and epicatechin. The results presented here are in accordance with the previous studies. The phenolic content of pitaya seed was found to be higher than many phenolic sources such as fruits and vegetables. Zulkifli *et al.* (2020) studied optimization of the extraction process for defatted pitaya (*Hylocereus polyrhizus*) seed extract using response surface

methodology. They reported the optimized extraction parameters as follows: extraction time of 45 min, extraction temperature of 70 °C, and ethanol concentration of 80%. They reported total phenol content as  $128.58 \pm 1.61$  mg gallic acid equivalent (GAE)/g defatted sample. Their result is higher than the results of this study, but they reported the results for the defatted sample, not for the extract. Huang *et al.* (2021) expressed the range of the total phenol content of the extracts of pitaya seeds from 4.51 to 8.54 mg GAE/g dry weight, which is lower than the present findings. Also, they expressed that the red pitaya seed has higher amounts of total phenolic acids, which also agreed with the present results.

The major phytoconstituents isolated from pitaya seed oil have health-promoting properties, such as antioxidant activity (Hao *et al.* 2022). The antioxidant ingredients contained in the flesh of the fruit are also found in pitaya fruit seeds (Safira *et al.* 2021). In the present work, *H. polyrhizus* and *H. undatus* seed extracts' DPPH values ranged from 46.9 to 54.1% inhibition. Çavdar *et al.* (2017) studied pomegranate seed oil and reported the DPPH IC50 value as  $17.00 \pm 0.21$  mg/mL. Boyapati *et al.* (2022) reported DPPH inhibition of around 69.6% and the polyphenol content as 96.7 mg GAE/g, in pitaya seed oil. The pitaya fruit may be thought to have a more significant amount of antioxidants when compared to other subtropical fruits like mango and pineapple (Farid Hossain *et al.* 2021). Seeds from other fruits, such as grape seeds, and berries, and from the Cactaceae family also *Opuntia* spp. and pitaya, are potent antioxidant sources (Adnan *et al.* 2011). Research on the antioxidant activity of pitaya components has mainly focused on the pulp and the pericarp, with only a few reports exploring the antioxidant capacity of pitaya seeds and their potentially bioactive compounds (Shi *et al.* 2022). Antioxidant-rich oil may be produced from the pitaya fruit's small black seeds, which are found throughout the fruit (Al-Radadi 2022). The antioxidant activities of the compounds, present in the extract from pitaya seeds, may depend on structural features, such as the number of phenolic hydroxyl or methoxyl groups and flavones hydroxyl groups (Al-Radadi 2022).

**Table 2.** Chemical Properties of Pitaya Seeds and their Antioxidant Properties

Species	N	Total phenol content (mg GAE/g dried extract)	% DPPH inhibition*	Induction time (hour)	$\alpha$ -tocopherol (g/kg oil)	$\gamma$ -tocopherol (g/kg oil)
<i>H. polyrhizus</i>	3	$12.81 \pm 0.36^{***}$	$46.90 \pm 1.53^b$	$5.35 \pm 0.09^a$	$0.367 \pm 0.15^a$	$0.129 \pm 0.05^b$
<i>H. undatus</i>	3	$11.90 \pm 0.24^b$	$54.07 \pm 1.22^a$	$5.07 \pm 0.09^b$	$0.275 \pm 0.09^b$	$0.164 \pm 0.11^a$

\*:500 $\mu$ g/ml solution of freeze-dried extract

\*\*Mean (three replicates)  $\pm$  standard deviation of each parameter

\*\*\*Significant ( $P < 0.01$ ) differences within a column are denoted by different superscript letters

The yield of total phenolic content from defatted pitaya seed extract ranged from 52 to 144 mg GAE/g sample, which were higher than from kiwi fruit seeds and defatted marigold *Tagetes erecta* L. residues. The total flavonoid content from defatted pitaya seed extract ranged from 2.69 to 10.67 mg QE/g sample, which was higher than grape by-products reported by Zulkifli *et al.* (2020). The polyphenol yield from grape seed oil with various treatments has been found to be between 49 and 83 mg/g (Dang *et al.* 2013; Boyapati *et al.* 2022). Adnan *et al.* (2011) detected total phenolic and ascorbic acid contents of the seed as  $13.56 \pm 2.04$  and  $0.36 \pm 0.01$  mg/g, respectively of red flesh pitaya (*Hylocereus polyrhizus*) seed. The total phenolic content of the pitaya seed was much lower

if compared to the grape seed, which was around 27.4 to 46.7 mg GAE/g dry weight, depending on the varieties. Regarding the identification and quantification of phenolic compounds in *H. undatus* and *H. polyrhizus*, those such as protocatechuic, p-coumaric, p-hydroxybenzoic, vanillic, caffeic, gallic, and syringic acids were reported in the seed oil of these two species (de Araujo *et al.* 2021). Catechin, epicatechin, and epigallocatechin were previously identified in the seeds of white and red pitaya fruits, grown in Thailand (Younis *et al.* 2023).

Seed oils of *H. undatus* and *H. polyrhizus* pitaya species have moderate levels of tocopherol compared to vegetable oils and contain 240 to 320 mg/kg  $\alpha$ -tocopherol and 116 to 127 mg/kg  $\gamma$ -tocopherol (Slover 1970; Frankel 1996; Lim *et al.* 2010). As shown in Table 2, the content of  $\alpha$ -tocopherol and  $\gamma$ -tocopherol content of *H. polyrhizus* species was higher than that of *H. undatus*, and the difference between the two species was statistically significant ( $P < 0.05$ ). Lim *et al.* (2010) reported  $\alpha$ -tocopherol content of *H. polyrhizus* and *H. undatus* oils as 0.319 g and 0.240 g per kg oil. They also reported  $\gamma$ -tocopherol content for both species as 0.116 g and 0.127 g, respectively. Lim *et al.* (2010) noted that *H. polyrhizus* pitaya seed oil contains more tocopherol, although it has a lower fat content and phenolic content than seed oil of *H. undatus*. In this study, the  $\alpha$ -tocopherol content of *H. polyrhizus* seed oil was higher than *H. undatus* seed oil, while the  $\gamma$ -tocopherol content was vice versa. The total phenol content of *H. undatus* dried extract was lower than *H. polyrhizus* dried extract. These findings are a little bit higher than the previously reported results. The difference may be due to soil, climate, and growing conditions.

The antioxidant activity (% inhibition of DPPH) of *H. undatus* specie seeds was higher than *H. polyrhizus* seeds (Table 2). The antioxidant activity difference between the two species was statistically significant ( $P < 0.05$ ). Pitaya seeds are thought to have an essential contribution to the antioxidant activity of the pitaya fruit. Liatrakoon (2013) reported the DPPH activity of pitaya fruit as 22.3  $\mu$ g GA equivalent for 1 g of pulp without seed and 63.5  $\mu$ g GA equivalent for 1 g of pulp with seed. Chemah *et al.* (2010) determined DPPH free radical scavenging activity of *H. polyrhizus* and *H. undatus* pitaya seed extracts (0.5-g seed in 20 ml of 50% aqueous methanol) as 46.6% and 44.5%, respectively. Therefore, pitaya seeds contribute to the antioxidant effect of the whole pitaya fruit. It may be offered to the consumer to chew and crush the seeds while eating the fruit.

The stability and quality of edible oils affect their acceptability and market value. The oxidative stability of the oils is an essential factor in maintaining their quality. One method used to determine the oxidative stability of oils is to find induction times. Induction time is when the oil begins to degrade with oxygen at the current temperature (Şimşek and Serindağ 2008). The Ransimat method determines the induction period and estimates the oxidative stability. The induction times of crude vegetable oils, which contain natural antioxidants such as tocopherol, are higher than those of refined oils (Güler 2009).

In this study, the difference between *H. polyrhizus* and *H. undatus* pitaya was statistically significant in induction period results ( $P < 0.05$ ). The induction period of sunflower oil is between 5.0 and 5.7 h at 110 °C (Silva *et al.* 2001; Judde *et al.* 2003). The means of both species of pitaya (5.07 to 5.53 h) are similar to sunflower oil.

The main fatty acid in both seed oils of pitaya species was linoleic acid (Table 3). Linoleic acid was higher in seeds of *H. undatus* than seeds of *H. polyrhizus* ( $P < 0.01$ ). The predominant fatty acid profile was composed of unsaturated fatty acids in both species. Oleic acid was the most common monounsaturated fatty acid. The palmitic and the stearic fatty acids were higher in *H. polyrhizus* than *H. undatus* ( $P < 0.01$ ).



**Table 3.** Main Fatty Acid Composition of Seed Oils

Fatty acids	<i>H. polyrhizus</i>	<i>H. undatus</i>
Myristic (C14:0)	0.20±0.02 <sup>*b**</sup>	0.25±0.02 <sup>a</sup>
Palmitic (C16:0)	15.51±0.37 <sup>a</sup>	13.22±0.07 <sup>b</sup>
Stearic (C18:0)	5.98±0.06 <sup>a</sup>	5.36±0.04 <sup>b</sup>
Arashidic (C20:0)	0.53±0.02 <sup>a</sup>	0.49±0.04 <sup>a</sup>
Palmitoleic (C16:1)	0.77±0.04 <sup>a</sup>	0.78±0.06 <sup>a</sup>
Oleic (C18:1)	25.60±0.28 <sup>a</sup>	24.57±0.77 <sup>a</sup>
Erusic (C22:1)	0.19±0.01 <sup>a</sup>	0.14±0.02 <sup>b</sup>
Linoleic (C18:2)	49.95±0.09 <sup>b</sup>	54.05±0.67 <sup>a</sup>
Linolenic (C18:3)	1.28±0.03 <sup>a</sup>	1.13±0.10 <sup>a</sup>
Saturated	22.21	19.33
Monounsaturated	26.56	25.49
Polyunsaturated	51.23	55.18

\*Mean (three replicates) ± standard deviation of each parameter

\*\*Significant (P < 0.01) differences within a column are denoted by different superscript letters.

Pitaya seed oil was mainly composed of unsaturated and saturated fatty acids, of which unsaturated fatty acids were more than 75%. Its fatty acid composition was mainly linoleic acid (40%), oleic acid (23%), and palmitic acid (15%). Linoleic acid plays a vital role in lipid metabolism in enzyme activity, the central nervous system, and other critical activities. Its linoleic acid content is higher than that of canola oil (20.4%), peanut oil, cashew nut oil (19.69%), and avocado oil (6 to 10%), which is another way to obtain linoleic acid (Liu *et al.* 2022). The linoleic acid in white pitaya seed oil was higher than that in red pitaya seed oil measured in this study as stated by Liu *et al.* (2022). Pitaya seed oil yield and composition of the oil varied greatly by species.

*H. undatus* and *H. polyrhizus* seed oils also consist of cholesterol, campesterol, stigmasterol, and β-sitosterol (Liu *et al.* 2022). Pitaya fruit is considered an excellent natural reservoir of omega-3 and omega-9 fatty acids, especially in the tiny black seeds (Younis *et al.* 2023). Pitaya seeds are known to have a high content of unsaturated fatty acids such as linoleic acid and linolenic acid, which were also found in grape seeds, flax seeds and other fruit seeds (Shi *et al.* 2022). Linolenic acid has been reported as the most abundant fatty acid in the seed oil of *Origanum* species, accounting for 34.4% to 67.4% (Matthäus *et al.* 2018). Górnaś (2015) reported the total tocopherol content of different apple seeds, ranging from 130 to 339 mg/100 g oil. Sicari *et al.* (2017) detected the maximum oil yield in bergamot seed as 35.0 g /100 g seed.

In different studies, polyunsaturated (48% to 50%) and monounsaturated (25% to 30%) fatty acids for *H. polyrhizus* and *H. undatus* species were reported. Linoleic acid and oleic acid are reported between 48% to 58% and 22 to 28%, respectively (Chemah *et al.* 2010; Le Bellec and Vaillant 2011; Lim *et al.* 2012; Liaotrakoon 2013). The high levels of unsaturated fatty acids and especially polyunsaturated fatty acids (linoleic acid) in pitaya seed oils add the pitaya seeds consumed together with fruit, an excellent nutritional value. To obtain the fatty acid content of seeds, consumers may chew and crush the seeds while eating the fruit.

## CONCLUSIONS

1. Although pitaya seeds are not used as an oil source, the high phenolic and linoleic acid content draws attention to the health care benefits and functions.
2. The phenolic content of both pitaya species harvested on the south coast of Turkey was higher than many phenolic sources such as fruits and vegetables.
3. Pitaya seeds contribute to the antioxidant activity of the whole pitaya fruit. To take advantage of the antioxidant activity of the seeds, the consumers may be offered to chew and crush the seeds while eating the fruit.
4. The oxidative stability of pitaya seed oil was found to be similar to sunflower oil but not as strong as olive oil.
5. Tocopherol content was slightly higher than the reported findings of the previous studies on pitaya.
6. Linoleic acid was detected to be the main fatty acid of the seed oil.
7. It was observed that there are no extreme changes in chemical composition and antioxidant activity by growing pitaya, whose original growing area is tropical regions, in a region with a Mediterranean climate.

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## Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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