



Effect of hot air drying process on color and antioxidant attributes of the flavedo of bitter orange (*Citrus aurantium* L.)

Sıcak hava kurutma prosesinin turunç (Citrus aurantium L.) flavedosunun renk ve antioksidan özellikleri üzerine etkisi

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ABSTRACT

This research investigated the effects of different drying temperatures (40, 50, and 60°C) on changes in color, total phenolic, total flavonoid, ascorbic acid, and antioxidant activity of bitter orange flavedo. In comparison to the fresh sample, the L* and a* values increased, while b* and h° values decreased at all air drying temperatures. The lowest total color change (TCC) values were achieved at drying temperatures of 40 and 60°C. Drying temperatures induced an increment in total phenolic and antioxidant activity, the highest total phenolic content (16.35±0.54 mg GAE g⁻¹) and DPPH radical scavenging activity (IC₅₀: 14.61±0.53 mg mg⁻¹) were determined in flavedo dried at 40°C, and the highest FRAP value (52.19±1.49 mmol TE g⁻¹) was achieved in flavedo dried at 50°C. However, the applied drying temperatures resulted in a decline in the flavedo's total flavonoid content (52.79–64.75%) and ascorbic acid content (36.63–45.54%). Based on the results obtained from this work, bitter orange flavedos can be dried at lower temperatures (40-50°C) to minimize color changes and enhance phenolic and antioxidative activity.

Key Words: *Citrus aurantium* L., drying temperature, total phenolic, IC₅₀, FRAP

ÖZ

Bu araştırmada, farklı kurutma sıcaklıklarının (40, 50 ve 60 °C) acı portakal flavedosunun renk, toplam fenolik madde, toplam flavonoid, askorbik asit ve antioksidan aktivitesindeki değişimlere olan etkileri incelenmiştir. Taze numuneyle karşılaştırıldığında, tüm hava kurutma sıcaklıklarında L* ve a* değerleri artarken, b* ve h° değerleri azalmıştır. En düşük toplam renk değişikliği değerleri 40 ve 60 °C kurutma sıcaklıklarında elde edilmiştir. Kurutma sıcaklıkları toplam fenolik madde ve antioksidan aktivitede artışa neden olmuş, en yüksek toplam fenolik madde içeriği (16.35±0.54 mg GAE g⁻¹) ve DPPH radikal süpürücü aktivitesi (IC₅₀: 14.61±0.53 mg mg⁻¹) 40 °C'de kurutulan flavedoda belirlenmiş ve en yüksek FRAP değeri (52.19±1.49 mmol TE g⁻¹) 50 °C'de kurutulan flavedoda elde edilmiştir. Ancak uygulanan kurutma sıcaklıkları flavedonun toplam flavonoid içeriğinde (%52.79-64.75) ve askorbik asit içeriğinde (%36.63-45.54) düşüşe neden olmuştur. Bu çalışmadan elde edilen sonuçlara göre, acı portakal flavedoları, renk değişimlerini en aza indirmek ve fenolik ve antioksidan aktiviteyi artırmak için daha düşük sıcaklıklarda (40-50°C) kurutulabilir.

Anahtar Kelimeler: *Citrus aurantium* L., kurutma sıcaklığı, toplam fenolik, IC₅₀, FRAP

Introduction

Citrus fruits, belonging to the Rutaceae family, are the most widely cultivated horticultural products worldwide, with a total production of 161.8 million tons. Türkiye ranks 8th in the world, with a cultivation area of 180 thousand hectares and a total quantity of 7.87 million tons (FAO, 2022; TÜİK, 2023). The coastlines of the Southern Aegean and Mediterranean regions of Turkey have favorable ecological conditions (climate, soil characteristics, etc.) for citrus cultivation. The main citrus species planted in Turkey are orange, mandarin, lemon, and grapefruit. Fewer amounts of other citrus fruits such as kumquat, bitter orange, lime, and bergamot are also cultivated.

Citrus fruits are mainly processed into juice in the food industry. They also make jams, jellies, soft drinks, essential oils, flavoring agents and bioactive extracts. The citrus processing industry generates considerable amounts of by-products such as peel, pulp, and seeds. These by-products are an important source of vitamins, minerals, essential oils, dietary fibers, and bioactive compounds, especially phenolic acids and flavonoids, which may possess anti-inflammatory, antioxidant, anti-infective, neuroprotective effects, and anticancer properties (Gómez-Mejía et al., 2019; Sharma et al. 2022). Therefore, citrus processing wastes have the potential to be employed as a source of ingredients, natural additives, and raw materials in cosmetics, dietary supplements, and food products. Furthermore, the conversion of citrus wastes into value-added products can provide an effective and environmentally friendly platform (Rafiq et al., 2018).

Bitter orange (*Citrus aurantium* L.) is a species of citrus fruit that originated in Southeast Asia. It is known by several names worldwide, including sour orange and Seville orange (Pawar et al., 2020). Turkey has witnessed a 65% increase in bitter orange production over the past decade, with a total yield of 3,581 tons in 2023. Approximately 77% of this production is accounted for by the Mediterranean region.

(TÜİK, 2023).

Bitter orange is typically employed as a rootstock for citrus cultivation. It exhibits morphological similarities to the orange but possesses a sour and bitter taste. Therefore; it is not suitable for consumption in its fresh form. In some regions, the juice of the bitter orange is utilized as a substitute for lemon juice in salads, imparting a sour taste. The flowers, leaves, and peels of the fruit have been used for a long time as a sedative, stomach stimulant, weight loss aid, eyelid inflammation, central nervous system disorders, muscle pain, and skin bruising. During the jam processing, the flavedo parts of fruits are removed to the debittering of peels. Several researchers have revealed that the flavedo parts of citrus fruits contain phytochemicals with health-related benefits and display mighty antioxidant activity. (Escobedo-Avellaneda et al., 2014; Muzykiewicz et al., 2019; Ramful et al., 2010; Badalamenti et al., 2022). Owing to its inherent moisture content, citrus flavedo is unsuitable for storage over extended periods. The drying process represents an efficacious preservation method to extend the shelf stability of flavedo (Farahmandfar et al., 2020). The principal objective of the drying operation is to diminish the moisture content of the material to a level that will inhibit microbial growth during storage. Furthermore, the drying process inactivates chemical reactions that cause deterioration (Suri et al., 2022).

Hot air drying is one of the most prevalent methods due to its simplicity, cost efficiency, operational suitability, and capability to provide hygienic products. Drying temperature and duration are the main factors affecting final product quality (Önal et al., 2019; Senadeera et al., 2020).

Dried flavedo can be offered for the formulation of various food products, including beverages, dairy, bakery, and confectionery, as well as for the extraction of valuable bioactive substances in cosmetic and pharmaceutical applications. The impacts of drying on the antioxidant attributes of citrus processing wastes

have been documented by numerous researchers, confirming that drying affects antioxidant activity and bioactive components. However, there is limited research (Farahmandfar et al., 2020) on the influence of drying on the antioxidant features of bitter orange flavedo.

This study aimed to effects of drying temperatures on color parameters, total phenolic, total flavonoid, ascorbic acid content, and antioxidant activity of the flavedo part of bitter orange fruit.

Material ve Method

Bitter oranges (Yerli F1 cultivar) were collected from citrus orchards in the Western Mediterranean Agricultural Research Institute, Antalya province, in winter season (January-2023). Then, the fruits were washed with water procured from a tap and wiped through a cloth. A kitchen grater was used to remove the flavedo layer from the fruit (20 pieces).

Hot air drying experiments

The flavedo parts were spread on glass petri dishes and dried in a pilot-scale hot-air oven (Eksis, Isparta, Türkiye) at temperatures of 40, 50 and 60° C, with a stationary air speed of 1.3 m s⁻¹. Approximately 100 g of samples were used for each drying experiment. Drying experiments were continued until a moisture content of 10% was reached and performed in triplicate. The drying treatments took about 185, 165, and 130 minutes for 40, 50, and 60°C, respectively. The moisture content was assessed using an oven at 105 °C until the sample weight was constant. The dried samples were kept in plastic bags at -18°C until analyses.

Analyses

Color measurements

L*, a*, b*, and hue angle parameters of flavedos were determined using a Minolta CR 400 (Osaka, Japan) color measurement device using a D65 light source from three different points. Total color changes were reckoned using the following equation 1 (Dağ et al., 2017).

$$TCC = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1)$$

Extraction Procedure

The extraction procedure of dried samples was conducted according to the method used by Ramful et al. (2010) with certain modifications. Approximately 5 and 2 g of fresh and dried flavedo samples were added to falcon tubes containing 25 mL of methanol solution (80%; v/v) and vortexed for 1 minute. The mixtures were subjected to extraction on an orbital shaker at 180 rpm and room temperature for 24 hours and subsequently, the sample was subjected to centrifugation at 7000 rpm for 5 minutes. After centrifuging, the upper phases were collected and the volumes were completed to 25 mL with extraction solvent. The extracts were conserved at a temperature of -18°C until the subsequent phytochemical analysis.

Total phenolic content

The total phenolic content of flavedo extracts was ascertained by the Folin–Ciocalteu colorimetric method suggested by Phuyal et al. (2020). Briefly, 950 µL of distilled water, 5 mL of Folin–Ciocalteu solution (10%; v/v), and 4 mL of Na₂CO₃ solution (7.5%; w/v) were added to 50 µL of extract, respectively. The mixture was vortexed and allowed to incubate for 90 minutes at room temperature in the absence of light. After incubation, the absorbance of the mixture was read to blank at 765 nm utilizing a spectrophotometer (Shimadzu UV-Vis 160A, Japan). Results were assessed as mg GAE (Gallic acid equivalent) g⁻¹ dm (dry matter) from the gallic acid calibration curve.

Total Flavonoid content

The total flavonoid content of the samples was analyzed using the aluminum chloride colorimetric method referred to as the Zhishen method (Zhishen et al., 1999). According to this method, 500 µL of the extract was transferred to a test tube, followed by the addition of 4.5 mL of distilled water and 300 µL of 5% (w/v) NaNO₂ solution. 600 µL of 10% (w/v) AlCl₃ was added

after five minutes. Then, at the 6th minute, 2 mL of 1M NaOH was added to the mixture and the total volume was achieved by the addition of 2.1 mL of distilled water. Following the thorough mixing of the solution, the absorbances of the samples were measured at a wavelength of 510 nm against the blank containing no extract. The total flavonoid values of the samples were presented as milligrams of catechin equivalent (CE) per gram of dry matter.

Ascorbic acid determination by HPLC

The analysis of ascorbic acid was conducted using a high-performance liquid chromatography (HPLC) device, as described by Sdiri et al. (2012), with alterations. For this purpose, flavedo samples were extracted with a 3% (w/v) metaphosphoric acid (HPO₃) solution. After centrifugation, the extracts were filtered through a 0.45 µm membrane filter and given to the HPLC (Shimadzu 2030 C 3 d Prominence-i, Japan) equipped with PDA (Photo-diode Array) detector. The separation was run on an Inertsil ODS3 C-18 column (5µm, 250x4.6 i.d.) (GL Sciences, Japan) at 25°C. The 25 mM potassium dihydrogen phosphate (KH₂PO₄; pH:2.3) was used as mobile phase with the flow rate of 0.6 mL min⁻¹, and the

detection wavelength was at 250 nm. The injection volume was set to 10 µL. Ascorbic acid was quantified as mg g⁻¹ dm according to the standard curve of ascorbic acid in the concentration range 1-200 mg L⁻¹ and the formula defining this curve.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity

The procedure for the DPPH method was as follows; 5 different volumes (25, 50, 75, 100, and 150 µL) of extracts were added to 600 microliters of 0.1 M DPPH solution in test tubes. Subsequently, the volume was brought to 6 mL with methanol and vortexed. The mixtures were left in the dark at room temperature for 15 minutes. The absorbance of the samples at 515 nm was read against methanol in a spectrophotometer. Methanol was used instead of extract as a control. With the help of the equation 2 below, the % inhibition data for different concentrations were calculated and the IC₅₀ value (the concentration required to reduce 50% of the initial DPPH concentration) was determined as mg mg⁻¹ from the curve prepared with these data (Cemeroğlu, 2010).

$$\text{Inhibition (\%)} = [(\text{Absorbance}_{\text{DPPH}} - \text{Absorbance}_{\text{extract}}) / \text{Absorbance}_{\text{DPPH}}] \times 100 \quad (2)$$

Ferric Reducing Antioxidant Power (FRAP) Assay

FRAP solution was generated by combining 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ), and 20 mM ferric chloride (FeCl₃ 6H₂O) at a ratio of 10:1:1 (v/v/v). 150 µL of extract and 2750 µL of FRAP reagent were blended and incubated in ambient conditions away from light for 30 minutes. Then, The absorbance was quantified by measuring the mixture against the blank in a spectrophotometer at 593 nm. The results were calculated from the calibration graph of standard Trolox and expressed as mmol Trolox equivalent (TE) g⁻¹ dm (Thaipong et al., 2006).

Statistical assessment

Drying studies were carried out in 3 repetitions and all values are given as “mean ± standard deviation”. Data were evaluated using one-way analysis of variance (ANOVA) using the SAS statistical program (Version 6.12). A comparison of the means were appraised by Duncan’s Multiple Range Test at P < 0.05 confidential interval.

Results and Discussion

As can be seen from Table 1, drying temperatures significantly affected the L*, a*, b*, and hue angle values of bitter orange flavedo (P ≤0.05). After drying, the L* value, indicating

lightness, and the a^* value, pointing to redness, increased compared to fresh flavedo. The closest L^* value to fresh flavedo was measured at drying temperatures of 50 and 60°C. However, the highest lightness was observed in flavedo dried at 40°C ($P<0.05$). The increment of a^* is related to browning reactions and carotenoid degradation (Ghanem et al., 2012; Özcan-sinir et al., 2018) and the highest increase occurred at 50°C drying temperature. Ghanem Romdhane et al. (2015) found an increase in the A^* value of lemon peels dried at 40 °C, 50 °C and 60 °C. The b^* value, an indicator of yellowness, increased after drying at 40°C and decreased at other drying temperatures. The decomposition of carotenoid pigments in the

samples may be responsible for the decrease in b^* (Ghanem Romdhane et al., 2015; Rafik et al., 2019). The hue angle closer to 90° indicates a more yellowish tone, and closer to 0° expresses a reddish tone (Wang et al., 2023). The h° value of fresh flavedo decreased with drying temperature and the lowest yellowness was recorded at 50 and 60°C drying. Hot air drying caused changes in the color of fresh flavedo and the lowest TCC occurred at 40 and 60°C. It is assumed that color alterations during the drying of plant materials arise due to various factors, including degradation of color pigments, oxidation and browning reactions (Ghanem Romdhane et al., 2015).

Table 1. Color values of fresh and dried flavedos*

Flavedo samples	L^*	a^*	b^*	h°	TCC
Fresh	68,97±0,85b	18,11±0,68d	50,5±3,19a	70,22±1,83a	-
40°C	74,18±1,16a	26,58±0,53b	52,01±1,45a	62,92±0,94b	15.53±0.45b
50°C	69,94±1,12b	28,56±0,31a	46,28±1,54b	58,31±0,87c	16.37±0.37a
60°C	69,85±0,39b	24,28±0,33c	42,14±0,41c	60,04±0,16c	15.70±0.12b

* Different exponential letters in the same line show that the means are significantly different ($P<0.05$)

The total phenolic and flavonoid contents of fresh and dried bitter orange flavedo are illustrated in Figure 1. The total phenolic content of fresh flavedo (11.84 ± 2.05 mg GAE g^{-1}) increased by about 38.1% and 26.69% drying at 40 (16.35 ± 0.54 mg GAE g^{-1}) and 50°C (15.00 ± 1.19 mg GAE g^{-1}), respectively. The increase in total phenolic content can be attributed to the temperature-induced release of some phenolic acids and flavonoids, which are mostly present in bound form in the plant matrix, and the decrease in the activity of polyphenoloxidase, an enzyme known to facilitate the oxidation of polyphenols (Papoutsis et al., 2017). Sultana et al. (2012)

observed an increase in total phenolics in oven-dried apricots and explained this with the release of high molecular weight phenolics due to heat treatment. Güçlü et al. (2022) reported that different drying methods increased the amount of phenolic substances in citrus peels. On the other hand, no significant difference was observed between fresh and dried flavedo at 60°C (11.99 ± 1.00 mg GAE g^{-1}) ($P\geq0.05$). A similar finding has been reported by Abd Rahman et al. (2018) for the pomelo flavedo.

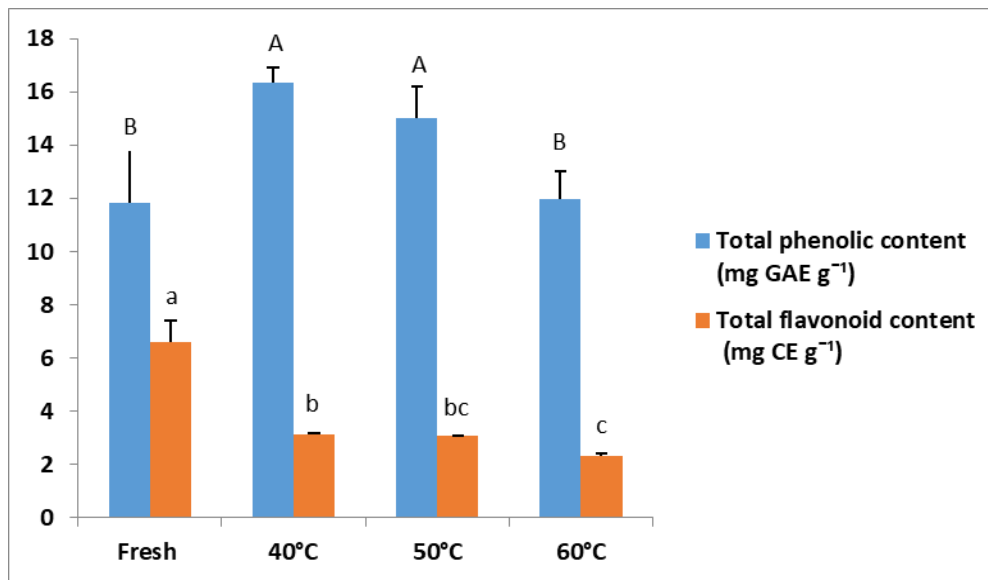


Figure 1. Total phenolic and flavonoid contents of fresh and dried flavedo samples*

*Different capital letters (A-B) on the bars show significant differences between total phenolic values and different lowercase letters (a-c) on the bars show significant differences between total flavonoid values ($P < 0.05$).

The contents of total flavonoids in fresh and dried flavedo varied between 2.33 ± 0.11 – 6.61 ± 0.78 mg CE g⁻¹ (Figure 1). In contrast to the total phenolic content, the total flavonoid content of fresh flavedo decreased by 52.79–64.75% with drying temperatures. This phenomenon is likely attributable to the chemical, enzymatic, or thermal deterioration of certain flavonoids (Rafiq et al., 2019). The highest total flavonoid content was found in fresh flavedo, followed by flavedo dried at 40°C (3.12 ± 0.05 mg CE g⁻¹), 50°C (3.07 ± 0.02 mg CE g⁻¹) and 60°C (2.33 ± 0.11 mg CE g⁻¹), respectively. Similarly, Lai et al (2022) found that total flavonoids reduced with increasing temperature during oven drying of orange peels, which could be attributed to the decomposition of some heat-sensitive flavonoids.

The ascorbic acid content of the flavedo samples exhibited a concentration range of 0.55

to 1.01 mg g⁻¹. The ascorbic acid content was highest in fresh flavedo (Figure 2). Drying temperatures caused losses of the ascorbic acid content of fresh flavedo by 36.63–45.54%. The lowest ascorbic acid losses were observed during drying at 50°C (0.64 ± 0.02 mg g⁻¹). Rafik et al. (2019) reported the reduction of ascorbic acid in kinnow peel after different drying techniques. The degradation of ascorbic acid during food processing can be attributed to several factors including pH, temperature, light, enzymes, oxygen, and metal ion catalyzers. The oxidation of ascorbic acid due to high drying temperatures or long drying times, as well as the depletion of this acid to prevent polyphenol oxidation, may be the cause of vitamin C losses during drying (Kamiloğlu et al. 2016; Yeasmin et al., 2021).

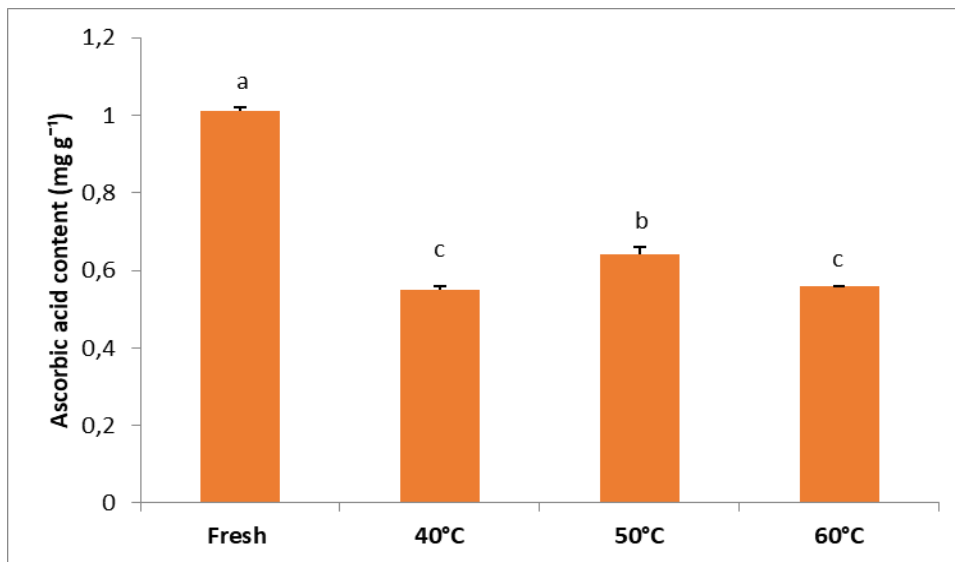


Figure 2. Ascorbic acid contents of fresh and dried flavedo samples*

*Different lowercase letters (a-) on the bars show significant differences between ascorbic acid values ($P < 0.05$).

Drying temperatures significantly decreased the IC_{50} value ($92.25 \pm 1.92 \text{ mg mg}^{-1}$) of fresh flavedo. In other words, DPPH radical scavenging activity increased. While the differences in IC_{50} values of the samples were inconsequential concerning drying temperatures ($P \geq 0.05$), the highest IC_{50} value was found in the samples dried at 60°C ($16.61 \pm 0.6 \text{ mg mg}^{-1}$), while the lowest IC_{50} value was achieved in the sample dried at 40°C ($14.61 \pm 0.53 \text{ mg mg}^{-1}$). The FRAP value of fresh flavedo ($40.28 \pm 1.22 \text{ mmol TE g}^{-1}$) increased with drying temperature. The FRAP values of the dried samples varied between 41.47 ± 2.21 and $52.19 \pm 1.49 \text{ mmol TE g}^{-1}$. The highest and lowest FRAP values were obtained at 50°C and 40°C ,

respectively (Figure 3). This trend is consistent with reports from previous studies conducted on various citrus cultivars. Abd Rahman et al. (2018) reported that the DPPH radical scavenging activity of pomelo flavedo was raised by oven drying at 50°C and 60°C . DPPH and FRAP values were significantly increased in dried grapefruit peel samples compared to fresh peels in the study by Castro-Vazquez et al. (2016). Chen et al., (2011) found that IC_{50} values of orange peels dried at 50°C , 60°C , and 70°C were higher than those of fresh ones. Özcan-Sinir et al. (2018) determined an increment in DPPH free radical scavenging activity of kumquats dried at 70°C and 80°C air temperatures.

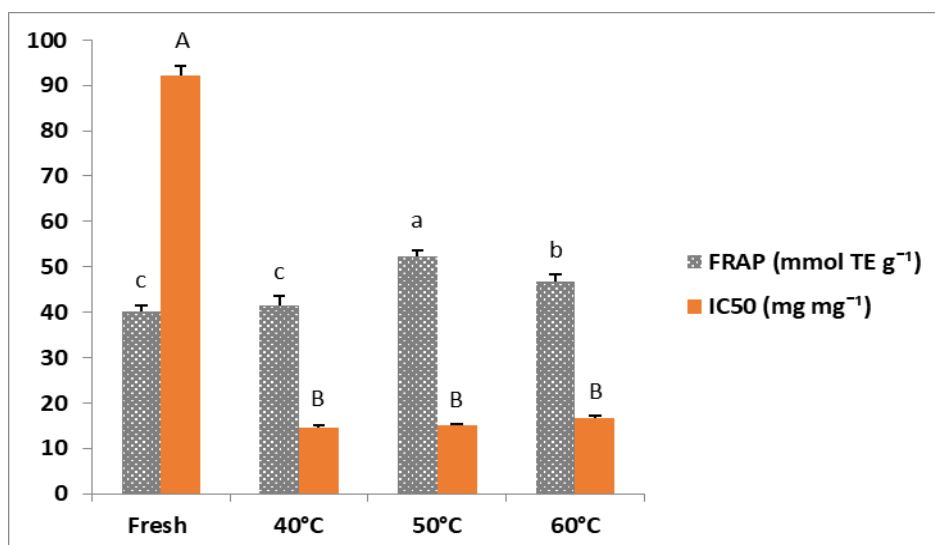


Figure 3. IC_{50} and FRAP values of fresh and dried flavedo samples*

*Different capital letters (A-B) on the bars show significant differences between IC_{50} values and different lowercase letters (a-c) on the bars show significant differences between FRAP values ($P < 0.05$).

It is thought that the antioxidant activities of dried flavedos are higher than the fresh sample, which may be due to the inactivation of the polyphenoloxidase enzyme during the drying process, as well as the decline in moisture content and the increment in dry matter content (Ghafoor et al. 2020). Moreover, high drying temperatures can stimulate Maillard reactions, resulting in the formation of Maillard products that possess antioxidant properties (Saikia et al., 2015). Previous studies have revealed that phenolic compounds and ascorbic acid are the primary contributors to the antioxidant activity observed in citrus fruits (Zou et al., 2016). However, our results showed that hot air drying increased the total phenolic and decreased the total flavonoid and ascorbic acid. Changes in antioxidant activity depend on individual phenolic and other antioxidant compounds, as well as their susceptibility to thermal and conformational modifications (Saikia et al. 2015).

Conclusion

The effect of the drying temperatures on the color, total phenolics, total flavonoids, ascorbic acid contents, DPPH radical scavenging activity and Ferric Reducing Antioxidant Power Assay of the flavedo parts of bitter orange was investigated in this work. Drying temperatures affected the color parameters measured. The drying temperatures were observed to reduce the brightness and redness values while simultaneously decreasing the h value, compared to those of the fresh flavedo. The flavedo subjected to drying at 40°C exhibited the highest b* value; however, the b* values decreased at the other drying temperatures. The total phenolic content was significantly higher at both 40°C and 50°C compared to the initial fresh sample. Nevertheless, the total flavonoid content of the bitter orange flavedo exhibited a decline as the drying temperature increased. As expected, drying temperatures caused the loss of ascorbic acid and a maximum loss of 45.54% was observed in samples dried at 40 °C. Hot air drying

significantly enhanced the antioxidant activity evaluated through the DPPH and FRAP assays. This study demonstrates that the drying temperatures applied have a significant influence on both the color and the antioxidant properties of bitter orange flavedos. It is recommended that bitter orange flavedos be dried at lower temperatures (40-50°C) to minimize color changes and enhance phenolic and antioxidative activity.

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Conflict of interest

The authors declare that they have no conflict of interest

Author contributions

DYT: Conducting experimental studies, data collection, analysis, writing original draft, review, and editing; MG: Project administration; conceptualization; supervision, analysis, review and editing; BB: Supervision, analysis, review, and editing; HT: Analysis, review and editing; OÇ: Analysis, review and editing; ET: Provision of material; review and editing.

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