

A Study on Quality Properties of Blackthorn (*Prunus spinosa* L.) Fruit Powder Obtained by Different Drying Treatments

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Abstract: In this study, the quality characteristics of blackthorn fruit (*Prunus spinosa* L.) powders obtained by convective hot air-drying (HAD) and freeze-drying (FD) treatments were investigated. The drying time was carried out equally (24 h) for both treatments. According to the results, the moisture (%) and water activity of powder samples obtained by HAD and FD treatments were found as 7.51% and 0.2471, 9.13% and 0.2718, respectively. Considering the pH and total ash parameters, there was no statistically significant difference between the powder samples ($p > 0.05$). However, both drying processes were effective on the color and changed the L^* , a^* , and b^* values of the powders compared to fresh fruit values. The biological and antioxidant results of the powder obtained by FD treatment were higher than the HAD treatment ($p < 0.05$). From this point of view, it was determined that the FD process had a minimal effect on the chemical content of fresh fruit, while the HAD technique applied at 40 °C combined with a fan system did not have an excessive negative effect on these values.

1 Introduction

The Rosaceae family is noted for its wide variety of edible fruits such as apples, pears, peaches, plums, cherries, almonds, strawberries, raspberries and more, which have economic value, and also nutritional and health benefits for human beings [1]. Among them, *Prunus spinosa* L. is a thorny perennial plant growing as a shrub on the slopes of wild uncultivated areas [2], on the edges of forests and open woodlands as part of Mediterranean thermophilus plant communities [3]. The fruits of the plant are spherical bluish-black with a single seed [4], commonly known as blackthorn or sloe, ripen in late summer and autumn, and are sometimes persistent on the plant through winter [5]. Blackthorn fruits (BFs) can be consumed fresh, but because of their astringent taste, they are more used to prepare syrups, juices, jams, compotes, spirits, pickled like olives, wine, and other traditional products [6]. The fruits are recently gaining attention as a functional food and an underutilized source of bioactive compounds for application in the food and pharmaceutical industry [7].

As is known, due to the perishable characteristics of fresh fruits, seasonality, and the increasing consumer demand for various food products, there is a growing interest in transforming them into new value-added products such as juice, jam and more stable dry powder [8]. In this context, "drying" is one of the oldest methods used to remove water for food preservation, as the lowest water potential (water activity) is achieved for food stability during storage [9]. Today, many different techniques are used for drying fresh fruits and vegetables. Among these techniques, convective hot air-drying (HAD) has advantages in terms of easy to use,

low operating cost and energy consumption [10]. However, this technique also has some disadvantages such as nutrient loss, color alteration, off flavor and shrinkage in the product [11]. Freeze-drying (FD), also known as lyophilization, is a process in which water in the form of ice under low pressure is removed from a material by sublimation [12]. It is a very gentle drying technique for heat sensitive foodstuffs compared to other conventional drying techniques. On the contrary, FD is a process with a high investment cost, energy consumption, and requires much time to reach the desired moisture content [13]. Therefore, it is very important to choose the best method that will cause minimum cost to the enterprise in industrial mass production and determine the parameters such as the time and/or temperature to be applied during the drying process in a way that will have the least effect on the quality of the final product.

As with many other fruits, the seasonality of ripening of BFs limits the availability of products obtained from these fruits in the market. In this regard, it requires processing of fruits after harvest or storing them under conditions that best preserve their nutritional value. On the other hand, it can be stated that the studies on BFs and its products, which have a significant potential in terms of bioactive components, are limited. From this point of view, in the present study, very mild convective HAD and FD treatments were applied to the homogenized BFs, and then the quality of the fruit powders were evaluated with different analyses and the results were compared with the control (fresh fruit puree) sample.

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2 Materials and methods

In the study, ripe and undamaged blackthorn (*Prunus spinosa* L.) fruits (BFs) were collected in October 2022 from Pınarhisar district of Kırklareli province, which is located in the North-western part of Türkiye. The fruits were kept in cooled bags (4 ± 0.5 °C) for transport to the laboratory and were stored in the refrigerator at 4 °C for no more than 3 days. All the chemicals used in the study were of analytical grade and purchased from Sigma-Aldrich (St Louis, MO, United States).

2.1 Drying Treatments

Before drying treatments, the seeds were manually separated from the fruits. Then, the fleshy and skin parts of the BFs were homogenized by using a laboratory blender (Waring laboratory blender, Conair Corporation, 7011G, Stamford, CT, USA) to make fruit puree (FP). Afterwards, the FP was immediately taken to the drying step. In Fig. 1, all steps performed within the scope of the study were tried to be schematized.

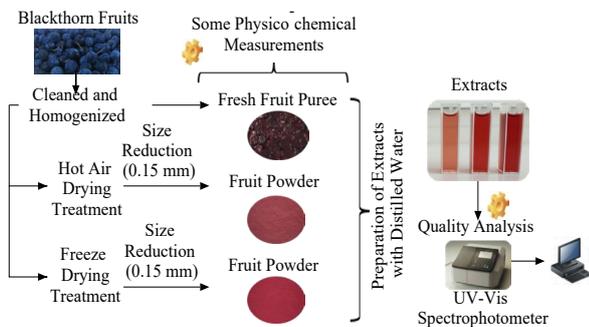


Fig. 1. All processes performed within the scope of the study.

According to the HAD, 100 g of FP was uniformly spread on non-stick baking paper with a stainless steel flat rubber spatula and placed in a single layer on the dryer trays (Atacama Pro, F77000, Tre Spade, Torino, Italy). The heat treatment conditions (time and temperature) that can give the best results in terms of energy consumption and product quality have been selected as a result of comprehensive literature review. In this regard, Pachura et al. (2022) [14] and Krzykowski et al. (2023) [15] stated that convective HAD at 40 °C gave the best results for preserving the volatile bioactive components of different plant-based materials, taking into account the energy requirements of treatment. Therefore, in the present study, a very mild HAD treatment was carried out in a dryer device (Atacama Pro, F77000, Tre Spade, Torino, Italy) at 40 °C with 2.0 ± 0.2 m/s airflow (just in front of the meshed sample tray of the fan) for 24 h. At the end of the treatment, dried samples easily separated from the non-stick baking paper were ground using a coffee grinder (Siemens MC 23200), immediately sieved with a 100 mesh (0.15 mm) stainless steel flour sifter, and then vacuum packed (100 g each) and stored at 4 °C until further analysis.

In the FD treatment, the FP (30 g) was placed in silicone freeze dryer trays and each tray was shock frozen in a freezer at -40 °C for 5 h. After this process,

the trays were immediately transferred into the drying chamber of a freeze dryer device (TRS4-4DS, Teknosem Corp., Istanbul, Türkiye). The FD process was carried out for 24 h (as in convective HAD) with condenser temperature and vacuum chamber pressure equal to -85 °C and 0.01 to 0.004 mbar, respectively [16]. At the end of the treatment, freeze-dried samples were ground using a closed domestic coffee grinder (Siemens MC 23200), immediately sieved with a 100 mesh (0.15 mm) stainless steel flour sifter, and then vacuum packed (100 g each) and stored at 4 °C until further analysis.

2.2 Physico-chemical measurements of the samples

The water activity values of FP and powder samples were determined using a_w meter (Decagon AquaLab, 4 TE). The moisture content (%) was determined by drying the samples at 70 °C for 24 h without air circulation until a constant mass was achieved [17]. The pH values of all samples were determined using a digital pH meter (Hanna, HI-2211, Romania) at room temperature. The pH value of FP was determined directly from the homogenized mixture, while the pH value of the powder samples were measured by making a 25% (w/v) suspension of the sample in distilled water [18]. Finally, the total ash content was measured by incinerating the samples in a muffle furnace at 550 °C until gray-white ash was obtained using AOAC (2010) [19] method and expressed as g/100 g dry matter (DM). All measurements were done in 3 replications.

2.3 Color analysis of the samples

The color parameters of all samples were determined by using Chromameter CR-400 Konica Minolta (Tokyo, Japan). As a result of the analysis, L^* (brightness), a^* (redness-greenness), b^* (yellowness-blueness) values of the samples were recorded. The hue angle ($^{\circ}h$) and chroma (C^*) parameters calculated from these values by using Equation (1) and Equation (2), respectively [20]. All measurements were done in 3 replications.

$$^{\circ}h = \arctan (b^*/a^*) \quad (1)$$

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (2)$$

2.4 Preparation of extracts from the samples

The extraction procedure was performed according to the method described by Gunes et al. (2019) [21] with some alterations. First, 1 g of FP and powder samples were mixed with 25 mL of distilled water in falcon tubes and the tubes were held in an ultrasonic bath for 30 min at 40 kHz and 25 ± 2 °C. Then, the tubes were centrifuged at 9500 rpm for 10 min and the supernatants were filtered using a 0.45 μ m filter. The application was performed in duplicate and each extract was transferred into dark glass bottles and kept at -18 °C for further analysis. All analyzes applied to the extracts were briefly described below.

2.5 Determination of total phenolic content of the extracts

The total phenolic content was measured by the Folin-Ciocalteu colorimetric method of Singleton et al. (1999) [22] with some modifications. The absorbance values of the samples were read at 720 nm by using UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan). The results were expressed as mg gallic acid equivalents (GAE)/100 g DM (dry matter) using the calibration curve of gallic acid ($R^2 = 0.9993$) and taking into account the dilution rates applied. Each measurement was performed in triplicates and results were shown as mean values with a \pm standard deviation.

2.6 Determination of total flavonoid content of the extracts

Total flavonoid was analyzed using the aluminum chloride colorimetric method with slight modification according to Shraim et al. (2021) [23]. The absorbance values of the samples were read at 420 nm by using UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) and quercetin solutions in pure methanol were used to make the calibration curve ($R^2 = 0.9981$). The results were expressed as mg quercetin equivalents (QE)/100 g DM using this curve and each measurement was performed in triplicates and results were shown as mean values with a \pm standard deviation.

2.7 Determination of total monomeric anthocyanin content of the extracts

Total monomeric anthocyanins (TMA) were quantified using the pH differential method [24]. The difference of absorbance at $\lambda_{vis-max}$ (530 nm) and 700 nm was measured by using a UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) versus a blank cell filled with distilled water. The absorbance of the dilute solutions were calculated using Equation (3) and the total amount of monomeric anthocyanin pigment in the original samples were determined using Equation (4). Cyanidin-3-glucoside (cyd-3-glu) was selected because it is the most common anthocyanin in nature.

$$A = (A_{\lambda_{vis-max}} - A_{700})_{pH\ 1.0} - (A_{\lambda_{vis-max}} - A_{700})_{pH\ 4.5} \quad (3)$$

$$TMA\ (mg/100\ g\ DM) = (A \times MW \times DF \times 1000) / (\epsilon \times l) \quad (4)$$

where MW (molecular weight) = 449.2 g/mol for cyd-3-glu, DF = dilution factor (previously recorded), l = path length in cm; ϵ = 26900 molar extinction coefficient for cyd-3-glu ($L\ mol^{-1}\ cm^{-1}$), and 10^3 = factor for conversion from g to mg. The results as monomeric anthocyanins were expressed as mg cyd-3-glu equivalents (CGE)/100 g DM. Each measurement was performed in triplicates and results were shown as mean values with a \pm standard deviation.

2.8 Determination of antioxidant activity of extracts using DPPH and ABTS methods

The DPPH method was conducted according to the method of Thaipong et al. (2006) [25] with some modifications. Sample extracts were mixed with 0.1 mM DPPH (prepared with methanol) solution. The cuvettes were measured against the blank (methanol) using the UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) at 517 nm. A calibration curve ($R^2 = 0.9995$) was obtained by using Trolox standard solution at concentrations ranging between 50 and 1000 μ M. The results were expressed as μ mol Trolox/g DM and each measurement was performed in triplicates.

The ABTS method was used according to Xu et al. (2016) [26] with some modifications. A known volume of sample extract was mixed with ABTS solution (diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm) into spectrophotometer cuvettes. The cuvettes were measured against the blank (methanol) with the UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan) at 734 nm. A calibration curve ($R^2 = 0.9971$) was obtained by using Trolox standard solution at concentrations from 50 to 4000 μ M. The results were expressed as μ mol Trolox/g DM and each measurement was performed in triplicates.

2.9 Statistical analysis

The data obtained as a result of the analysis studies were determined by using the Windows-based SPSS 17.0.1 (SPSS Inc., Chicago, Illinois, USA) statistical package program, and one-way ANOVA & Tukey's test was used to determine whether there was a statistical difference between the samples ($p < 0.05$).

3 Results and discussion

3.1 Physico-chemical measurement results of the samples

The results of some physico-chemical properties of fresh BFs and powder samples produced with two different drying techniques within the scope of the research were given in Table 1. Considering the results, it was determined that the moisture, water activity, pH, and total ash values of fresh BFs were 69.73%, 0.9691, 3.31, and 4.50%, respectively. According to the literature, Marakoğlu et al. (2005) [27] was detected the moisture, ash, and pH values of BFs growing on the bushes in Konya (Türkiye) as 69.37%, 2.72%, and 3.53, respectively. In another study, Barros et al. (2010) [28] found the moisture and ash values of BFs to be 60.86% and 6.65%. In the study of Babalau-Fuss et al. (2020) [5], the moisture, total ash, and pH values of BFs were found to be 68.5%, 2.81%, and 3.46, respectively. Based on these results, there are values that are very close to the results determined in the current study, and the differences in some values may be related to the climatic conditions and regional differences in which the BFs were obtained.

Table 1. Some physico-chemical properties of the samples.

Sample	Moisture (%)	Water activity	pH	Total ash
HAP	7.51 ±0.45 ^C	0.2471 ±0.0035 ^C	3.34 ±0.03 ^A	4.72 ±0.04 ^A
FDP	9.13 ± 0.17 ^B	0.2718 ±0.0031 ^B	3.35 ± 0.03 ^A	4.68 ±0.01 ^A
FS	69.73 ±1.46 ^A	0.9691 ±0.0008 ^A	3.31 ±0.07 ^A	4.50 ±0.04 ^A

Data represent average values ± standard deviation. There is no statistical difference between the results shown with the same exponential capital letter in the same column ($p > 0.05$). HAP: Hot air-dried powder. FDP: Freeze-dried powder. FS: Fresh sample. Total ash values were expressed as g/100 g DM.

As is known, the most important factor that restricts the shelf life of fresh fruits and triggers various reactions that cause deterioration of freshness is their high water content [29,30]. Therefore, the water activity and moisture values of the dried products are very important for a long shelf life period. In this regard, it was stated that the minimum water activity for all microbial growth is 0.60 and spoilage of foods would not be of a microbiological nature below this value [31]. In addition, it was reported that the activity of most enzymes was inhibited when the water activity was lower than 0.80. In particular, amylase, polyphenol oxidase and peroxidase in the food were strongly inhibited or lost their activity when the water activity value fell to the range of 0.25-0.30 [32]. As seen in Table 1, the moisture and the water activity values of the powders obtained at the end of the drying period were 7.51% and 0.2471 for the HAP sample, 9.13% and 0.2718 for the FDP sample. The water activity of powders in all cases was below 0.6, hence the samples might be deemed to be safe against common microbial damage as well as enzymatic activity. Considering the pH and total ash parameters, there was no statistically significant difference between all samples ($p > 0.05$).

3.2 Color analysis results of the samples

Drying processes usually cause changes in the color of the dried product. In this regard, the color of dried products is an important quality parameter that decides consumer acceptance [33,34]. In the present study, the color properties of fresh BFs and powder samples were given in Table 2. According to the results, L^* values increased in the powder samples compared to the fresh BFs, and the highest value was detected in the HAP sample ($p < 0.05$). The higher L^* value of the HAP sample may be due to lightening of the color due to further degradation of the pigments in the presence of oxygen during HAD treatment. Drying also caused an increase in $+a^*$ (redness) and $+b^*$ (yellowness) values of the powder samples compared to the FS. In this regard, the highest $+a^*$ value was detected in the FDP sample ($p < 0.05$), and it was seen that the $+b^*$ values of both powder samples were not different from each other ($p > 0.05$). Based on these results, FD treatment resulted in the production of fruit powder having more intense red color.

Table 2. The color properties of the samples.

Sample	L^*	a^*	b^*	$^{\circ}h$	C^*
HAP	35.57 ±0.96 ^A	21.42 ±0.07 ^B	7.77 ±0.02 ^A	19.9±0 .04 ^A	22.78 ±0.07 ^B
FDP	30.79 ±0.21 ^B	26.36 ±0.08 ^A	7.97 ±0.03 ^A	16.84 ±0.06 ^B	27.54 ±0.07 ^A
FS	12.66 ±0.16 ^C	8.01 ±0.34 ^C	1.23 ±0.16 ^B	8.11 ±0.36 ^C	8.73 ±0.67 ^C

Data represent average values ± standard deviation. There is no statistical difference between the results shown with the same exponential capital letter in the same column ($p > 0.05$). HAP: Hot air-dried powder. FDP: Freeze-dried powder. FS: Fresh sample.

The same trend was seen in hue angle ($^{\circ}h$) and chroma (C^*) values, and drying caused increase in these values of the powder samples compared to the FS. Hue angle is defined as the color intensity degree, starting at $^{\circ}0$, which indicates $+a$ (redness), $^{\circ}90$ indicates $+b$ (yellowness), $^{\circ}180$ indicates $-a$ (greenness) and $^{\circ}270$ indicates $-b$ (blueness) [35]. In this case, a lower hue value means a more red product [36]. Chroma is defined as the chromaticity of an area that appears white or highly transparent, evaluated as a ratio of the brightness of a similarly lit area. The words describing the high chroma colors are also used to describe extroverts such as lively, active, and energetic [37]. According to the results, the highest $^{\circ}h$ value was determined in the HAP sample, while the highest C^* value was found in the FDP sample. In the literature, according to the results of color analysis of the powders obtained by applying different drying treatments to various fruit products, the common interpretation is that the drying applications significantly change the color coordinates [38,39]. However, this situation can turn into an advantage in order to give different colors in products intended to use these fruit powders obtained in the research.

3.3 Total phenolic (TPC), flavonoid (TFC), and monomeric anthocyanin (TMA) content results of the samples

The TPC, TFC, and TMA contents of the water extracts of the FS and powder samples were given in Table 3. According to each analysis result, the highest values were detected in the FS sample, followed by the FDP and HAP samples, respectively. Previous studies have shown that BFs contain a variety of bioactive polyphenolic compounds, including phenolic acids, flavonoids, and anthocyanins. In this regard, it has been determined that aqueous (water) or alcoholic extracts of BFs have significant antioxidant, antibacterial, and anti-inflammatory activities [40]. For instance, in a study, the TPC, TFC, and TMA contents of BFs water extracts were determined as 1217 mg GAE/100 g, 42 mg QE/100 g, and 120 mg CGE/100 g fresh material, respectively [4]. In another study, Marčetić et al. (2022) [7] determined the TPC, TFC, and TMA contents of water extracts of BFs collected from different regions of Serbia as 1522.2-1645.7 mg GAE/kg, 342.4-460.6 mg hyperoside equivalents/kg, and 6.8-22.2 mg CGE/kg fresh material, respectively.

Table 3. The total phenolic, flavonoid, and monomeric anthocyanin pigment contents of the samples.

Sample	TPC	TFC	TMA
HAP	3759.23 ±189.86 ^C	2467.78 ±74.75 ^C	640.71 ±47.49 ^C
FDP	4401.81 ±226.91 ^B	3023.04 ±67.54 ^B	756.42 ±45.49 ^B
FS	4991.62 ±118.00 ^A	3872.74 ±96.13 ^A	851.69 ±49.73 ^A

Data represent average values ± standard deviation. There is no statistical difference between the results shown with the same exponential capital letter in the same column ($p > 0.05$). HAP: Hot air-dried powder. FDP: Freeze-dried powder. FS: Fresh sample. TPC: Total phenolic content (mg gallic acid equivalents (GAE)/kg dry matter). TFC: Total flavonoid content (mg quercetin equivalents (QE)/kg dry matter). TMA: Total monomeric anthocyanin pigment content (mg cyd-3-glu equivalents (CGE)/kg dry matter).

However, there are limited studies on the characterization of powders or other types of products obtained from BFs by different processing techniques and the changes that fresh fruits undergo depending on the production process. In this perspective, according to the results of the present study, the biological activity of the FDP sample obtained with FD treatment was quite close to the FS values. This is an expected result, because FD treatment stands out as one of the important methods in the preservation of sensitive compounds among drying techniques [41,42]. On the other hand, HAD treatment, is a simpler and more cost-effective method. In this regard, the temperature (40 °C) and fan-assisted application that preferred in the present study did not cause the TPC, TFC, and TMA values of the HAP sample to decrease much (Table 3).

3.4 Antioxidant activity results

The antioxidant activity results of the water extracts of the samples investigated by two distinct methods (ABTS and DPPH) were given in Table 4. It was determined that FS had the highest antioxidant activity compared to powder samples. In powder samples, the highest values belong to the FDP sample, which was very consistent with the previous analysis results. BFs, which are among the wild fruits, have high antioxidant activity and show the potential to be used for various purposes. In the literature, there are studies that determine the antioxidant activity of the extracts of fresh BFs obtained with different solutions by various methods. In different studies, the antioxidant activity of methanol and water extract of BFs were determined as 43.6 μM Trolox/g fresh material [43] and 55.1 μM Trolox/g fresh material [44] according to ABTS method, respectively. In another study, the ABTS and DPPH antioxidant activity of combined methanol and acetone extracts of freeze-dried BFs, collected during three consecutive seasons, were found to vary at 18.3-76.4 μmol Trolox/g fresh weight and 9.2-13.9 μmol Trolox/g fresh weight, respectively [45]. Marčetić et al. (2022) [7] determined that the ABTS and DPPH values of methanol extracts of BFs collected from two sites in central and western Serbia were 398.8 and 33.8 μmol

Trolox/g fresh fruit, respectively. As can be seen, results close to the values obtained in the present study are available in the literature, however, there are also studies with different results arising not only from the diversity of the biological material (BFs), but also from the analysis method, especially in relation to the extraction medium and the calculation differences of the results.

Table 4. The antioxidant activity results of the samples depending on DPPH and ABTS methods.

Sample	ABTS (μmol Trolox/g)	DPPH (μmol Trolox/g)
HAP	28.05 ± 1.59 ^C	20.60 ± 0.89 ^C
FDP	32.17 ± 1.31 ^B	28.77 ± 1.01 ^B
FS	36.15 ± 1.06 ^A	32.57 ± 0.72 ^A

Data represent average values ± standard deviation. There is no statistical difference between the results shown with the same exponential capital letter in the same column ($p > 0.05$). FDP: Freeze-dried powder. HAP: Hot air-dried powder. FS: Fresh sample.

On the other hand, considering the fruit powders, it is possible to compare the antioxidant activity results of dried different fruits or powders obtained by HAD and FD techniques in other studies with the current results. For instance, ABTS results of methanolic extract of sour cherry fruits dried by HAD (50 °C) and FD techniques were found to be 477.80 and 604.47 μmol Trolox/g dry matter, respectively [11]. In a different study, Oszmianski et al. (2015) [46] found that ABTS and DPPH values of methanolic extracts of freeze-dried cranberry fruits were 194.46 and 144.84 μmol Trolox/g dry weight, respectively. In another study, DPPH results of methanolic extracts of freeze-dried and convective-dried (60 °C) date slices were determined as 1.48 and 1.87 μmol Trolox/g dry weight, respectively ([47]). Sadowska et al. (2019) [48] determined the antioxidant activity values (ABTS) of water extracts of the powders using FD and convective drying (70 °C, 48 h) applications as 583.6 and 489.5 μmol Trolox/g dry matter, respectively.

4 Conclusion

In the present study, the quality of fruit powders obtained as a result of processing wild blackthorn fruits (BFs) with convective hot air-drying (HAD, 24 h) and freeze-drying (FD, 24 h) treatments were investigated. According to the results, both treatments provided suitable results in terms of water activity and moisture content of the powders. Both drying treatments altered the color parameters of the obtained powders compared to the fresh fruit puree. On the other hand, the FD treatment provided the production of powder with higher bioactive content and antioxidant activity ($p < 0.05$). In this regard, it is very important to choose the most suitable method and parameters depending on the end product quality, cost and usage purpose. As is known, HAD offers the advantages of lower equipment costs and it is always a much quicker process. On the other hand, FD is more expensive than air drying because of the specialist equipment and the energy costs, but it provides a reasonable added value by

dehydrating the product as close as possible to its fresh condition. Finally, BFs, their processed products such as powder forms, or different extracts have high potential in terms of natural food ingredients, providing the color and the stability of the products. For this reason, it is thought that further studies should be given importance to re-evaluate BFs as a safe and cost-effective source of antioxidants. In addition, this study contributes not only to a better knowledge of BFs but also to their valorisation and the rich bioactive compounds of the studied fruit powders make them a very special supplement for food fortification.

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