RESEARCH ARTICLE

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# Bioactive compounds of hawthorn powders produced by convectional and lyophilized foam mat drying method

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**Abstract**

Fruit powders produced with drying technologies have a wide range of uses in the food industry. The fruit powders have the potential to be used as a food supplement or natural colorant thanks to their health-promoting functional properties. Hawthorn is one of the fruits that has attracted attention in recent years with its positive effects on health. In this study, hawthorn powder was produced by convective (C) and lyophilized (L) foam mat drying methods. In preliminary experiments, the best foam properties were obtained with 1% egg white powder. The foams were dried until their moisture content decreased to 4±0.5%. Three different temperatures (60-65-70°C) were used in convective foam mat drying. Total phenolic content (TPC), total flavonoid content (TFC), antioxidant activity (ABTS and DPPH) and phenolic composition were determined in the powders. The convective foam mat dried at 60 °C (C-60) and lyophilized foam mat dried (L) samples exhibited higher TPC and ABTS values than other samples. In powder and fresh samples, gallic acid, protocatechuic acid, chlorogenic acid, epicatechin, and catechin were detected with the liquid chromatographic method. Epicatechin and chlorogenic acid were the most abundant phenolic compounds in the samples. In the C-70 sample, epicatechin and protocatechuic acid were significantly lower ($p<0.05$). According to the results of the study, it was determined that the samples that applied the foam mat drying technique at 60 °C showed similar results with lyophilized foam mat drying. The foam mat drying method at 60 °C can be recommended as a preferred method in hawthorn powder production due to the reduction in drying time, low investment and operating costs.

**Keywords:** Hawthorn, Powder, Foam Mat Drying, Lyophilization, Phenolic compounds, Antioxidant

## INTRODUCTION

Many fruit species are grown wild or culturally in Turkey, which is very rich in plant diversity. One of these fruits, the Hawthorn fruit, belongs to the genus *Crataegus* of the *Rosaceae* family. The hawthorn grows in the Northern Hemisphere, and it mainly grows in Western and Southern Anatolia in our country; it generally grows on slopes overlooking streams, in bushes, in rocky and stony places, in forests, or spread over mountainous areas (González-Jiménez et al., 2018).

Hawthorn contains high amounts of pectin (~9.94%), sugar compounds (~5.50%), and phenolic compounds such as anthocyanins (~0.03%) and flavonoids (~1.93%) (Cuevas-Bernardino et al., 2016; He et al., 2013). Among the phenolic compounds (+) catechin and (-)-epicatechin, procyanidin B2, procyanidin B5, procyanidin C1, and procyanidin D1, hyperoside, apigenin, quercetin, chlorogenic acid, gallic acid, vitexin, hesperetin, coumaric acid, caffeic acid, naringenin, cratenacin were

detected in hawthorn fruit, which has high antioxidant activity due to the phenolic compounds it contains (Coklar & Akbulut, 2016).

The awareness of hawthorn fruit is increasing day by day all over the world, especially because it has been used for therapeutic purposes for many years for medical purposes. Different parts of the plant (leaves, flowers, shoots, roots) and medicines prepared from them are used in traditional and complementary medicine practices and have been treated as medicine since ancient times (Chang et al., 2002). It is reported that it is widely used as a herbal medicine in various parts of the world for the treatment of gastrointestinal complaints, high blood pressure, sore throat, cough, flu, asthma, colds, skin diseases, nephritis, hemorrhoids, especially cardiovascular diseases (Dahmer & Scott, 2010; Nazhand et al., 2020). Many of which are wild, have recently been under the spotlight worldwide due to growing requests for natural and sustainable eco-compatible remedies for pathological conditions with beneficial health effects that are able to support/supplement a daily diet or to support and/or replace conventional pharmacological therapy. The main requests for these products are: safety, minimum adverse unwanted effects, better efficacy, greater bioavailability, and lower cost when compared with synthetic medications available on the market. One of these popular herbs is hawthorn (Crataegus spp.). These positive health effects are also associated with the flavonoids in hawthorn (Rigelsky & Sweet, 2002) pharmacology, clinical efficacy, dosage and administration, adverse effects, and drug interactions of hawthorn are discussed. Hawthorn (Crataegus oxyacantha) in animal studies, hawthorn has been shown to have positive effects on blood pressure, and blood lipids; antioxidant and anti-inflammatory effects. In addition, it has been reported that it may have protective effects on heart health and can be listed as a functional food against cardiovascular diseases (Kisioglu & Nergiz-Unal, 2018).

Despite the stated health benefits, fruits and vegetables, are easily perishable products with a moisture content of over 80%. It can be difficult to implement low-temperature storage techniques, which are the best way to keep the product fresh, during the distribution chain. Drying can be applied as the most appropriate post-harvest processing technique, especially in regions where cold chain applications and processing facilities are insufficient (Dev & Raghavan, 2012). In addition, uncultivated hawthorn-like fruits are offered for sale in small local markets in a short period of a year, especially for people living in big cities which are not easy to reach.

Physical and biochemical changes occur with the reduction and the mobility of water from food during the dehydration process. In addition to physical changes such as shape and structure changes, encrustation, and regional dry matter accumulation in the fruit structure (Cemeroğlu, 2003), changes occur in the color, flavor, and nutritional properties of the dried product (Nijhuis et al., 1998). The changes uniquely occur in each product, and the temperature intensity applied in the drying process significantly affects the level of these changes (Cemeroğlu, 2003).

In the dried foods group, dry powder foods have become preferred by consumers and manufacturers in recent years due to their various advantages. With the increasingly changing living conditions, consumers expect a single product they buy to meet all their requirements and provide ease of use. On the other hand, food manufacturers have expectations from the product such as long shelf life, easy processing, reduced packaging, storage, and transportation costs due to reduced volume/weight. For this reason, food manufacturers and consumers especially tend to foods in powder form. For powder food production, spray drying can be carried out by vacuum drying, freeze-drying and foam mat drying methods (Koç & Ertekin, 2016). However, various difficulties the use of spray drying poses due to the adhesiveness problem in drying foods such as fruit juices with low glass transition temperatures due to components such as sugars and organic acids. In vacuum drying and freeze-drying methods, due to drying at low temperatures, a sticky structure is formed with the removal of the vacuum, as well as preserving the nutrient content (Jiang et al., 2013). Also, freeze-drying and vacuum-drying methods have additional disadvantages such as high installation and operating costs due to low temperature and high vacuum applications long drying times, working in batch systems, low temperature and high vacuum applications (Sangamithra et al., 2015; Türker et al., 2018).

Foam mat drying is the process of converting a liquid or semi-liquid product into a stable foam and then drying it generally convectively. This drying technique gives good results especially in drying foods with high sugar content, viscous and low glass transition temperature. It is stated as an economical and practical alternative to other drying methods for food powder production (Qadri et al., 2020). The foam mat drying method has additional advantages of increasing food quality such as preservation of nutritional quality and bioactive components as a result of drying at a lower temperature and in a shorter time, and easy reconstitution (Sangamithra et al., 2015). Despite the advantages mentioned, in some products, low-quality products can be obtained compared to traditional powder product production methods such as spray drying with FMD. Therefore, research is carried out on hybrid drying methods that are alternatives to hot air drying methods in FMD. Hybrid methods are developed by applying FMD together with other drying methods such as vacuum drying, freeze drying and microwave drying (Qadri et al., 2020). Compared to other conventional drying methods, freeze-drying (FD) is

accepted as the method that minimizes the loss of taste, aroma and nutritive elements. However, due to the very high cost of production, the use of FD is restricted to high-value-added and sensitive products (Ratti, 2013). It is thought that the use of FD in combination with FMD as a hybrid method may provide an additional advantage as it can reduce the drying time of foamed and frozen products. There is a limited number of studies conducted with Foam Mat Freeze Drying (FMFD). This method has been applied in previous studies for drying egg white (Muthukumaran et al., 2008), apple juice (Raharitsifa & Ratti, 2010), dates (Seerangurayar et al., 2018), and blueberry juice (Darniadi et al., 2018).

In line with all these objectives, it was aimed to produce hawthorn powder by convectional foam mat drying (C) and lyophilized foam mat drying (L), and to examine the bioactive properties which provide antioxidant effects of the produced hawthorn powders.

## MATERIALS AND METHODS

### Foam Preparation and Drying

The hawthorn fruit used was obtained from the local market in October 2020 and 2021. The fruits were kept in the refrigerator at 4 ± 2 °C until used in the experiments. According to the results of preliminary experiments, the conditions to obtain stable foams were determined. After washing and sorting, boiled water at a ratio of 1:2 (w:v) was added to the fruits and left for 30 minutes. The fruit flesh obtained after the sorting process was crushed with a blender for about 1 minute. To minimize possible bioactive component losses, the amount of water-soluble dry matter (Brix) was adjusted to 3.5 by adding its boiling water. As a foaming agent, 1% (w/v) egg white powder (Gusto, İstanbul-Turkey) was used. After the foaming agent was added, a hand mixer (Arzum Ar1017 Prostick 1000 W, Turkey) was mixed at maximum speed for 5 minutes and foam was obtained. The foam obtained was homogeneously spread on 20 cm diameter stainless steel trays with a thickness of 5 mm.

Laboratory type digital tray convective dryer was used for drying (Dalle Lt-10, China). Three temperatures, 60, 65 and 70 °C (C-60, C-65, C-70) were chosen as the drying temperature. The foams obtained for freeze-drying (L) were frozen and then lyophilized in a freeze-drying device (Xianou-12 N Freeze Dryer) under a vacuum of 100 Pa. The drying was continued until the moisture content of the powders was 4 ± 0.5%. The samples that reached the desired humidity level were removed from the dryer and scraped with the help of a plastic spatula and homogeneous particle size was obtained by using a grinder (Fakir Hausgeräte, Germany). The production flow chart is given in Figure 1.

### Determination of moisture and Water activity (aw)

An IR moisture analyzer (Kern & Sohn Gmbh, Germany) was used in the moisture analysis of powder samples. Temperature mode was selected as step (60°C, 70°C, 80°C). The water activity values of the powder samples were determined with a digital water activity meter (Novasina Lachen, Switzerland).

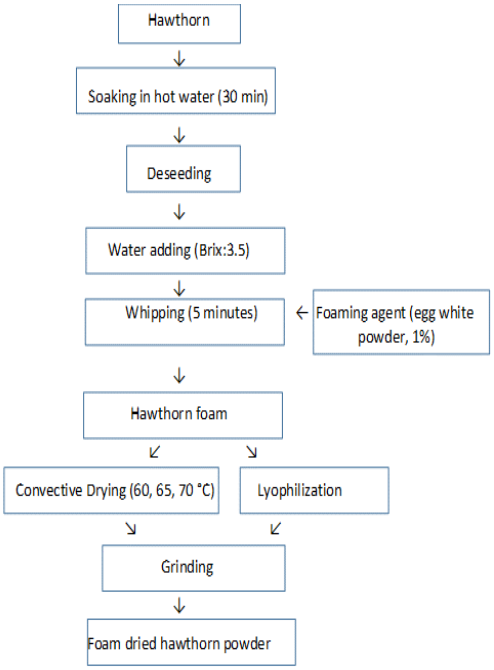


**Figure 1.** Flow chart for production of foam mat dried hawthorn powder

### Total phenolic content (TPC) analysis

Total phenolic content (TPC) was determined according to the colorimetric Folin-Ciocalteu method (Singleton et al., 1999). After adding sodium carbonate solution (%20) and foline reagent (2 N) to the diluted sample, the tubes were kept in the dark for 120 minutes. The total amount of phenolic substance in the samples was calculated as gallic acid equivalent (GAE) from the gallic acid standard curve. The absorbance values were measured at 760 nm (Shimadzu Scientific Instruments, Inc., Tokyo, Japan). The concentration of total phenolic compounds in samples was calculated as gallic acid equivalent (GAE) using an equation obtained from the standard gallic acid curve ($R^2$ =0.993).

### The total flavonoid content (TFC) analysis

The TFC of samples was determined using the spectrophotometric method as reported by (Zhishen et al., 1999). The absorbance of the mixture was measured at 510 nm after the addition of sodium nitrite (NaNO2), aluminum chloride (AlCl3) and NaOH solutions in the samples diluted with distilled water at appropriate ratios. A catechin calibration curve was created using a catechin solution in the range of 0-400 mg/L. Total flavonoid content was calculated from the calibration curve as catechin equivalent.

### Determination of Antioxidant Activity (ABTS$^+$ and DPPH)

ABTS$^+$ (2,2'-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt) radical scavenging activity was spectrophotometrically measured at 734 nm (Re et al., 1999). The diluted and homogenized sample at appropriate concentrations was added to the absorbance-adjusted ABTS solution. The absorbance value after completion of the reaction at 30 °C for 6 minutes. ABTS$^+$ values were expressed as Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) equivalents (mmol TE/100 g).

To measure the DPPH radical scavenging activity, the diluted samples were added to the DPPH solution and kept in the dark for 30 minutes. The absorbance was measured at 517 nm. The linear regression equation was obtained from the standard curve prepared from Trolox concentrations. The value was given as mmol TE/100 g (Brand-Williams et al., 1995)

### Liquid Chromatographic Analysis of Phenolic compounds

To determine the effects of different foam mat drying methods on phenolic compounds, liquid chromatographic analysis (HPLC) was used (Shimadzu SCL-10A, Scientific Instruments, Inc., Tokyo, Japan). Phenolic compounds including quercetin ($R^2$=0.995), catechin ($R^2$=0.993), chlorogenic acid ($R^2$=0.997), caffeic acid ($R^2$=0.988), rutin ($R^2$=0.986), p-coumaric acid ($R^2$=0.999), gallic acid ($R^2$=0.990), protocatechuic ($R^2$=0.998), epicatechin ($R^2$=0.989) were separated. The compound amounts were calculated by the external standard method and shown as µg/g dry weight (d.w.). min flow rate, column temperature 30°C and injection volume 20 µl. The gradient procedure started at 7% of solvent B for 10 min and increased linearly during 90 minutes of analysis time.

### Statistical analysis

Experimental data were analyzed with OneWay ANOVA of $p<0.05$ significance level. Duncan's multiple comparison tests were used to assess differences between samples. Statistical evaluation was carried out using SPSS 22.00 statistical package program (SPSS Inc., Chicago, IL). All the experiments were carried out in duplicate and repeated three times.

## RESULTS AND DISCUSSION

In food powders, the moisture content is critical in processing and storage. It is especially associated with physical properties such as cohesiveness, and caking problems (Barbosa-Cánovas et al., 2019). Water activity is related to food stability and the aw values of dried products are relatively low (Perera, 2005).

The moisture value of fresh hawthorn samples was 73.25 ±1.9 % (Table 1). After the drying process, the moisture content of the samples was between 3.97-4.58%. The water activity values of the samples were 0.129-0.162.

In the literature, there is no study on foam drying of the hawthorn. Therefore moisture and aw values of different fruit powders produced with foam mat drying methods have been reviewed. The moisture content of Spray dried and FMFD blueberry powders were 2.2-4 % (Darniadi et al., 2018). Water content and water activity of freeze-dry, convectional, microwave and lyophilized FMD apple juices were reported as 1.81-3.81% and 0.097-0.171 (Jakubczyka et al., 2011).

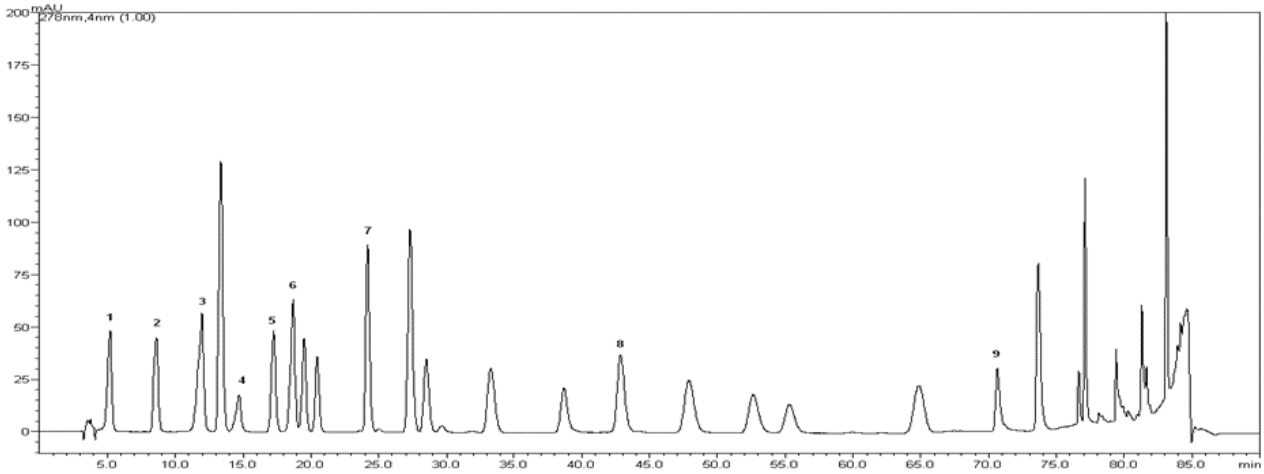


**Figure 2.** Standard chromatogram of phenolic compounds (1:gallic acid, 2:protocatechuic acid, 3:catechin, 4:chlorogenic acid, 5:caffeic acid, 6:epicatechin, 7:p-coumaric acid, 8:rutin, 9:quercetin)

The standard chromatogram was given in Figure 2. Agilent Eclipse XDB-C18 (250x4,60 mm, 5µm) was used as column and chromatographic conditions were gradient phase (A: %3 acetic acid, B: Methanol) 0,8 ml/

Fruits and vegetables show positive effects on health because they contain high amounts of phenolic compounds with bioactive properties. They are the most important sources of phenolic substances, including

flavonoids and anthocyanins (Lin & Tang, 2007).

TPC and TFC of fresh samples were 23975 mg GAE/kg and 13062 mg CE/kg, respectively. TPC and TFC of powder samples are shown in Figure 3. The highest total phenolic content was found in the samples produced by foam mat drying and C-60 and L samples. The total phenolic content of the convective FMD samples decreased with increasing temperature. The TPC content of the C-70 sample was found to be significantly lower than the other samples ($p<0.05$). Total flavonoid content (TFC) was between 26999-29224 CE mg/kg. TFC was the highest in the L sample, however, differences were not statistically significant.

**Table 1.** Moisture and water activity values (aw) values of hawthorn powder samples

| Powder samples | Moisture (%) | aw |
|---|---|---|
| C-60 | 3.97±0.58 | 0.162±0.01 |
| C-65 | 4.12±0.33 | 0.127±0.01 |
| C-70 | 4.13±0.55 | 0.129±0.01 |
| L | 4.58±0.68 | 0.139±0.01 |

C-60: Convective foam mat dried hawthorn powder at 60 °C, C-65: Convective foam mat dried hawthorn powder at 65 °C, C-70: Convective foam mat dried hawthorn powder at 70 °C, L:Lyophilized foam mat dried hawthorn powder

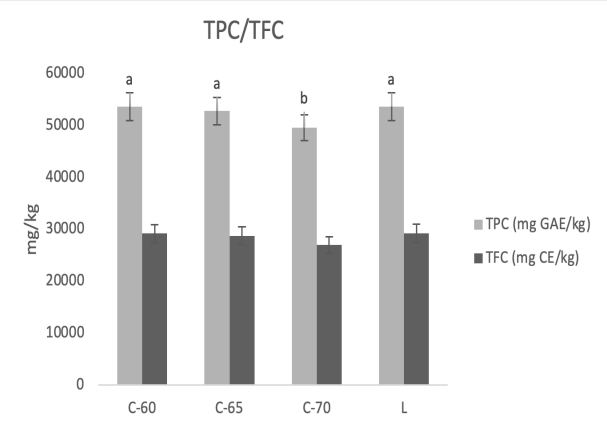


**Figure 3.** Total phenolic content (TPC) and total flavonoid content (TFC) of foam mat dried hawthorn powders
Different lowercase letters indicate a significant difference among different samples ($p<0.05$)

González-Jiménez et al. (2018) reported that the amount of TPC and TFC in fresh hawthorn (*Crataegus pubescens*) was 168.6 mg GAE/g and 55.89 g Quercetin Equivalent/g. In hawthorn slices dried by different methods, TPC was found the highest in lyophilized and dried at 60°C samples, while the flavonoid content was highest in the lyophilized sample In hawthorn slices, TPC increased in the FD sample and samples applied hot air drying at 60 °C. However, TPC decreased with increasing drying temperatures. TFC values of freeze-dried hawthorn were higher than fresh and hot air-dried samples (Liu et al., 2019). Li et al. (2020) found the amount of TPC and TFC in convective dried (60 C-16 hours) hawthorn were 2982 mg GAE/100 g and 51 mg RT/100 g and they stated that TPC and TFC were 3158 mg GAE/100 g and 79 mg RT/100 g in lyophilized samples, respectively. They emphasized that thermal treatment caused a reduction in soluble and total phenolic components of hawthorn. Darniadi, (2017) dried blueberry juice with foam mat drying method using Maltodextrin and Whey Protein Isolate, and he reported higher TPC and Total Monomeric Anthocyanin amounts in dried powders than in spray drying.

Flavonoids, flavone-C-glycosides, catechins, triterpene saponins, and oligomeric procyanidins in hawthorn have antioxidant effects (Rigelsky & Sweet, 2002). The antioxidant activity of such nutrient-derived antioxidant molecules found in fruits can be measured by various methods. Antioxidant activity values of hawthorn powders were determined by ABTS and DPPH methods (Figure 4). ABTS values were between 1,345-1,605 mmol TE/100 g and L and C-60 samples exhibited higher ABTS radical scavenging activity than powders produced at 65 and 70 C temperatures ($p<0.05$). DPPH radical scavenging activities of samples were in the range 5.88-6.37 mmol TE/100 g. Although L and C-60 showed high radical scavenging activity, no statistical difference was found. The DPPH values of soluble and insoluble phenolics in dried hawthorn were found to be 0.15 and 5.10 mg/ml loss, respectively, while ABTS values were reported as 0.06 and 4.46 mg/ml loss for soluble and insoluble phenolics, respectively (Li et al., 2020). Liu et al., (2019) reported that DPPH values decreased significantly in hot air-dried hawthorn slices.

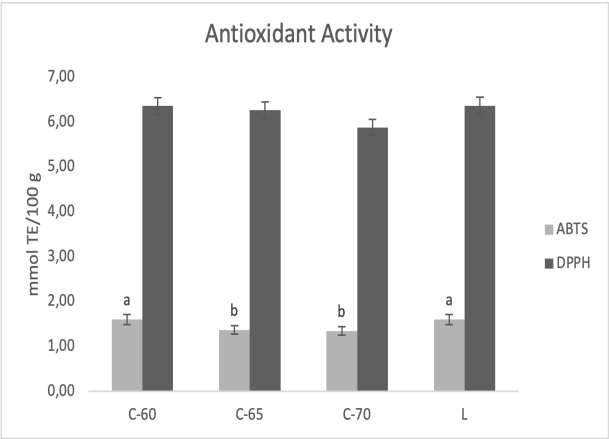


**Figure 4.** Antioxidant activity (ABTS and DPPH) values of fam mat dried hawthorn powders
Different lowercase letters indicate a significant difference among different samples ($p<0.05$)

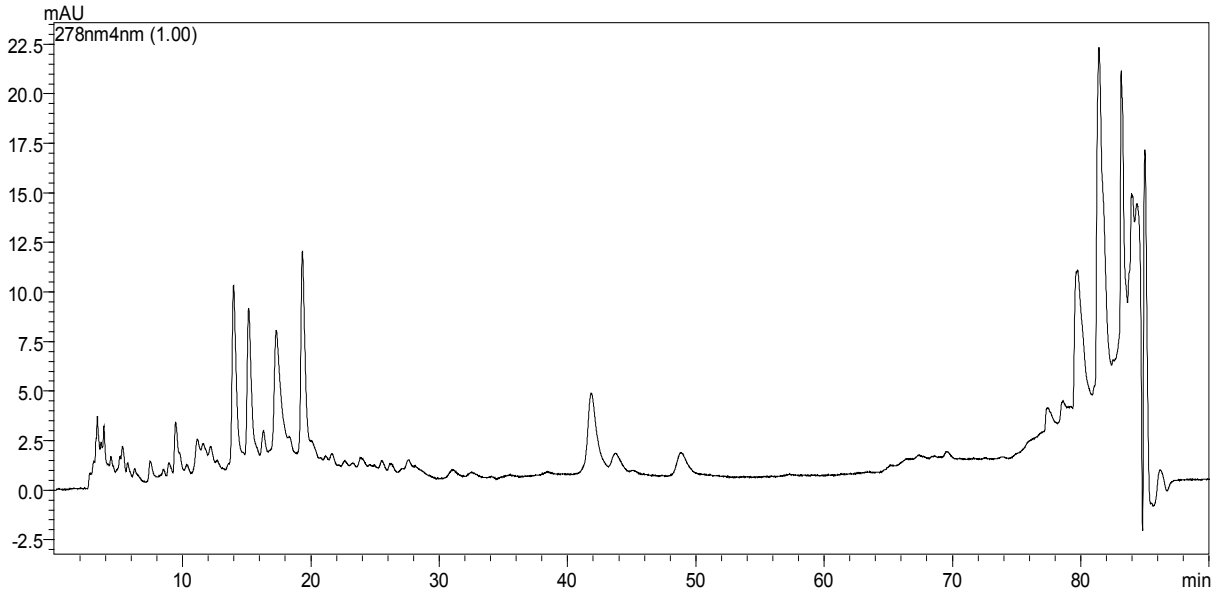


**Figure 5.** A sample phenolic chromatogram of the hawthorn powder (C-70)

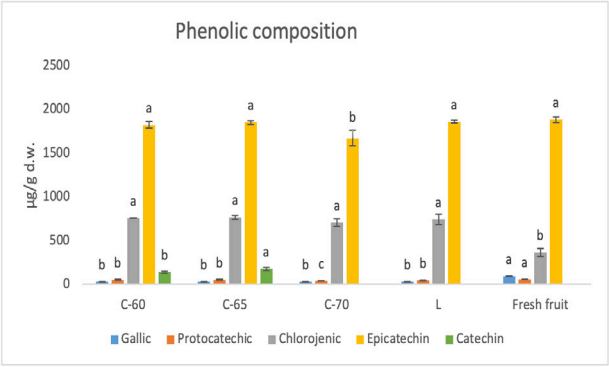


**Figure 6.** Phenolic compound profile of hawthorn powders and fresh fruit
Different lowercase letters indicate a significant difference among different samples ($p<0.05$)

Figure 5 shows a sample chromatogram of the C-70 sample. Five phenolic compounds in FMD hawthorn powders were separated. Major phenolic compounds in hawthorn powders were chlorogenic acid and epicatechin (Figure 6). Similar to total phenolic matter results, epicatechin and protocatechuic acid content of C-70 was statistically lower than other powder samples.

Gallic acid content significantly decreased in dried samples and gallic acid content of the fresh sample was higher than in dried samples ($p<0.05$). In another study examining the thermal stability of gallic acid, it was reported that it decreased by 15% and 25% with the application of 60 and 80C temperatures for 4 hours (Volf et al., 2014).

All powder samples contained higher chlorogenic acid content than fresh samples. The protocatechuic acid content of the fresh sample was the highest ($p<0.05$). Catechin was only found in C-60 and C-65 samples.

Procyanidin B2, Epicatechin, and Rutin, among 11 phenolic compounds detected in ripe hawthorn fruit, were reported as three major phenolic compounds (Coklar & Akbulut, 2016). In another study, catechin, chlorogenic acid, procyanidin B2, epicatechin, quercetin, paracoumaric acid, hyperoside, and isoquercitrin phenolics were identified in fresh hawthorn (Zhang et al., 2020). In dried hawthorn slices, amounts of Procyanidin B2; epicatechin and chlorogenic acid, which were major phenolic compounds, were 2.67; 1.93; 1.34 mg/g, respectively. Other detected phenolics were vitexin rhamnoside, rutin, hyperoside and isoquercetin. It was found that the decrease in the amount of epicatechin and chlorogenic acid in hawthorn, which was applied to hot air drying at 60 °C for 16 hours, was significant (Li et al., 2020). Liu et al., (2019) dried hawthorn slices with various methods and reported that the amounts of chlorogenic acid, epicatechin, rutin, ellagic acid and protocatechuic acid in freeze-dried samples were 482, 517, 143, 21 and 32 mg/100 d.w., respectively. In this study, epicatechin was determined as the most abundant phenolic component, which was consistent with our results. The amount of chlorogenic acid increased in the FD sample compared to the fresh sample, similar to our results. The difference between the chlorogenic acid content of the samples dried at 60 and 80 °C and the chlorogenic acid content of the fresh sample was not found significant. The amount of epicatechin decreased with increasing drying temperature. While protocatechuic acid decreased in the lyophilized sample, it increased in the hot air-dried (60 and 80°C) samples and decreased at higher temperatures (100-120°C).

Generally, fresh plant products are thought to have a higher phenolic content than dried products due to the degradation of phenolics during drying. However,

it has been stated that dried products such as tomatoes and shiitake mushrooms have higher phenolic content compared to fresh ones (Suvarnakuta et al., 2011). Heat treatment can lead to the release of phenolic acids accumulating in vacuoles due to the breakdown of cellular components (Dewanto et al., 2002). Most antioxidant compounds in plants are covalently bound to insoluble polymers. It is suggested that heat treatment breaks down the cell wall, liberates the antioxidant components from the insoluble part, and increases the number of bioavailable antioxidant components (Choi et al., 2006). In our study, it is thought that the reason why the amount of chlorogenic acid in the powder samples was higher than in the fresh sample may be that the heat treatment liberates the phenolics, which are bound in the plant cell walls, which are complex, porous polysaccharide structure.

In the study examining the phenolic stability in apple pomace, apple pomace was dried at temperatures of 50, 60, 70 and 80 °C. In the samples dried at 80 °C, the decrease in the amount of epicatechin was found to be significant compared to the samples dried at 50 C, the change in the amount of chlorogenic acid was found to be insignificant for all four drying temperatures. The amounts of chlorogenic acid, epicatechin and catechin in the dried samples increased compared to the pre-drying sample (Heras-Ramírez et al., 2012).

Phenolic compounds are significantly affected by foam mat drying conditions. It has been reported in some studies that high foam mat drying temperature reduces phenolic compounds. On the other hand, the type and concentration of the foaming agent can be effective in the loss or preservation of the bioactive components. Depending on the foaming and stabilizing agent and the type of dried product, variable results can be seen promoting either the preservation of phenolic substances or their degradation (Reis et al., 2021)

## CONCLUSION

Foam mat drying is a drying method applied after foaming liquid or semi-liquid foods using various foaming and stabilizing agents. It provides an advantage because it is an easy-to-apply method with low investment and operating costs. Another advantage of this method is that the drying time is shortened and the thermal degradation of bioactive components in foods is less. To increase product quality and energy efficiency, there are searches for the application of hybrid methods in which the FMD technique is applied together with different drying methods. In this study, foam drying methods were used in the production of powder from hawthorn fruit, which is one of the fruits that have been emphasized more in recent years due to its positive effects on health, and the effects of the methods and different drying temperatures on the antioxidant bioactive components of the powders were determined.

The freeze foam mat dried samples showed similar bioactive content to the samples that were convective foam mat driedat 60 °C. According to these results, convective foam mat drying application at the appropriate temperature for the product can be considered a preferable method in terms of bioactive component content. Hawthorn powders produced by foam mat drying methods, whose bioactive content is highly preserved, can be used as a natural functional ingredient or food supplement in the food industry. Dried fruit and vegetable products are valuable foodstuffs as a concentrated source of nutrients and bioactive compounds. Optimizing the process conditions is important to maximize both their quality properties and their bioactive content.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The author declared that for this research article, they have no actual, potential, or perceived conflict of interest.

### Author contribution

The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

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### Data availability

Not applicable.

### Consent for publication

Not applicable.

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