

Drying Effects on Anthocyanins and Technological Properties of Berry Powders (*Rubus* and *Vaccinium* spp.): Exploring Alternatives to Artificial Colorants

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Abstract—Fruits from the *Rubus* and *Vaccinium* genus have a short shelf life because of their high moisture content. Due to their high anthocyanin content and antioxidant capacity, the application of methods for preserving them and preventing food waste is essential. This study aimed to assess the impact of the drying time and temperature on anthocyanins' concentration and the particle size characteristics of the obtained powders. Samples of raspberries, blackberries, and blueberries were cleaned and stored until use. Dehydration kinetics were obtained at 1-12 h and 60°C, 70°C, and 80°C in a convection stove. Total anthocyanin contents were quantified during dehydration, and technological properties from the powders, such as particle size distribution and index were evaluated. Blueberries displayed the highest moisture loss (87.69 ± XX % at 80°C) and the highest anthocyanins' retention, being temperature the most critical parameter affecting anthocyanins' concentration. The 24 h dehydration aiming to improve powdering properties of the berries impacted on their color but allowed all berries to be screened through 20-60 mesh. Results indicated that blackberries are the most suitable berries to be dehydrated and powderd, preserving most of its anthocyanins.

Keywords—Berries, powders, anthocyanins, dehydration, particle size.

I. INTRODUCTION

Food waste problem is on the rise in Mexico. Despite more than 28 million Mexicans grappling with food insecurity, there has been a 40% surge in food wastage in the country, as reported by the Mexican Food Banks Network (BAMX), mainly in the fruits and vegetables group [1].

Berries from the *Rubus* and *Vaccinium* species contain high amounts of anthocyanins, which are water-soluble polyphenolic-based glycosides derived from poly-hydroxyl and poly-methoxyl variations of 2-phenyl-benzopyrylium and are commonly referred to as flavylum salts. These compounds also have a high antioxidant capacity that has been linked to improvements in some biomarkers from non-communicable diseases [2]. Moreover, anthocyanins are some of the most critical responsible for the berries' color. The most abundant

anthocyanin in fruits is cyanidin-3-glucoside, exhibiting some of the highest anti-inflammatory activities [3].

Among berries, raspberries (*Rubus idaeus* L.), blackberries (*Rubus fruticosus* L.), and blueberries (*Vaccinium corymbosum* L.) are representative groups of berries of high production in Mexico. Raspberries consist of numerous convex, depressed, and roughly textured drupes clustered together, forming a distinctive pineapple-like arrangement that stands out effortlessly. While the most common color is red or yellowish, varieties of fruits come in white and black hues. It has been reported that raspberries contain around 9.27 mg of anthocyanins (cyanidin and pelargonidin derivatives, 32:1) per gram of fruit [4].

Blackberries comprise many closely clustered and interconnected small drupes (multidrupe), initially red and transforming into black as they ripen. In the ripening stage, blackberries may contain up to 1.02 mg per gram. Some anthocyanin types reported in blackberries are cyanidin-3-*O*-glucoside, cyanidin-3-*O*-sophoroside, pelargonidin-3-*O*-glucoside, petunidin-3-*O*-glucoside, peonidin-3-*O*-galactoside and peonidin-3-*O*-glucoside [5]. Finally, blueberries contain malvidin 3-galactoside, delphinidin 3-galactoside, petunidin 3-galactoside, malvidin 3-arabinoside, and cyanidin 3-glucoside among others, and fruits are black-blue berries measuring 0.5-1 cm with numerous seeds. Studies reported a content of 7.2 mg of anthocyanins per gram [6].

In this sense, foods with high water activity/content, such as berries, have a short shelf-life. The indicated limitation confers just two days under standard storage conditions, making berries utilized not only as fresh fruit, but predominantly frozen fruit, jam, juice, wine, and other products [5]. Nonetheless, controlling the moisture content of a product is a key tool for its preservation. This can be achieved through drying, which removes moisture and other volatile substances to produce a solid and dry product (dehydrated products) [7]. An inconvenience of this process is that anthocyanins are

thermolabile molecules degraded at relatively higher temperatures $>60^{\circ}\text{C}$, causing color changes.

Optimizing dehydration methods while balancing polyphenolic preservation and antioxidant capacity in dehydrated blackberry fruits is challenging and requires careful attention to maintaining desirable qualities [8]. Once dehydrated, milling and particle size have critical applications during food processing [9] since producing these dry powders could be an alternative to replacing artificial colorants, an urgent industrial trend [10]. This study aimed to assess the impact of drying time and temperature on the concentration of anthocyanins and the particle size characteristics of dry powders obtained from three different berries.

II. MATERIALS AND METHODS

A. Biological material

Three types of fruits were obtained from a commercial market *Rubus idaeus*, *Rubus fruticosus*, and *Vaccinium corymbosum* species, corresponding to common names such as raspberries, blackberries, and blueberries. The fruits were washed, disinfected, and left dry for 3 hours at room temperature ($25 \pm 1^{\circ}\text{C}$) to remove excess water.

B. Drying process

Several preliminary tests were conducted to determine the different temperatures and drying times to which the fruits would be subjected to achieve the desired characteristics. To perform the dehydration process, a device prototype coupled to a drying oven (Yamato®, model No. DX602, Scientific America Inc., Japan) and an analytical balance (AND®, model No. GR-202, Japan) with a sensitivity of 0.0001 g was employed. The fruits were individually placed on mesh trays to ensure uniform air distribution (Figure 1). The fruits were subjected to three different temperatures (60, 70, and 80°C) until dehydration was achieved. Samples were weighed at different times (0, 4, 8, and 12 hours) to conduct the kinetics. The balance registered the sample weight without opening the oven to avoid temperature fluctuations.



Fig. 1. Raspberry fruits in an aluminum mesh at the initial stage of drying process.

C. Anthocyanin extraction from dehydrated fruits

Individual fruit pieces were extracted at 0, 4, 8, and 12 hours, placed in a 50 mL plastic tube, and stored in a freezer (Sanyo MPR-215F, USA) at 4°C until further use. Exposure to light was avoided to prevent the degradation of anthocyanins. A sample of 3 grams from each stored tube was weighed, ground in a mortar, and placed in a 125 mL Erlenmeyer flask with acidified ethanol (ethanol 85%, HCl 1N, 15%) [11]. The flasks were refrigerated for 48 hours for subsequent anthocyanin quantification, stored in complete darkness, and sealed with Parafilm to prevent solvent evaporation.

D. Total monomeric anthocyanins content assessed by spectrophotometry UV-vis

The contents in the flasks were transferred to 5 mL centrifuge tubes. After that, they were centrifuged for 10 minutes at $2500 \times g$ (SOLBAT J-40, Puebla, Mexico) at 4°C . This process ensured a proper separation of the solid and liquid phases. Once the centrifuged extract was obtained, the supernatant was compared to a blank reagent in a quartz cell to determine the total amount of anthocyanins present. The quantification was performed using spectrophotometry (Thermo Scientific, Evolution 300 UV-Vis) at a wavelength of 520 nm to measure these compounds.

Samples exceeding the spectrophotometer's detection limit were diluted at a 1:10 ratio. Anthocyanin quantification was determined based on the spectrophotometer's absorbance according to the method proposed by Escalante-Aburto [12]. The calculation of the total monomeric anthocyanins concentration (mg/kg) in the fruits was done using the equation (1) proposed by Abdel-Aal & Hucl [13]:

$$C = (A/25,965) \times (50/1,000) \times 449 \times (1/3) \times 10^6 \quad (1)$$

Quantifications were performed in triplicates.

E. Milling process of the dehydrated fruits to obtain powders

For this step, fruits were removed from the oven after 24 hours of dehydration and left to cool for 1 hour in a dehydrator to avoid moisture recovery due to the high hygroscopicity of the samples. Then, a knife mill (A11 Basic model, IKA-Werke, Staufen, Germany) was used to obtain the powder; samples of 5 grams were placed inside the equipment and milled three times for 30 sec. The obtained powders were kept in polyethylene-sealed bags and stored at 5°C in darkness until use.

F. Particle size analysis

Particle size distribution (PSD) was obtained using a sieve separator, passing the ground material through the following sieves: No. 20 ($841 \mu\text{m}$), 30 ($595 \mu\text{m}$), 40 ($420 \mu\text{m}$), 60 ($250 \mu\text{m}$), and lid. The powders retained on each sieve were weighed

to determine the particle size distribution (PSD %) using equation (3):

$$\text{PSD (\%)} = (\text{grams retained on each mesh}) / (\text{grams of initial sample}) \times 100 \quad (3)$$

The result of this determination was reported as the percentage of weight retained on each sieve for each sieve number. The analysis was performed in duplicate.

For the particle size index (PSI), the higher the ITP, the greater the quantity of fine particles in the evaluated material. It was calculated according to the method reported by Bedolla & Rooney [14] using the following formula (3):

$$\text{PSI} = \sum[(\text{FNOM}_i)(\% \text{DTP}) + \dots + (\text{FNOM}_n)(\% \text{DTP})] \quad (3)$$

Where:

FNOM: Sieve Number Factor

PSD: Particle Size Distribution (%).

The sieve number factor was assigned according to the U.S. series: 0.2 for No. 20 sieve; 0.3 for No. 30 sieve; 0.4 for No. 40 sieve; 0.6 for No. 60 sieve, and 1 for the lid. The percentage retention for each sieve was obtained in the PSD analysis [9]. The determination was carried out in duplicate.

F. Experimental design and statistical analysis

A 3x3x4 factorial design was employed, where the evaluated factors were temperature (60, 70, and 80 °C) and drying time (0, 4, 8, and 12 h). For the statistical analysis, a variance analysis with a 95% confidence level was conducted to observe the effects of processing factors on the percentage of water loss, total anthocyanin concentration, particle size distribution, and index of the obtained powders. Tukey-Kramer's multiple range test was also performed to identify statistically significant differences among the evaluated variables ($p < 0.05$). The statistical analysis was conducted using the Minitab® version 21.4.2 software package.

III. RESULTS

The results of the analysis of variance (ANOVA) for the effect of temperature and drying time on fruit moisture loss are presented in Table 1, and according to the F -value, time and temperature and their interaction significantly affected the evaluated parameters.

TABLE I
ANALYSIS OF VARIANCE, F -VALUE FOR THE EFFECT OF TEMPERATURE AND DRYING TIME ON FRUIT WEIGHT LOSS.

Source of variation	Degrees of freedom	F
Time	12	66.40**
Temperature	2	297.90**
Time*Temperature	24	3.83**

**Significant ($p < 0.0001$)

Figures 2a, 2b, and 2c present the dehydration kinetics of the fruits conducted in the convection oven at 60, 70, and 80 °C.

The percentage of weight lost for each fruit, based on the initial weight and drying time, is shown. The highest moisture loss achieved after drying was observed in the raspberry at 80 °C (Figure 2c), at 87.69 ± 0.13 % at 8 hours. Lower percentages of weight loss were obtained for blackberries and blueberries.

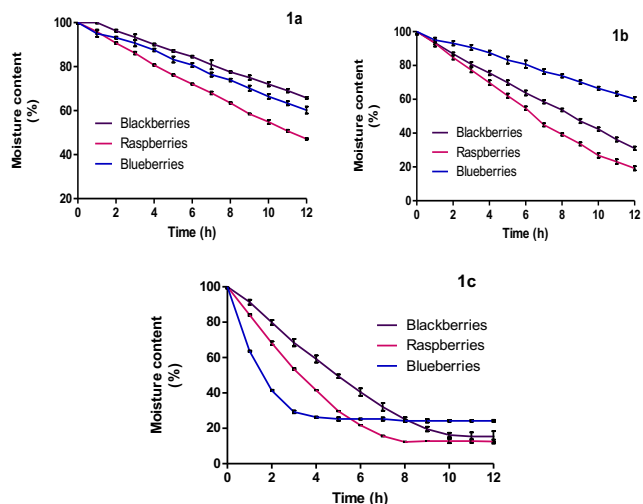


Fig. 2. Moisture content loss (%) during the dehydration process of three berries at different temperatures (1a, 60°C; 1b 70°C; and 1c 80°C) and times (0-12 h).

The results of the analysis of variance (ANOVA) for the effect of temperature and drying time, as well as their interaction on the concentration of anthocyanins in the evaluated fruits, are presented in Table II.

According to the F -value, time and temperature statistically significantly affected the total anthocyanin content. The interaction of time*temperature did not present a statistically significant effect.

TABLE II
ANALYSIS OF VARIANCE, F -VALUE FOR THE EFFECT OF TEMPERATURE AND DRYING TIME ON TOTAL ANTHOCYANINS CONTENT.

Source of variation	Degrees of freedom	F
Time	16	3.38*
Temperature	2	7.68*
Time*Temperature	10	1.71 ^{ns}

*Significant ($p < 0.05$), ^{ns} non-significant ($p > 0.05$)

Figure 3 displays the average concentration of total anthocyanins per fruit during the dehydration process. It was observed that blueberries had the highest anthocyanin concentration (756.51 mg/kg), almost double compared to raspberry (305.16 mg/kg) and blackberry (363.26 mg/kg).

The highest concentration of total anthocyanins was observed in blueberries at 70°C-80°C at 8 h and 12 h of the dehydration process. All three evaluated temperatures were observed to reach a peak concentration of anthocyanins before a significant loss occurred.

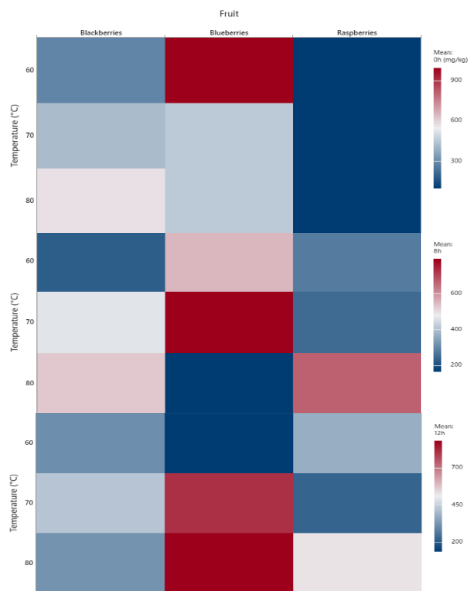


Fig. 3. Total anthocyanins concentration (mg/kg) evaluated in different berries at three temperatures (60°C, 70°C, and 80°C) and three dehydration times (0, 8 and 12 hours). The results are an average of three replicates.

Results from the milling process are depicted in Fig. 4. After 12 h of dehydration, all the berries have an inadequate consistency for the powder's obtention. Thus, blueberries were dehydrated 80°C, 24 h; 70°C, 44 h, and 60°C, 60 h; blackberries 80° and 70°C, 36 h and 60°C, 52 h; and raspberries up to 24 h. As expected, some samples showed color degradation due to anthocyanin losses.

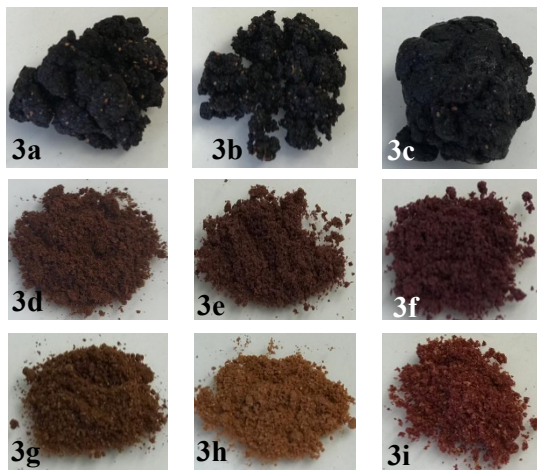


Fig. 4.

Powder obtained after different drying temperatures (60°C, 70°C, and 80°C) and times. 3a, 3b and 3c correspond to blueberries; 3d, 3e and 3f correspond to blackberries; 3g, 3h, and 3i correspond to raspberries.

Fig. 5 shows the PSD (%) from the obtained powders after the milling process. Tukey's comparison demonstrated that the berry variety did not affect the PSD (%) values ($p>0.05$).

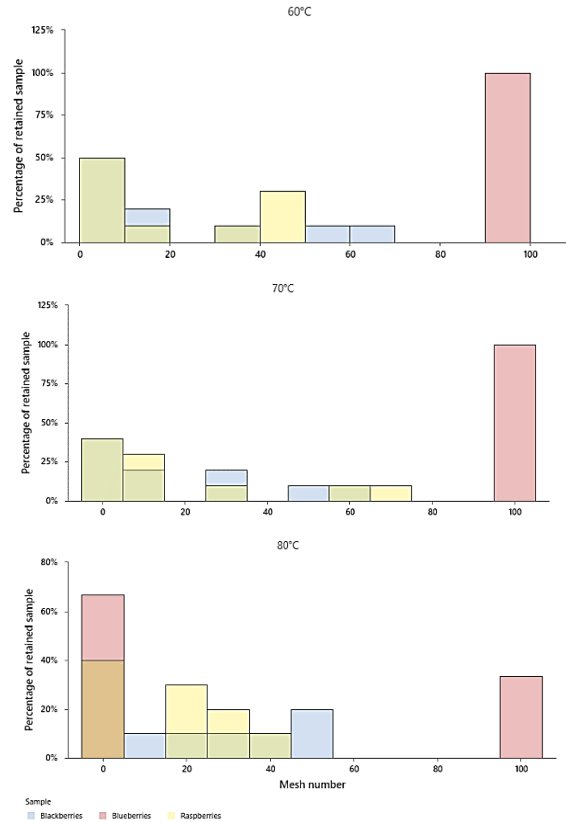


Fig. 5. Particle size distribution (PSD %) analysis from berry powders obtained at three dehydration temperatures (60°C, 70°C, and 80°C). The results are an average of duplicates.

Nevertheless, the results of the analysis of variance (ANOVA) showed that according to the F -value, the mesh size (mesh number) and the interaction of fruit type*mesh number showed a highly significant statistical effect ($p<0.0001$). Furthermore, the triple interaction of fruit type*temperature*mesh number demonstrated statistically significant effects ($p<0.05$) on this parameter.

Also, according to the Tukey grouping, there were no statistically significant differences in the mean particle size distribution among raspberry, blackberry, and blueberry powders. Blueberry powder exhibited the highest retention, displaying a 100% weight retention, indicating that it did not pass through the No. 20 sieve (841 μm) due to the resulting product from grinding not being a powder but rather a material with a hard pasty consistency. However, the powders obtained from blackberry and raspberry fruits successfully passed through all the sieves, including No. 20 (841 μm), 30 (595 μm), 40 (420 μm), and 60 (250 μm), indicating a lower percentage retention value (Fig. 4) for the three dehydration temperatures.

The results of the analysis of variance (ANOVA) for the effect of temperature, drying time, and their interaction on the PSI of the powders showed that according to the F -value, the fruit variety exhibited a statistically significant effect ($p<0.05$) on this value. In Fig. 5, it was observed that the powder with

the lowest PSI was blueberry (20.71), and this difference was statistically significant ($p < 0.05$) compared to raspberries and blackberries. Raspberry and blackberry were the powders with the statistically ($p < 0.05$) highest PSI, indicating finer particle grinding (29.55 and 26.03, respectively), as it had the greatest moisture loss among the fruits. Consequently, higher dehydration leads to easy manipulation during grinding and uniformity among its particles.

Among the evaluated powders, the highest TPI (finest) was recorded for materials obtained at 60 °C. Nevertheless, none of the temperatures showed statistically significant differences according to the Tukey grouping. Blueberries obtained the coarse particle size index compared with the other varieties regarding the dehydration temperature.

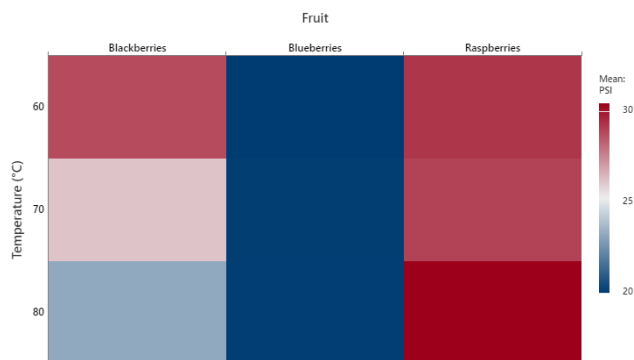


Fig. 5. Particle size index (PSI) analysis from berry powders obtained at three dehydration temperatures (60°C, 70°C, and 80°C). The results are an average of duplicates.

IV. DISCUSSION

Doymaz & Ismail [15] conducted a cherry dehydration study at 60°, 70°, and 75°C, concluding that temperature significantly affected drying time. On the other hand, Kim & Kerr [16] performed a study evaluating the influence of drying on blueberry powder's physical and quality properties analyzed at 80°, 95°, and 110°C. The authors concluded that higher temperatures led to faster dehydration and reduced drying time. These findings are consistent with this research, as the three fruits dehydrated more quickly at 80°C. In blackberries and blueberries, lower weight loss percentages were observed, reflecting significant variability based on the fruit type and temperature, as highlighted by García Pastor [17]. This variability indicates that each fruit possesses a different contact surface, leading to diverse dehydration patterns. Consequently, fruits subjected to higher temperatures will require less drying time.

It was observed that there is a specific dehydration time when the anthocyanin content in berries is at its highest, after which the compounds begin to degrade at an accelerated rate due to various factors. According to Patras et al. [18], the impact of temperature on anthocyanin concentration occurs through two mechanisms: 1) the hydrolysis of the glycosidic bond, leading to the formation of free sugars and aglycones

(anthocyanidins), or 2) hydrolytic breakdown resulting in compounds such as chalcones. Another factor is light, which affects anthocyanins in two different ways, being essential for the biosynthesis of these compounds, but also accelerating their degradation [19]. Additionally, enzymes commonly known as *anthocyanases*, play a role in anthocyanin degradation. In fruits such as berries, the polyphenol oxidase (PPO) acts on anthocyanins in the presence of *O*-diphenols through an oxidation mechanism. PPO, typically found in plant tissue, catalyzes the oxidative transformation of catechol and other *o*-dihydroxyphenols into *O*-quinones, which can subsequently react with amino acids or other phenolic compounds, including anthocyanins [6]. According to Zielinska et al. [20], berries exhibit surface crust, reduced bioactive compounds, undesired chemical reactions, off-flavors, shrinkage, and poor rehydration when processed by convection drying.

In Fig. 4, it was observed that blueberries resulted in coarse particles, and milling them to obtain a fine powder was not possible. Nevertheless, they preserved their coloration compared with powders from raspberries, with higher intense coloration (Fig 3h). Powders from blackberries resulted in more uniform materials regarding the temperature and time of dehydration.

Results from the PSD analyses demonstrated that blueberries were the most difficult to dehydrate to obtain fine particulate powders. The latter observations may be attributed to the waxy layer surrounding the blueberry that should not be damaged during handling, as it benefits its preservation. Associated with cutin are the waxes or soluble cuticular lipids; these are either embedded within the cuticular matrix, known as intracuticular waxes, or deposited on the outermost surface of the cuticle, known as epicuticular waxes. Additionally, the cuticle contains non-lipid components such as polysaccharides (mainly cellulose and pectin), polypeptides, and phenolic compounds [21]. While the cuticle is a barrier, it is not entirely impermeable to water. This may be one of the reasons why the blueberry exhibited a pasty consistency. However, powders obtained from blackberry and raspberry fruits successfully passed through all sieves, including No. 20 (841 μm), 30 (595 μm), 40 (420 μm), and 60 (250 μm), allowing the obtention of powders that could be further incorporated into different food matrices.

Regarding PSI, the blueberry showed a lower value since their consistency did not allow proper milling. This could be attributed to inadequate dehydration and consistency. Consequently, uniform powder was not achieved during the milling process due to its inappropriate consistency, rendering it unsuitable for classification as a powder. Raspberry emerged as the powder with the highest PSI, indicating finer particle grinding, as it had the highest moisture loss among the fruits. Therefore, increased dehydration leads to a milling process that is easy to manipulate, resulting in uniformity among its particles.

V. CONCLUSIONS

The study emphasizes the significant impact of drying parameters on the weight loss of fruits as well as the

concentration of anthocyanins. It is crucial to optimize the drying and milling conditions to achieve the highest anthocyanin content in the powders. The study observed that there is an optimum dehydration time for achieving high phenolic contents, low color degradation, and particle size. Although blueberries posed a challenge during milling, raspberries and blackberries proved to be suitable for producing finely ground products with potential food applications. Further studies are needed to evaluate the antioxidant activity of the powders and their functionality when are incorporated into specific food matrices.

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