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Graduation Research Project

**Extraction of anthocyanins from blueberry (*Vaccinium spp*) residues  
and evaluation of their coloring and antioxidant capacity in ice cream.**

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### Abstract

Blueberry residues are a valuable source of antioxidants, which can positively affect human health. The ethanolic extract of blueberry residue exhibits substantial antioxidant activity, as evidenced by its high antioxidant capacity. This study aimed to extract anthocyanins from blueberry residues for coloring the ice cream instead of using synthetic colorants avoiding harmful effects on health. Also, the study aimed to quantify the extraction of total anthocyanins from different ethanol: water concentrations and evaluate the antioxidant capacity as well as the application of blueberry residues in ice cream by measuring its physicochemical characteristics. A Completely Randomized Design was used; means obtained from color, pH, hardness, overrun, and antioxidant capacity were analyzed through ANOVA with Duncan test at  $p < 0.05$ . Total anthocyanins for different ethanol: water ratios (80:20, 60:40, 40:60, 20:80) of blueberry residues were evaluated, using an HPLC method, to determine which of them had the most anthocyanin content, which later was used to develop the ice cream. The antioxidant capacity of the various extracts was determined using the DPPH free radical scavenging assay, and the absorbance at 517 nm was measured. All the evaluated ethanol: water ratios showed scavenging activity above 90%. The different physicochemical characteristics measured did not have many significant differences among them; this occurred because of the little variations in the amounts of extract used. It is recommended to increase the amount of blueberry residue extract for the analyses of color, pH, overrun, and hardness.

*Keywords:* color, free radicals, HPLC, upcycling,.

## Resumen

Los residuos de arándano son una valiosa fuente de antioxidantes, que pueden afectar positivamente a la salud humana. El extracto etanólico de residuos de arándanos presenta una actividad antioxidante sustancial, como demuestra su elevada capacidad antioxidante. El objetivo de este estudio era extraer antocianinas de residuos de arándanos como fuente de color para el helado en lugar de utilizar colorantes sintéticos, evitando así efectos nocivos para la salud. Asimismo, el estudio pretendía cuantificar la extracción de antocianinas totales a partir de diferentes concentraciones de etanol: agua y evaluar la capacidad antioxidante, así como la aplicación de los residuos de arándanos en helados midiendo sus características fisicoquímicas. Se utilizó un Diseño Completamente Al Azar; las medias obtenidas de color, pH, dureza, aireado y capacidad antioxidante se analizaron mediante ANOVA con test de Duncan a  $p < 0,05$ . Se evaluaron las antocianinas totales para diferentes relaciones etanol: agua (80:20, 60:40, 40:60, 20:80) de los residuos de arándano, mediante un método HPLC, para determinar cuál de ellos tenía mayor contenido de antocianinas, que posteriormente se utilizó para elaborar el helado. La capacidad antioxidante de los distintos extractos se determinó mediante el ensayo de eliminación de radicales libres DPPH, y se midió la absorbancia a 517 nm. Todas las proporciones de etanol y agua evaluadas mostraron una actividad de barrido superior al 90%. Las diferentes características fisicoquímicas medidas no presentaron muchas diferencias significativas entre ellas; esto ocurrió debido a las pequeñas variaciones en las cantidades de extracto utilizadas. Se recomienda aumentar la cantidad de extracto de residuo de arándano para los análisis de color, pH, aireado y dureza.

*Palabras claves:* color, HPLC, radicales libres, reciclaje.

## Introduction

Globally, fruit waste is the largest and losses can occur at any stage along the entire value chain, from the agricultural production stage to consumption by the final customer. It is estimated that between 24% and 30% of the food produced is lost at the production stage, while around 20% is lost in post-harvest and 30% - 35% is lost at the consumption stage, while fruits and vegetables (44%) are the foods that are lost the most (Vilariño et al., 2017). Thus, according to Food and Agriculture Organization (2019) food loss is the decrease in the quantity or quality of food resulting from decisions and actions by food suppliers in the chain, excluding retail, food service providers and consumers. Food waste is the decrease in the quantity or quality of food resulting from decisions and actions by retailers, food services, and consumers. Food waste is initially intended for consumption and is discarded or used alternatively, either by choice or because it has been allowed to expire through neglect (FAO., 2015).

Upcycling is a new trend that seeks to reduce food waste by using processed food waste to create new products. This trend consists of avoiding food waste and losses by creating high-quality products from food that would otherwise go to waste. Through fruit surpluses, it is possible to improve the physicochemical characteristics of foods that are formulated with them, such as flavor, color, texture, fiber, and preservatives (García, 2022). Likewise, upcycling counters the argument that a product has no value once it is discarded or that it must be destroyed before it can re-enter a new circle of production and value creation (Wegener, 2016).

*Vaccinium ssp.*, better known as blueberries, are wild berries from the Ericaceae family, known for being very low in sugar content and very high in antioxidants. Currently, there are more than 450 different species of blueberries. This fruit is native to North America, where it grows throughout forests and mountainous regions of the U.S. and Canada. Although the habitat of this species is mainly colder regions of the northern hemisphere, nowadays, many of these species are

also cultivated in the southern hemisphere (Instituto de Investigación y Desarrollo de Comercio Exterior [IDEXCAM], 2017).

In the fruit sector, blueberry harvesting can result in approximately 21% of residues per ton harvested, while the other 79% is destined for agroindustry and home consumption. This is due to microbiological degradation processes, physical damage, dehydration, and overripening in the supply chain (Lagos-Alvarez et al., 2022). The industrial processing of these fruits inevitably generates a large amount of blueberry waste and by-products (press cake residues), consisting mainly of skins and seeds. Since blueberry press cake retains large amounts of anthocyanins and other phenolic antioxidants, it represents a potential source of natural colorants (Pataro et al., 2017). Thus, a way is being sought to find an optimal use for blueberry waste and to give it added value.

Different studies have found that blueberries are rich in bioactive compounds (Kalt et al., 2020). Blueberries have a broad spectrum of bioactive substances such as vitamin C, vitamin A,  $\beta$ -carotene, and phenolic compounds. Among the phenolic compounds, the predominant metabolites are flavonoids, particularly anthocyanins (Garrido & Pérez, 2014). Due to the high antioxidant activity compared to other fruits, the health benefits of blueberry became widely accepted after different studies reported that blueberries had the highest antioxidant activity of 42 fruits and vegetables evaluated. These sparked numerous investigations into the health benefits that blueberries provided, including that they reduced the risk of certain types of cancer, cardiovascular diseases, and other chronic diseases (Lee & Wrolstad, 2006).

Anthocyanins are responsible for the taste and flavor of fresh fruit and for the brilliant red color and its different hues in many fruits and berries. The attractive color is one of the main sensory characteristics of fruit and berry products (Song et al., 2018). Compared to other small fruits such as raspberries, strawberries, and cherries, blueberries contain higher antioxidant activity and higher levels of phenols, flavonoids, anthocyanins, and carotenoids.

Therefore, the objectives of the present study are:

To quantify the content of total anthocyanins, present in the blueberry residue extracts.

To evaluate the antioxidant capacity of the ethanolic extract of blueberry residue.

To evaluate the physicochemical properties of blueberry residue extracts in ice cream.

## **Materials and Methods**

### **Location of the Study**

This research was conducted at the Louisiana State University, Baton Rouge, Louisiana, United States of America. The preparation of the raw material, extraction of anthocyanins, evaluation of the antioxidant capacity, and physicochemical analyses were performed at the Animal and Food Science Laboratory. While the ice cream was produced at the Dairy Science Building.

### **Research Description**

The study was developed in three phases. The first phase consisted of the extraction of anthocyanins with different concentrations of ethanol: water. The second phase consisted of measuring antioxidant properties. Finally, the third phase consisted of making the ice cream with the extracts and evaluating different physicochemical characteristics of the resulting ice cream.

### **Methods**

#### ***Blueberry Residue Preparation***

Frozen blueberries were obtained from a local grocery store. The blueberries were ground in a blender and filtered using a cheesecloth and cleaned with water to obtain the blueberry residue. The rinsing step was repeated until the water became clean and colorless.

#### ***Anthocyanin Extraction***

For the extraction of anthocyanins, a solid-liquid extraction solvent was prepared, controlling the concentration variables according to each treatment. Two grams of the blueberry residue were placed in each test tube and the different ethanol to water ratios (treatments) were added in a 25 ml centrifuge tube. Then each treatment was shaken in the Vortex for one minute and was left in a water bath for 2 and 4 hours at 60 °C so that later it could be analyzed using HPLC (High-Performance Liquid Chromatography). Subsequently, the extracted solution was centrifuged at 5000 rpm for 15 minutes to remove any of the sediments.

### **Total Anthocyanins**

To obtain the concentration of total anthocyanins, the method of High-Performance Liquid Chromatography was performed using a Thermo Scientific Ultimate 3000 HPLC and an Ultimate 3000 Diode Array Detector set at 520 nm for monitoring anthocyanins as well as a Luna C18 column (250 × 4.60 mm, 5 μm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of 10% acetic acid (A) and acetonitrile (B) and was run in a gradient mode. The flow rate was 0.800 ml/min.

For the preparation of the samples the same solid-liquid extraction solvent as stated above was prepared. Followed by using the different ethanol to water ratios (treatments). The only difference was that when pouring the samples into the HPLC screw neck, 1.5 mL, flat bottom, amber vials a Thermo Scientific Nalgene Syringe Filter 0.2 μm was used to prevent any possible sediment of the residues to go through the HPLC analysis.

### **Antioxidant Capacity**

The antioxidant activity of the extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl), it was prepared following the methodology reported by Trandafir et al. (2013) with some modifications. A methanolic stock solution of DPPH (0.1mmol/L) was prepared. Next, 0.2 mL of encapsulated extract was added to 1.8 mL of methanolic DPPH solution and shaken vigorously. The samples were left to incubate for 30 min at 27 °C. After this time, each sample was poured into a spectrophotometer cuvette for the immediate absorbance measurement in a UV/VIS spectrophotometer (Cary 8454, Agilent Technologies, USA) at a wavelength of 517 nm. The ability to scavenge the DPPH radical was expressed as a percentage of inhibition and calculated using the following equation (Adebisi et al., 2017), Equation 1:

$$\text{Scavenging Activity} = \left( \frac{A_0 - A_1}{A_0} \right) * 100 \quad [1]$$

Where  $A_0$  was the absorbance of the control (DPPH solution at 0 min), and

$A_1$  was the absorbance of the sample and DPPH after 30 min incubation.

Additionally, a standard gallic acid curve was constructed by preparing concentrations of 2, 4, 6, and 7 ppm of gallic acid, dissolved in deionized water ( $R^2 = 0.9974$ ). The results were expressed in mg gallic acid equivalents per gram of extract (mg EAG/g of extract).

### ***Colorimetry***

The color analyses were evaluated by means of the Hunter Lab MiniScan XE Plus colorimeter with 10° standard observer and D 65 illuminant. Using the  $L^*a^*b^*$  scale (CIE-LAB), where  $L^*$  indicates lightness on a scale from 0 (black) to 100 (white). Likewise, the values  $a^*$  and  $b^*$  indicate chromaticity, where the positive value of  $a^*$  (+60) indicates red color tendency, negative  $a^*$  (-60) indicates green color, the positive value  $b^*$  (+60) indicates yellow color tendency and negative  $b^*$  (-60) indicates blue color. Each treatment was evaluated using 16 ounces of ice cream samples.

### ***pH***

pH of ice cream was measured using a Thermo Scientific® potentiometer calibrated with buffer solutions of 4 and 7. All measurements were done in triplicate. The ice cream samples were adjusted to room temperature. The electrode of pH meter was immersed in ice cream sample and reading was recording after stabilization of pH meter reading.

### ***Texture Analyses***

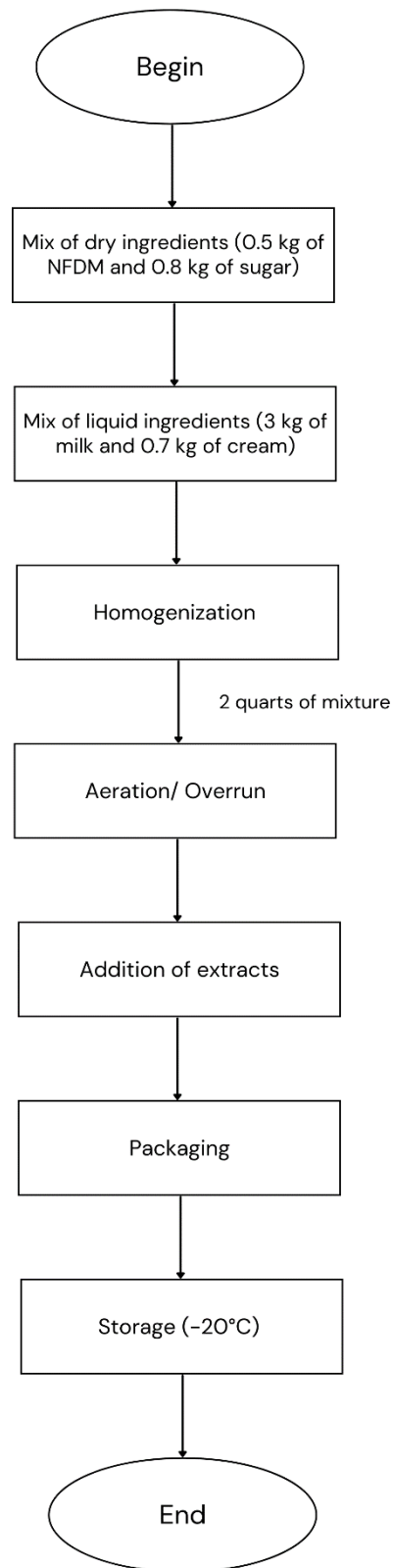
The texture properties of the ice cream such as hardness were determined using a texture analyzer (TA.XTplusC Texture Analyser, Stable Micro System). The ice cream samples were filled in a 16-ounce container for g-force measurement to prevent disturbing its physical and textural states. The speed was set to 2mm/s. The depth of penetration was inversely proportional to the hardness. Three measurements were taken per sample.

### ***Ice Cream Preparation***

As shown in Figure 1, the ice cream making was done by adding all the dry ingredients, 0.5 kg. of NFDM (Non-Fat Dry Milk) and 0.8 kg. of sugar followed up by adding the liquid ingredients, 3 kg. of milk and 0.7 kg. of cream in a 2 quarts cuvette. Next, all the ingredients were homogenized together

to create the mixture for the ice cream. Afterward, the aeration/ overrun was measured, followed by the addition of the anthocyanin extracts.

Finally, packaging and storage at -20 °C took place to end the preparation of the ice cream mixture used in the different analyses.

**Figure 1***Flowchart of ice cream*

## Overrun

Two overrun measurements were taken per sample by comparing the weight of the ice cream mix and ice cream in a fixed-volume container. Overrun (in %) was calculated as follows (Muse & Hartel, 2004).

$$\text{Overrun} = \left( \frac{\text{weight of mix} - \text{weight of ice cream}}{\text{weight of ice cream}} \right) \quad [2]$$

## Experimental Design and Statistical Analysis

Phase I consisted of determining which of the following treatments (Table 1) had the most amount of anthocyanin content using the HPLC method.

**Table 1**

*Ethanol: water ratio levels, treatments to be used for phase I*

Treatments	Description of ethanol: water ratio levels	
	Ethanol	Water
T1	80	20
T2	60	40
T3	40	60
T4	20	80

For phase II the concentration of antioxidant capacity of the anthocyanins in the ethanolic extracts was evaluated (Table 1). There were 4 treatments with 3 repetitions each, for a total of 12 experimental units (EU).

Phase III consisted of the application of the treatments (Table 2) that showed the most amount of anthocyanin content from Phase I on the dairy product. The dairy product chosen was ice cream. The addition of two concentrations of extract (1% and 3%) was studied, in addition to a control (without extract). For this phase, there was a total of three treatments with three repetitions each, for a total of 9 experimental units (EU). For the purposes of this experiment, the following treatments were identified:

**Table 2***Treatments for Phase III*

Treatment	Amount of extract
Treatment 1 (Control)	No extract
Treatment 2	1% of phase I extract
Treatment 3	3% of phase I extract

In which color, hardness, pH and overrun was evaluated for the 3 treatments.

Experimental Units for color, hardness, and pH: three treatments x three repetitions = 9 EU (Experimental Units).

Experimental Units for overrun: 3 treatments x 2 repetitions = 6 EU (Experimental Units)

**Statistical Analysis**

The study was based on four physicochemical analyses. For the analysis of color, pH, hardness, and overrun, as well as antioxidant capacity, a Completely Randomized Design (CRD) was used, using three repetitions. The separation of means was executed by using a Duncan means separation at a significance level of 95%. Statistical analysis was performed using the Statistical Analysis System (SAS version 9.4).

## Results and Discussion

### Total Anthocyanins

High Performance Liquid Chromatography (HPLC) is a frequently used analytical separation technique due to its high sensitivity, easy adaptation to exact quantitative determinations, its suitability for separating non-volatile species and its wide application in industry, science, engineering, and society in general, are some of the reasons for the great popularity of this technique (Ropero & Soledad, 2020).

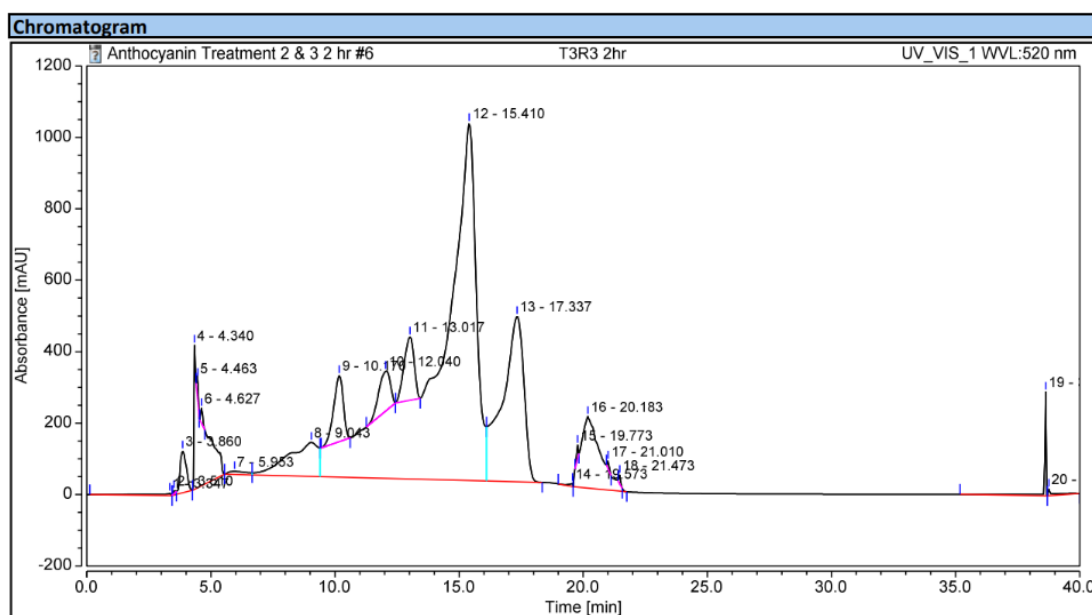
It has been widely used in the structural characterization of anthocyanin pigments and is therefore the most preferred procedure for the isolation and identification of anthocyanins. The experimental conditions are usually in reversed phase columns, with water-methanol or acetonitrile-methanol mixtures and with a detection of the components at 520 nm (Marcano & Hasegawa, 2018).

Figure 2 and Figure 3 are chromatograms of treatment 3 (ethanol-water ratio, 40:60) which shows that at both times (two and four hours) the anthocyanins were measured, Treatment 3 showed the greatest number of anthocyanins present. The anthocyanins found in the chromatogram at 2hr were Delphinidin-3-arabinoside, Petunidin-3-glucoside, Peonidin-3-galactoside, Malvidin-3-galactoside, Peonidin-3-arabinoside and Cyanidin-3-glucoside. And the anthocyanins found in the chromatogram at 4 hr were Delphinidin-3-arabinoside, Petunidin-3-glucoside, Peonidin-3-galactoside, Malvidin-3-galactoside, Peonidin-3-arabinoside and Malvidin-3-glucoside. In both chromatograms, the anthocyanins that had the highest peaks were Malvidin-3-galactoside and Peonidin-3-arabinoside with a retention time of 15 min and 18 min, respectively. According to Lin et al. (2020), Malvidin-3-galactoside is the major and most common anthocyanins in blueberry anthocyanins. It possesses great antioxidant capacity with excellent free radical scavenging properties (Huang et al., 2016) and contributes to reducing the risk of diabetes and cancerous cells (Herrera-Balandrano et al., 2021). While Peonidin 3-arabinoside has been studied extensively in scientific research due to its potential health benefits. It has been studied as an antioxidant, anti-inflammatory, and antimicrobial agent

(Faria et al., 2005). This differs with what was reported by Lončarić et al. (2020) where they used green extracted methods for extraction of polyphenolic compound from blueberry pomace where they found that Peonidin 3- glucoside was the most abundant anthocyanin in their study. According to these results, treatment 3 was the one used to develop phase III.

**Figure 2**

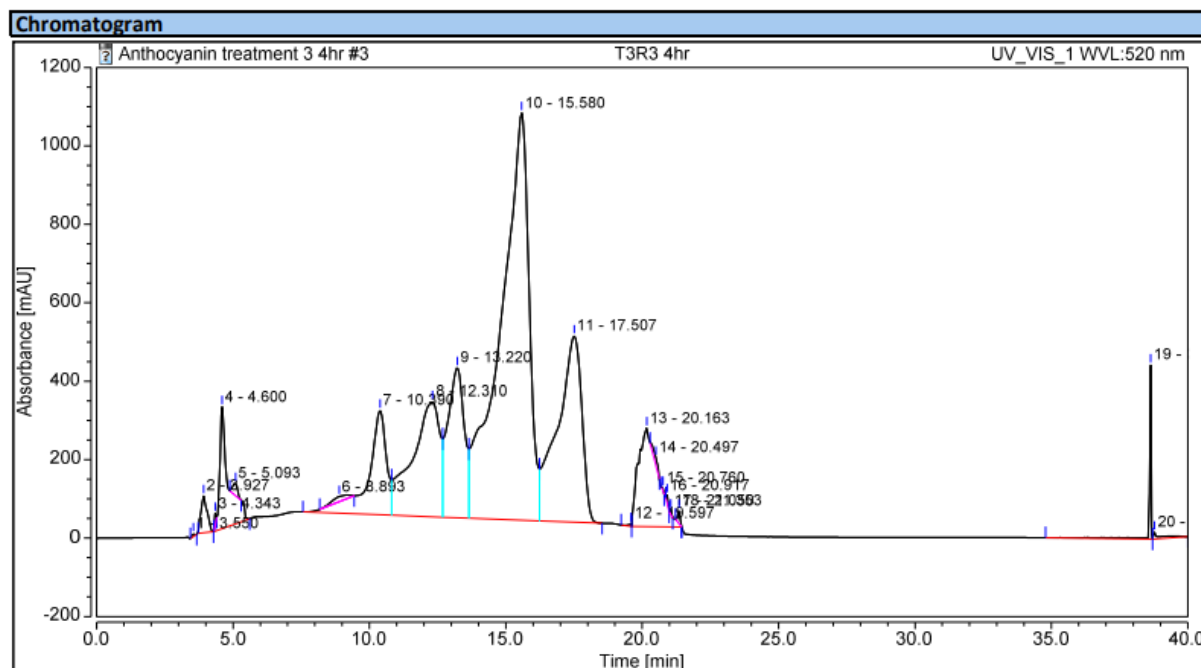
*HPLC chromatogram (520 nm) of anthocyanins of blueberry residues at 2 hr.*



	Retention time (min) 2hr					
	Delphinidin-3- arabinoside	Petunidin-3- glucoside	Peonidin-3- galactoside	Malvidin-3- galactoside	Peonidin-3- arabinoside	Cyanidin-3- glucoside
<b>Standard</b>	10.5	12.3	13.3	15.4	18.2	10.1
<b>Sample</b>	9	12	13	15.4	17.3	10.2

**Figure 3**

HPLC chromatogram (520 nm) of anthocyanins of blueberry residues at 4 hr.



	Retention time (min) 4hr					
	Delphinidin-3-arabinoside	Petunidin-3-glucoside	Peonidin-3-galactoside	Malvidin-3-galactoside	Peonidin-3-arabinoside	Malvidin-3-glucoside
Standard	10.5	12.3	13.3	15.4	18.2	20.4
Sample	10.3	12.3	13.2	15.5	19.5	19.5

### Antioxidant Capacity

There are different methods to quantify antioxidant capacity. The quantification of the antioxidant capacity of the blueberry residues was carried out by the spectrophotometric method and the assay using free radical 2,2-diphenyl-1-picrylhydrazyl, most known as DPPH. When DPPH reacts with radical scavenger, the purple color changed into pale purple or light yellow (Baliyan et al., 2022). The absorbance reduction of the stable radical DPPH at 517 nm was used to assess the free radical scavenging activity. Total antioxidant capacity is defined as the potential of a substance or compound to inhibit or hinder the oxidation of a substrate up to very small amounts (< 1%, commonly 1-1,000 mg/L) (Benítez-Estrada et al., 2020).

Gallic acid was employed as an antioxidant standard (Figure 4) to assess the antioxidant activity of the different ethanol: water ratios.

**Figure 4**

*Gallic acid standard curve*

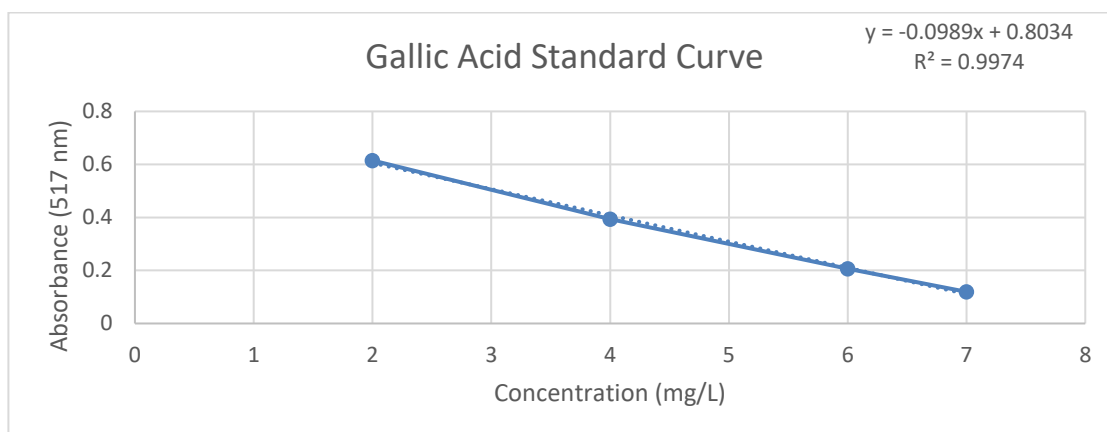


Table 3 shows four different treatment conditions. Each treatment represented a different ratio of ethanol to water used. The results reveal that blueberry residue extracts obtained from various ethanol: water ratios exhibited high antioxidant activity, with values ranging from approximately 90.27 to 90.80 %. In which according to its P value there are no significant differences in antioxidant activity among the tested ethanol-water ratios. However, there was a slightly trend towards higher antioxidant activity with increasing ethanol concentrations. Nevertheless, even at low concentrations, anthocyanins are powerful natural antioxidants (Lv et al., 2022). This agrees with what was reported by Thu Thao et al. (2015) where the ratio with the most amount of ethanol also presented the highest amount of antioxidant activity. This trend suggests that ethanol may be a better solvent for extracting antioxidant compounds from blueberries than water. Furthermore, because of their economic affordability, ethanol-water combinations are one of the most commonly utilized solvents (Hidalgo & Almajano, 2017). This can also be observed in the results of Ekin et al. (2017) where they measured the antioxidant activity of aqueous and ethanol extracts of *Crataegus meyeri* pojark leaves and concluded that the ethanolic extract showed more antioxidant activity, with higher percentage of inhibition, than the aqueous extracts. Antioxidants are tremendously important

substances, which possess the ability to protect the body from damage caused by free radical-induced oxidative stress. Plant polyphenols act as reducing agents and antioxidants by the hydrogen-donating property of their hydroxyl groups (Phuyal et al., 2020). Numerous research have been conducted to compare the performance of various solvents for the extraction and recovery of antioxidant compounds, and ethanol has been found to be the most successful when compared to water, acetone, hexane, ethyl acetate, and methanol (Hidalgo & Almajano, 2017).

**Table 3**

*Scavenging Activity of the different ethanol: water ratios of blueberry extracts.*

Treatment	Ethanol: Water ratios	Scavenging ability (%) $\pm$ S. D. <sup>NS</sup>
1	80:20	90.80 $\pm$ 0.29
2	60:40	90.43 $\pm$ 0.22
3	40:60	90.27 $\pm$ 0.36
4	20:80	90.35 $\pm$ 0.28
P		0.0988
C. V		0.25

Note. P : Probability. S.D.= Standard Deviation. CV = Coefficient of variation. NS: Not significant

### Color

Recently, the scientific community has shown a growing interest in exploring natural alternatives to food additives, with the goal of improving people's access to healthier diets. One noteworthy area of research is the development of natural pigments from various food industry residues (Montibeller et al., 2018). Color as describe by Simons (2018) is a sensory attribute of food that can be used to determine its quality and consumer acceptability. Color measurements using a spectrophotometer allow food manufacturers and retailers to understand and meet food quality standards (Phillips, 2023). According to Wrolstad and Smith (2017), color can also be defined as the sensation that is experienced by an individual when radiant energy within the visible spectrum (380-770 nm) falls upon the retina of the eye.

In Table 4, the results obtained for lightness show that each treatment presented no significant difference in lightness values. The L\* values for all treatments are similar, indicating that the ice cream samples are relatively light in color. This trend seems to relate with the results found by

Montibeller et al. (2018), for which anthocyanins were obtained from skin grape and applied in kefir as natural colorant, the results showed that the parameter  $L^*$  also reported a high value indicating lightness in the kefir.

In the case of  $a^*$  values, all treatments are negative indicating a slightly green hue (-60). The negative values show that ice cream samples tend toward green, but there are no significant differences between the treatments. The presence of anthocyanin extracts did not shift the samples toward a red color, which is desired. This change in color can be caused due to anthocyanin degradation, this behavior was also observed in a study by Rosso and Mercadante (2007).

In  $b^*$  values, there were no significant differences between the treatments. The values reported indicated a slightly yellow hue, regardless of the addition of anthocyanins extracts. Therefore, comparing the results obtained in Table 4 and the results agreed with those found by Narváez (2020), where color was also measured from blackberry and strawberry anthocyanins extract in semi-cured cheese showed that there were no significant differences present, for which the treatments measured didn't have a change in color when adding the anthocyanins extracts.

Unlike this study, Montibeller et al. (2018) did find a significant difference between their treatments for values  $a^*$  and  $b^*$ . This color loss is typical in food matrices with additional natural extracts; this behavior has already been observed in other studies and is one of the main limitations in relation to the application of these products on a large scale. As a result, more research is needed to extend the stability of color (Kammerer et al., 2007).

**Table 4**

*Color parameters of ice cream using anthocyanins extracts from blueberry residues.*

Treatments	Extract	$L \pm S.D$	$a \pm S.D$	$b \pm S.D$
1	No extract	95.85 $\pm$ 0.58	-1.23 $\pm$ 0.08	12.64 $\pm$ 0.15
2	1% of phase I extract	95.82 $\pm$ 0.26	-1.30 $\pm$ 0.08	13.10 $\pm$ 0.30
3	3% of phase I extract	95.19 $\pm$ 1.13	-1.25 $\pm$ 0.11	13.17 $\pm$ 0.49
P		0.8088	0.7900	0.6094
C.V		0.90	0.91	3.22

Note. Each measurement of L, a and b represents the mean of three replicates. TRT: Treatment. P: Probability. C.V: Coefficient of Variation.

P < 0.05 indicate significant differences.

## pH

Small adjustments to pH levels can influence almost everything that is done in the food manufacturing process. The effects on food quality and safety are significant (Bekermeier, 2021). The average pH value of ice cream is 6.0 to 6.5 (Dervisoglu et al., 2005).

In Table 5 the results show that the pH of the ice cream decreases slightly with increasing concentrations of blueberry residue extract this is due to the inherent acidic properties of anthocyanins and their interactions with other components in the ice cream mixture. However, the differences in pH between the treatments are not statistically significant. This means that the pH of the ice cream is not affected by the addition of blueberry residue extract.

This is important because pH is a critical factor in the quality and shelf life of ice cream. A pH that is too low can cause the ice cream to become sour, while a pH that is too high can cause the ice cream to become sandy as a result of crystallization (Rosy, 2023). The fact that the addition of blueberry residue extract does not affect the pH of ice cream means that it can be safely added to ice cream without affecting the quality or shelf life of the product.

The findings of the research contradict those reported by Shamshad et al. (2023), who also found a decrease in pH values, but observed significant differences because they evaluated pH at different storage days. The decrease in pH values in their study was attributed to the enrichment of ice cream with microencapsulated anthocyanins powder. Bajwa et al. (2003) found no significant differences in pH over the storage time of ice cream made with different concentrations of strawberry pulp, but also confirmed a decreasing trend in pH due to the presence of ascorbic acid in strawberries.

**Table 5***Effect of pH of ice cream with extracts*

Treatments	Extract	pH $\pm$ S. D
1	No extract	6.28 $\pm$ 0.03
2	1% of phase I extract	6.26 $\pm$ 0.06
3	3% of phase I extract	6.21 $\pm$ 0.03
P		0.59
C. V		0.86

Note. P: Probability. S.D. : Standard Deviation. CV: Coefficient of variation. P <0.05 indicate significant differences.

**Overrun**

Overrun is a measurement that relates to an increase in the volume of an ice cream product during processing. Soukoulis et al. (2010) defines it as the structure of the final product, also known as the presence of air, that gives ice cream an enjoyable light texture. According to Aloglu et al. (2018) overrun does not only affect the consistency of the ice cream, but also concerns the quality of the food, yield and nutritional value.

Table 7 shows the effect of blueberry residue extract on the overrun of ice cream. Overrun is the amount of air incorporated into the ice cream during freezing, and it is expressed as a percentage of the total volume of the ice cream. The amount of overrun in the soft-frozen desserts is commonly 50% (Goff & Hartel, 2013). The air cell structure of ice cream is one of the primary determinants of its melting rate, shape retention during the melting process, and rheological properties in the molten state, which are all correlated with creaminess (Bahramparvar et al., 2011) .

The results show that the overrun of ice cream is significantly increased by the addition of blueberry residue extract. The overrun of ice cream with 1% blueberry residue extract is 53.90%, which is significantly higher than the overrun of ice cream without extract (48.88%). The overrun of ice cream with 3% blueberry residue extract is 50.49%, which is also significantly higher than the overrun of ice cream without extract. Overall, an overrun of 50% is a good balance for ice cream it results in a soft and creamy ice cream that is still flavorful and has a good shelf life. In accordance to Singo and Beswa (2019) reported that an ice cream with high overrun melted slowly than ice cream

with low overrun. The findings of the present study are in general agreement with the results reported by Shamshad et al. (2023) who also evaluated overrun in ice cream enriched with microencapsulated anthocyanins from black carrots and also reported values between 44.34% to 50.72% for overrun.

**Table 6**

*Means for overrun (%) of ice cream with antocyanin extract.*

Treatments	Extract	Overrun (%) $\pm$ S. D. <sup>NS</sup>
1	No extract	48.88 $\pm$ 1.04
2	1% of phase I extract	53.90 $\pm$ 2.62
3	3% of phase I extract	50.49 $\pm$ 2.47
P		0.1107
C. V		3.66

Note. P: Probability. S.D. : Standard Deviation. CV: Coefficient of variation. NS: Not significant.

### Hardness

Ice cream hardness is a measure of its resistance to deformation when an external force is applied. It is affected by several factors, including overrun, ice crystal size, ice phase volume, and extent of fat destabilization. Muse and Hartel (2004) observed a direct relationship between hardness and overrun, indicating that ice cream with more air incorporated is typically softer. This is because air bubbles act as a cushion between the ice crystals, preventing them from growing too large and making the ice cream harder.

Both ice crystal size and ice phase volume also contribute to hardness, with larger ice crystals and a higher percentage of ice phase resulting in a harder ice cream (Sulejmani & Demiri, 2020). These factors can be manipulated to control the hardness of ice cream for desired sensory and functional properties. For example, ice cream manufacturers may reduce overrun to produce a harder ice cream that is more resistant to melting. Conversely, they may increase overrun to produce a softer ice cream that is easier to scoop.

It is important to note that the relationship between hardness and these factors is complex and can be influenced by other factors, such as the type of fat used, the presence of stabilizers, and the freezing process (Sofjan & Hartel, 2004).

In table 6 the results show that the extract had a significant effect on hardness ( $P \geq 0.05$ ). The hardness of the treatments with the extract was significantly lower than the hardness of the treatment without the extract. This suggests that the extract can be used to reduce the hardness of the ice cream. The higher the concentration of the extract, the softer the samples. According to Michue et al. (2015) the more solids dispersed in ice cream, the lower the depth of penetration, and therefore the harder the ice cream. For which according to Sofjan and Hartel (2004) the presence of air (high % overrun) decreased the hardness of ice cream. This results differ with those found by Yeon et al. (2017) were they found no significant difference, instead an inverse relationship between hardness and overrun was found, differing as well from other studies who reports that ice cream with high overrun and low hardness tends to be softer.

**Table 7**

*Texture analysis for hardness (g force) of ice cream with anthocyanins extracts.*

Treatments	Extract	Hardness (g force) $\pm$ S. D
1	No extract	13684 $\pm$ 2701.44 <sup>a</sup>
2	1% of phase I extract	6808 $\pm$ 1188.54 <sup>b</sup>
3	3% of phase I extract	4230 $\pm$ 774.48 <sup>b</sup>
P		0.015
C. V		20.79

Note. P : Probability. S.D.= Standard Deviation. CV = Coefficient of variation. Different letters in each column indicate significant differences

( $P < 0.05$ ) between treatments.

### **Conclusions**

The HPLC UV-VIS method was optimal for the quantification of total anthocyanins in blueberry residue extracts using ethanol as a solvent. Malvidin-3-galactoside and Peonidin-3-arabinoside were identified as the main anthocyanins present in the extracts.

The ethanolic extracts of blueberry residues exhibited a high radical scavenging ability, which was not affected by the ethanol concentration, suggesting that these residues are a promising source of natural antioxidants.

The incorporation of blueberry residue extracts in dairy products, such as ice cream, did not show significant differences in color, pH, overrun, and antioxidant activity.

### **Recommendations**

Increase the amount of blueberry residue extract for the analyses of color, pH, and overrun.

Conduct a sensory analysis to evaluate the acceptability of the ice cream with blueberry residues extracts.

Perform a cost-utility analysis to determine the benefits of the natural dye compared to the artificial dye.

Study the stability of anthocyanins when applied to different products.

## References

- Adebiyi, O. E., Olayemi, F. O., Ning-Hua, T., & Guang-Zhi, Z. (2017). In vitro antioxidant activity, total phenolic and flavonoid contents of ethanol extract of stem and leaf of *Grewia carpinifolia*. *Beni-Suef University Journal of Basic and Applied Sciences*, 6(1), 10–14. <https://doi.org/10.1016/j.bjbas.2016.12.003>
- Aloglu, H. S., Ozcan, Y., Karasu, S., Cetin, B., & Sagdic, O. (2018). Influence of transglutaminase treatment on the physicochemical, rheological, and melting properties of ice cream prepared from goat milk. *EurekaMag*. <https://eurekamag.com/research/070/866/070866474.php>
- Bahramparvar, M., Mazaheri, & Tehrani, M. (2011). Application and Functions of Stabilizers in Ice Cream. *Food Reviews International*, 27(4), 389–407. <https://doi.org/10.1080/87559129.2011.563399>
- Bajwa, U. A., Huma, N., Ehsan, B., Jabbar, K., & & Khurrama, A. (2003). Effect of Different Concentrations of Strawberry Pulp on the Properties of Ice Cream. *International Journal of Agriculture and Biology*. [https://www.researchgate.net/profile/Muhammad-Arshad-10/publication/242108917\\_Effect\\_of\\_Different\\_Concentrations\\_of\\_Strawberry\\_Pulp\\_on\\_the\\_Properties\\_of\\_Ice\\_Cream/links/00b4953c980c146757000000/Effect-of-Different-Concentrations-of-Strawberry-Pulp-on-the-Properties-of-Ice-Cream.pdf](https://www.researchgate.net/profile/Muhammad-Arshad-10/publication/242108917_Effect_of_Different_Concentrations_of_Strawberry_Pulp_on_the_Properties_of_Ice_Cream/links/00b4953c980c146757000000/Effect-of-Different-Concentrations-of-Strawberry-Pulp-on-the-Properties-of-Ice-Cream.pdf)
- Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R. P., & Chang, C.-M. (2022). Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of *Ficus religiosa*. *Molecules (Basel, Switzerland)*, 27(4). <https://doi.org/10.3390/molecules27041326>
- Bekermeier, C. (2021). Importance of pH in Food Production | PacMoore. <https://www.pacmoore.com/blog/importance-of-ph-in-food-production/>
- Benítez-Estrada, A., Villanueva-Sánchez, J., González-Rosendo, G., Alcántar-Rodríguez, V. E., Puga-Díaz, R., & Quintero-Gutiérrez, A. G. (2020). Determinación de la capacidad antioxidante total de alimentos y plasma humano por fotoquimioluminiscencia: Correlación con ensayos fluorométricos (ORAC) y espectrofotométricos (FRAP). [https://www.scielo.org.mx/scielo.php?script=sci\\_arttext&pid=S1405-888X2020000100103](https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-888X2020000100103) <https://doi.org/10.22201/fesz.23958723e.2020.0.244>
- Dervisoglu, M., Yazici, F., & Aydemir, O. (2005). The effect of soy protein concentrate addition on the physical, chemical, and sensory properties of strawberry flavored ice cream. *European Food Research and Technology*, 221(3-4), 466–470. <https://doi.org/10.1007/s00217-005-1207-3>
- Ekin, S., Bayramoglu, M., Goktasoglu, A., Ozegoke, F., & Kisiltas, H. (2017). Antioxidant Activity of Aqueous and Extracts of *Crataegus Meyer* Leaves and Contents of Vitamin, Trace Element. *Journal of the Chilean Chemical Society*, 62(4), 3661–3667. <https://doi.org/10.4067/s0717-97072017000403661>
- FAO. (2015). Iniciativa mundial sobre la reducción de la pérdida y el desperdicio de alimentos: Food and Agriculture Organization. <https://www.fao.org/3/i4068s/i4068s.pdf>
- Faria, A., Oliveira, J., Neves, P., Gameiro, P., Santos-Buelga, C., Freitas, V. de, & Mateus, N. (2005). Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *Journal of Agricultural and Food Chemistry*, 53(17), 6896–6902. <https://doi.org/10.1021/jf0511300>

- Food and Agriculture Organization (2019). El estado mundial de la agricultura y la alimentación. Progresos en la lucha contra la pérdida y el desperdicio de alimentos. <https://www.fao.org/3/ca6030es/ca6030es.pdf>
- García, G. (2022). Food Upcycling, una tendencia con gran potencial. *THE FOOD TECH*. <https://thefoodtech.com/insumos-para-empaque/food-upcycling-una-tendencia-con-gran-potencial/>
- Garrido, C. M. V., & Pérez, E. (2014). Arándano rojo I (*Vaccinium macrocarpon* Ait.). 1989-3620. <https://docta.ucm.es/entities/publication/1e1ac2af-e9ab-4b3f-923b-d12ea954b19b>
- Goff, H. D., & Hartel, R. W. [Richard W.]. (2013). *Ice cream* (Seventh edition). Springer. <https://doi.org/10.1007/978-1-4614-6096-1>
- Herrera-Balandrano, D. D., Chai, Z., Hutabarat, R. P., Beta, T., Feng, J., Ma, K., Li, D., & Huang, W. (2021). Hypoglycemic and hypolipidemic effects of blueberry anthocyanins by AMPK activation: In vitro and in vivo studies. *Redox Biology*, 46, 102100. <https://doi.org/10.1016/j.redox.2021.102100>
- Hidalgo, G.-I., & Almajano, M. P. (2017). Red Fruits: Extraction of Antioxidants, Phenolic Content, and Radical Scavenging Determination: A Review. *Antioxidants (Basel, Switzerland)*, 6(1). <https://doi.org/10.3390/antiox6010007>
- Huang, W., Zhu, Y., Li, C., Sui, Z., & Min, W. (2016). Effect of Blueberry Anthocyanins Malvidin and Glycosides on the Antioxidant Properties in Endothelial Cells. *Oxidative Medicine and Cellular Longevity*, 2016, 1591803. <https://doi.org/10.1155/2016/1591803>
- Instituto de Investigación y Desarrollo de Comercio Exterior (2017). Oportunidades y retos en la exportación de arándanos. <https://apps.camaralima.org.pe/RepositorioAPS/0/0/par/ESTUDIO8/Oportunidades-y-retos-en-la-exportaci%C3%B3n-de-ar%C3%A1ndanos.pdf>
- Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F., & Zamora-Ros, R. (2020). Recent Research on the Health Benefits of Blueberries and Their Anthocyanins. *Advances in Nutrition (Bethesda, Md.)*, 11(2), 224–236. <https://doi.org/10.1093/advances/nmz065>
- Kammerer, D. R., Schillmöller, S., Maier, O., Schieber, A., & Carle, R. (2007). Colour stability of canned strawberries using black carrot and elderberry juice concentrates as natural colourants. *European Food Research and Technology*, 224(6), 667–679. <https://doi.org/10.1007/s00217-006-0356-3>
- Lagos-Alvarez, Y. B., Díaz-Ramírez, L. M., Melo-Velasco, J. M., & Hurtado Bermudez, J. J. (2022). Residuos de plaguicidas en mora (*Rubus glaucus* Benth.) en el Valle del Cauca, Colombia. *Agronomía Mesoamericana*, 47538. <https://doi.org/10.15517/am.v33i2.47538>
- Lee, J., & Wrolstad, R. E. [R. E.] (2006). Extraction of Anthocyanins and Polyphenolics from Blueberry Processing Waste. *Journal of Food Science*, 69(7), 564–573. <https://doi.org/10.1111/j.1365-2621.2004.tb13651.x>
- Lin, J., Tian, J., Shu, C., Cheng, Z., Liu, Y [Yunen], Wang, W [Weisheng], Liu, R., Li, B., & Wang, Y. (2020). Malvidin-3-galactoside from blueberry suppresses the growth and metastasis potential of hepatocellular carcinoma cell Huh-7 by regulating apoptosis and metastases pathways. *Food Science and Human Wellness*, 9(2), 136–145. <https://doi.org/10.1016/j.fshw.2020.02.004>

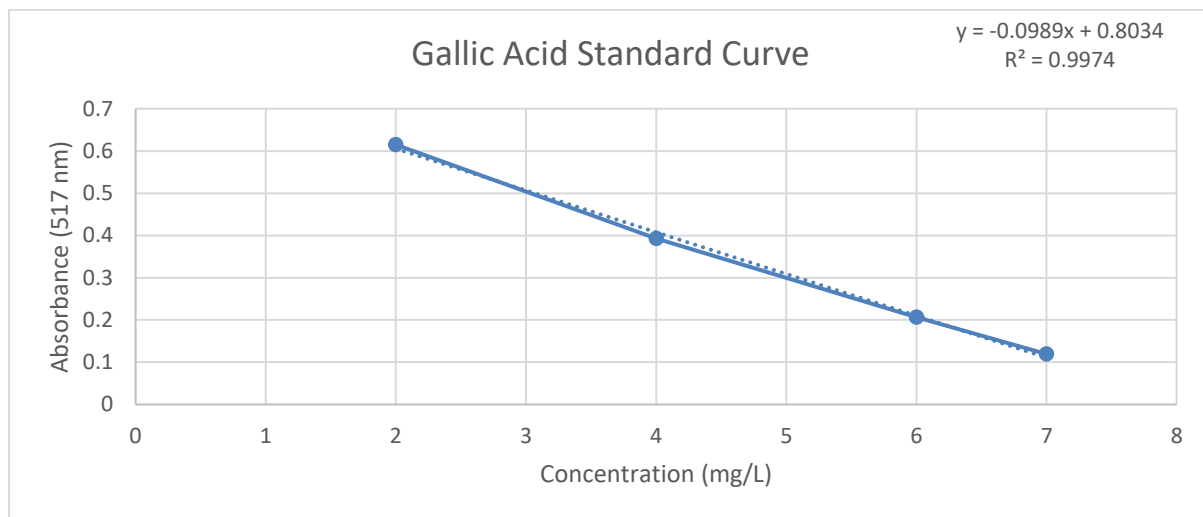
- Lončarić, A., Celeiro, M., Jozinović, A., Jelinić, J., Kovač, T., Jokić, S., Babić, J., Moslavac, T., Zavadlav, S., & Lores, M. (2020). Green Extraction Methods for Extraction of Polyphenolic Compounds from Blueberry Pomace. *Foods (Basel, Switzerland)*, 9(11). <https://doi.org/10.3390/foods9111521>
- Lv, X., Li, L., Lu, X., Wang, W [Wenxiu], Sun, J., Liu, Y [Yaqiong], Mu, J., Ma, Q., & Wang, J. (2022). Effects of organic acids on color intensification, thermodynamics, and copigmentation interactions with anthocyanins. *Food Chemistry*, 396, 133691. <https://doi.org/10.1016/j.foodchem.2022.133691>
- Marcano, D., & Hasegawa, M. (2018). *Fitoquímica Orgánica*. <http://saber.ucv.ve/omp/index.php/editorialucv/catalog/view/18/10/56-1>
- Michue, J., Encina, C., & Ludeña, F. (2015). *Optimización del overrun (aireado), de la dureza, la viscosidad y los costos de un helado mediante el diseño de mezclas*. Universidad Nacional Agraria La Molina. Lima, Perú. [https://revistas.ulima.edu.pe/index.php/Ingenieria\\_industrial/article/download/543/516/](https://revistas.ulima.edu.pe/index.php/Ingenieria_industrial/article/download/543/516/)
- Montibeller, M. J., Lima, M. P. de, Tupuna-Yerovi, D. S., Rios, A. d. O., & Manfroi, V. (2018). Stability assessment of anthocyanins obtained from skin grape applied in kefir and carbonated water as a natural colorant. *Journal of Food Processing and Preservation*, 42(8), e13698. <https://doi.org/10.1111/jfpp.13698>
- Muse, M. R., & Hartel, R. W [R. W.] (2004). Ice cream structural elements that affect melting rate and hardness. *Journal of Dairy Science*, 87(1), 1–10. [https://doi.org/10.3168/jds.S0022-0302\(04\)73135-5](https://doi.org/10.3168/jds.S0022-0302(04)73135-5)
- Narváez, E. (2020). Efecto de la adición de extractos antociánicos de mora de castilla (*rubus glaucus* B.) y fresa (*Fragaria x ananassa* W.) sobre la capacidad antioxidante y aceptabilidad organoléptica en queso semi-maduro. <http://repositorio.espe.edu.ec/xmlui/bitstream/handle/21000/22056/T-IASAI-005592.pdf?sequence=1&isAllowed=y>
- Pataro, G., Bobinaité, R., Bobinas, Č., Šatkauskas, S., Raudonis, R., Visockis, M., Ferrari, G., & Viškelis, P. (2017). Improving the Extraction of Juice and Anthocyanins from Blueberry Fruits and Their By-products by Application of Pulsed Electric Fields. *Food and Bioprocess Technology*, 10(9), 1595–1605. <https://doi.org/10.1007/s11947-017-1928-x>
- Phillips, K. (2023). *The Importance of Food Color Measurement*. <https://www.hunterlab.com/blog/importance-of-food-color-measurement/>
- Phuyal, N., Jha, P. K., Raturi, P. P., & Rajbhandary, S. (2020). Total Phenolic, Flavonoid Contents, and Antioxidant Activities of Fruit, Seed, and Bark Extracts of *Zanthoxylum armatum* DC. *TheScientificWorldJournal*, 2020, 8780704. <https://doi.org/10.1155/2020/8780704>
- Ropero, M., & Soledad, B. (2020). Estudio del perfil de antocianinas en *Rubus Niveus* utilizando cromatografía líquida (HPLC) y pH diferencial. *EDUCAB*, 11, 147–156. <https://revistasenlinea.saber.ucab.edu.ve/index.php/educab/issue/download/483/119>
- Rosso, V. V. de, & Mercadante, A. Z. (2007). The high ascorbic acid content is the main cause of the low stability of anthocyanin extracts from acerola. *Food Chemistry*, 103(3), 935–943. <https://doi.org/10.1016/j.foodchem.2006.09.047>

- Rosy. (2023). *Is ice cream acidic, alkaline or neutral before we consume it?* [https://moobearsicecream.com/is-ice-cream-acidic-alkaline-or-neutral-before-we-consume-it/#What\\_makes\\_ice\\_cream\\_slightly\\_acidic](https://moobearsicecream.com/is-ice-cream-acidic-alkaline-or-neutral-before-we-consume-it/#What_makes_ice_cream_slightly_acidic)
- Shamshad, A., lahtisham-Ul-Haq, Butt, M. S., Nayik, G. A., Al Obaid, S., Ansari, M. J., Karabagias, I. K., Sarwar, N., & Ramniwas, S. (2023). Effect of storage on physicochemical attributes of ice cream enriched with microencapsulated anthocyanins from black carrot. *Food Science & Nutrition*, *11*(7), 3976–3988. <https://doi.org/10.1002/fsn3.3384>
- Simons, C. (2018, October 10). Color Determination in Food. *Food Science Toolbox*. <https://cwsimons.com/color-determination-in-food/>
- Singo, T. M., & Beswa, D. (2019). Effect of roselle extracts on the selected quality characteristics of ice cream. *International Journal of Food Properties*, *22*(1), 42–53. <https://doi.org/10.1080/10942912.2019.1567535>
- Sofjan, R. P., & Hartel, R. W [Richard W.] (2004). Effects of overrun on structural and physical characteristics of ice cream. *International Dairy Journal*, *14*(3), 255–262. <https://doi.org/10.1016/j.idairyj.2003.08.005>
- Song, H.-N., Ji, S.-A., Park, H.-R., Kim, H.-H., & Hogstrand, C. (2018). Impact of Various Factors on Color Stability of Fresh Blueberry Juice during Storage. *Preventive Nutrition and Food Science*, *23*(1), 46–51. <https://doi.org/10.3746/pnf.2018.23.1.46>
- Soukoulis, C., Lyroni, E., & Tzia, C. (2010). Sensory profiling and hedonic judgement of probiotic ice cream as a function of hydrocolloids, yogurt and milk fat content. *LWT - Food Science and Technology*, *43*(9), 1351–1358. <https://doi.org/10.1016/j.lwt.2010.05.006>
- Sulejmani, E., & Demiri, M. (2020). The effect of stevia, emulsifier and milk powder on melting rate, hardness and overrun of ice cream formulations during storage. *Mljekarstvo*, *70*(2), 120–130. <https://doi.org/10.15567/mljekarstvo.2020.0206>
- Thu Thao, N. L., Dao, T. K., Thang, L. P., Than, T. U., & Mai, D. S. (2015). *Effect of Ethanol on the Anthocyanin Extraction from the Purple Rice of Vietnam* (Vol. 3). [https://www.researchgate.net/publication/341480196\\_Effect\\_of\\_Ethanol\\_on\\_the\\_Anthocyanin\\_Extraction\\_from\\_the\\_Purple\\_Rice\\_of\\_Vietnam](https://www.researchgate.net/publication/341480196_Effect_of_Ethanol_on_the_Anthocyanin_Extraction_from_the_Purple_Rice_of_Vietnam)
- Vilariño, M. V., Franco, C., & Quarrington, C. (2017). Food loss and Waste Reduction as an Integral Part of a Circular Economy. *Frontiers in Environmental Science*, *5*, Article 21. <https://doi.org/10.3389/fenvs.2017.00021>
- Wegener, C. (2016). Upcycling. *Creativity: A New Vocabulary*, 181–188. [https://doi.org/10.1057/9781137511805\\_22](https://doi.org/10.1057/9781137511805_22)
- Wrolstad, R. E [Ronald E.], & Smith, D. E. (2017). Color Analysis. In *Food Analysis* (pp. 545–555). Springer, Cham. [https://doi.org/10.1007/978-3-319-45776-5\\_31](https://doi.org/10.1007/978-3-319-45776-5_31)
- Yeon, S.-J., Kim, J.-H., Hong, G.-E., Park, W., Kim, S.-K., Seo, H.-G., & Lee, C.-H. (2017). Physical and Sensory Properties of Ice Cream Containing Fermented Pepper Powder. *Korean Journal for Food Science of Animal Resources*, *37*(1), 38–43. <https://doi.org/10.5851/kosfa.2017.37.1.38>

**Appendices**

**Appendix A**

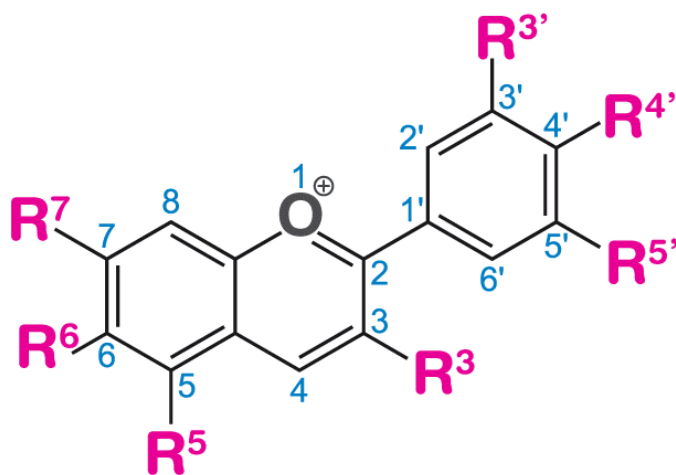
*Gallic Acid Standard Curve*

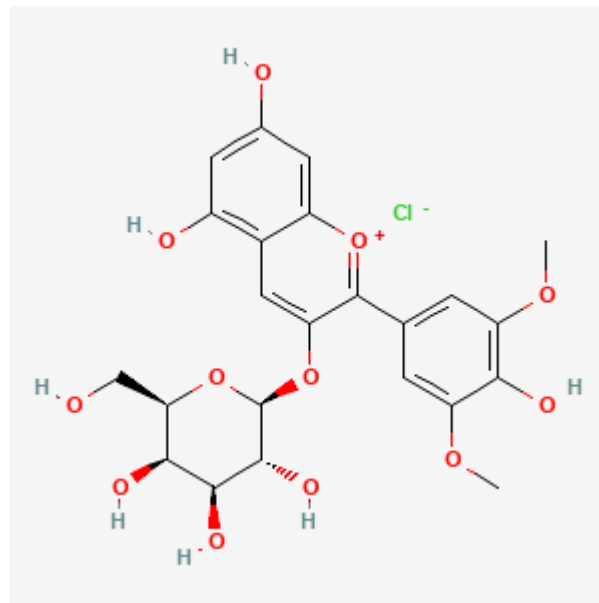


## Appendix B

## Anthocyanins Structure

## ANTHOCYANIN STRUCTURE

BYJU'S  
The Learning App

**Appendix C***Malvidin-3-galactoside*

**Appendix D***Peonidin-3-arabinose*