



SOLVATOCHROMIC BEHAVIOR OF THE NATURAL COLORANT OF BLUEBERRY (*Vaccinium floribundum* Kunth)

COMPORTAMIENTO SOLVATOCRÓMICO DEL COLORANTE NATURAL DE MORTIÑO (*Vaccinium floribundum* Kunth)

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Abstract

The solvatochromic effect is the modification of the absorption spectrum of a solute by varying the solvent. This research evaluated the solvatochromic characteristics of Malvidin-3-glucoside hydrochloride. The method to obtain maximum wavelengths was by spectral scanning. In primary standard CAS No. 7228-78.6, the variation was determined in binary mixtures of ethanol-water: 40,55, and 70 %v/v. The solvatochromic effect when modifying the pH of the solvent (water) was evaluated in natural dye and stabilized. The results suggest that the intermolecular hydrogen connections between Malvidin-3-glucoside and the ethanol-water binary mixtures are responsible for the solvatochromic changes: $565.2586 \pm 3.2784\text{nm}$, $472.5498 \pm 2.5128\text{nm}$ and $457.3589 \pm 6.2586\text{nm}$, produced by the analyzed combinations. When anthocyanins are stabilized in a chelating matrix, the solvatochromic changes produced by varying the pH of the water solvent are not significant compared to the unstabilized natural dye.

Keywords: malvidin-3-glucoside, secondary metabolites, dye, natural products, solvatochromism, anthocyanins, pectin.

Resumen

El efecto solvatocrómico es la modificación del espectro de absorción de un soluto al variar el solvente. El presente trabajo evaluó las características solvatocrómicas de Malvidina-3-glucósido clorhidrato. El método para obtener longitudes de onda máxima fue por barridos espectrales. En estándar primario CAS N° 7228-78.6, la variación fue determinada en mezclas binarias de etanol-agua: 40,55 y 70 %v/v. El efecto solvatocrómico al modificar el pH del solvente (agua) fue evaluado en colorante natural y estabilizado. Los resultados indican que los enlaces hidrógeno intermoleculares entre Malvidina-3-glucósido y las mezclas binarias de etanol-agua son responsables de los cambios solvatocrómicos: $565,2586 \pm 3.2784\text{nm}$, $472,5498 \pm 2.5128\text{nm}$ y $457,3589 \pm 6.2586\text{nm}$, producidos por las combinaciones analizadas. Al estabilizar antocianinas en una matriz quelante los cambios solvatocrómicos producidos al variar

el pH del solvente agua, son no significativos en comparación con el colorante natural sin estabilizar.

Palabras clave: Malvidina-3-glucósido, metabolitos secundarios, colorante, productos naturales, solvatocromismo, antocianinas, pectina.

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1 Introduction

Vaccinium floribundum Kunth is a fruit native of Ecuador (Torres and Pulgar, 2017). During ripening, this berry undergoes color changes (Xu et al., 2010): initially it is green when the fruit is immature, pink when it reaches physiological maturity and finally black when it reaches full maturity (Arteaga et al., 2014). Cyanidine, malvidine and delphinidine are anthocyanins typically in this type of fruit (Jin et al., 2020). Narváez and Suárez (2016) reported that they obtained 3.92 mg of cyanidine-3-o-glucoside for each gram of dry extract of this fruit (Rahman et al., 2021).

Plant-origin anthocyanins are natural dyes (Yépez and Suaáñez, 2019) with protective properties

for plants against UV light, oxidants and free radicals (Enaru et al., 2021). Its usefulness in the cosmetic, food and pharmaceutical industries has increased by its bioactive properties (Buchweitz et al., 2013) among which are: antidiabetic, antitumor, anti-inflammatory and anticancer effects (Garzón, 2008). In addition, they provide a variety of colors ranging from red to blue in various fruits (Nguyen et al., 2018). One of the reasons for this variety of colors is the structure of the B-ring present in the Malvidine 3-O-Glucoside structure (Sánchez, 2013). This ring has variations in its radicals (Table 1), in which greater methoxylation can be differentiated with displacements towards blue colorations, on the other hand, fewer methoxylations are related with red tones (Rahman et al., 2021).

Table 1. Anthocyanins present in nature. Modified by Rahman et al. (2021).

anthocyanins	Radical		λ_{\max} [nm]	Color
	R1	R2	Visible spectrum	
Pelargonidin	H	H	494	orange
Cyanidin	OH	H	506	orange-red
Delphinidin	OH	OH	508	blue-red
Peonidin	OCH ₃	H	506	orange-red
Peninidin	OCH ₃	OH	508	blue-red
Malvidin	OCH ₃	OCH ₃	510	blue-red

To increase their solubility anthocyanins have glycosidic replacements in radicals 3 and/or 5 (Putra et al., 2023). For example, malvidine in nature presents as Malvidine 3-glucoside (Ayala et al., 2018) see Figure 1.

Aromatic acylations can substitute glycosidic groups, producing purple shades (Kader et al., 1998). In addition, the colored pigments are found in four different chemical forms depending on the pH of the medium (Castañeda-Ovando et al., 2009). Thus, the flavilio cation at $pH = 1 - 3$ is formed, which is soluble in water and is also responsible for the colors red and purple (Vasco et al., 2009). When the pH increases between 8 and 10, the quinoidal blue species is abundant (Belmonte et al., 2016) while the pseudobase of carbinol and a colorless chalcona appear at pH between 12 and 14. However, all four compounds are soluble in polar solvents and can coexist in a wide pH range (Enaru

et al., 2021).

Solvatochromism is commonly used in many fields to study global and local polarity in macrosystems (Reichardt, 1994). Its study includes phenomena involving intermolecular and dynamic forces coupled to the solvent (Marini et al., 2010).

Anthocyanins under experimental conditions have solvatochromic properties by contact with solvents that could produce hydroxylations, methoxylations, changes in pH or aromatic substitutions (Iosub et al., 2014). Based on spectral data Iosub et al. (2014) correlations were established between the solvatochromic properties and the polarity parameters of the solvent. The study determined that anthocyanin extracts are useful in the study of the solvatochromic effect in solvents of different polarity.

The importance of studying solvatochromic behavior, specifically in natural dyes, with structure similar to anthocyanins lies in obtaining the quantification method (Klymchenko, 2017). Emphasizing spectral data, "Beer's law explains the quantitative aspects of absorption measurements by the linear dependence of solute concentration" [p. 1386] Linying2022. The Lorentz-Lorenz and Clausius-Mosotti equations are known for determining dipolar moments, and the purpose "is to predict a strong coupling between the solute and solvent oscillators" [p. 2] Mayerhofer2020. When intermolecular forces are involved with the solvent, it is necessary to establish variation ranges, specifically with regard to polarity (Lee et al., 2013).

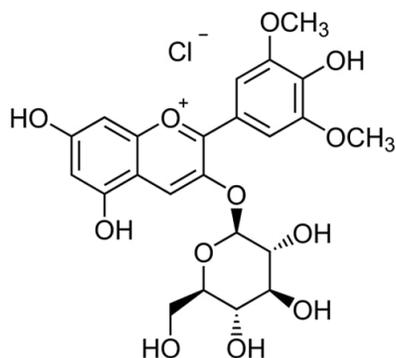


Figure 1. The structure of Malvidine 3-O-Glycoside. Modified by Pubchem (2022).

Codex Alimentarius, (2021), during the manufacturing process it is essential that the natural dye is not affected by wavelength variations when in contact with the ingredients of the formulation (Loving et al., 2010). The solvatochromic effect of anthocyanins in a pharmaceutical, cosmetic or food product is reflected as a degradation (Cai et al., 2020).

Based on the latter, this study evaluates the solvatochromic behavior of Malvidine -3-glycoside hydrochloride primary standard CAS N°. 7228-78.6, produced by the solute-solvent coupling, by varying the molar fraction of the solvent. This analysis determined the maximum wavelength for each molar fraction of solvent.

The solvatochromic behavior was also evaluated by the diversification of the maximum wavelength of Malvidine -3- glycoside in natural and stabili-

zed dye, by modifying the pH of the solvent (water) to 4 and 6, with HCl and NaOH respectively. The analysis evaluated whether the solvatochromic behaviors in these pigments are statistically significant, by varying the pH of the solvent.

2 Materials and Methods

2.1 Solvatochromic study: primary standard

The solvatochromic behavior was evaluated in Malvidine-3-glycoside hydrochloride primary standard CAS N°. 7228-78.6, with variation in the proportion of the solvent (ethanol-water: 40,55,70 %v/v). During the experimental determination of spectral sweeps with primary standard the pH was maintained at the value of 2, with the addition of a solution of HCl 1M to preserve the flavylium ion structure, which is consistent with the literature (Iosub et al., 2014).

The maximum wavelength change of Malvidine 3-O-Glycoside was analyzed in duplicate during three days in a VARIAN 50Bio spectrophotometer. The variation of the data was analyzed by standard deviation at each solvent concentration.

2.2 Solvatochromic study: natural dye

2.2.1 Extraction

The extraction method was a modification of the research (Almachi, 2018). Obtaining the natural dye of *Vaccinium floribundum* Kunth began with degreasing the previously dried and ground mortiño fruit, using Soxhlet with n-hexane for 8 hours, followed by maceration in filter paper caps with ethanol 96% for 24 hours. Each cap was individually percolated at a rate of 20 drops per minute until the test for phenolic compounds with 5% ferric chloride was negative. Each ethanolic extract was concentrated in the RapidVap equipment: Evaporation heat 205 Kcal/Kg, Speed 45% and Vacuum 175 Mbar. The dry extracts were stored in a desiccator protected from light.

2.2.2 Stabilization

The natural dye from dry extract of *Vaccinium floribundum* Kunth was stabilized in a commercial pec-

tin matrix (Ceampectin RS 4710), by the absorption method with the following conditions: ethanol concentration 60%v/v, extract concentration 5%w/v and contact time 25 hours.

2.2.3 Solvatochromism

The solvatochromic behavior was evaluated by the maximum wavelength variation of Malvidine -3-glycoside in natural and stabilized dye, by modifying the pH of the solvent (water) to 4 and 6 with HCl and NaOH, respectively. The study was based on ANCOVA multiple covariance analysis of two factors, each with 2 levels on 1 response variable, i.e., $K=2$ and $n=1$. Four experimental runs were developed with a complete replica of the design to determine the reproducibility of the model, giving a total of 8 experimental runs.

3 Results and Discussion

3.1 Evaluation of the solvatochromic behavior of Malvidine -3- glycoside hydrochloride

The absorption maxima of Malvidine with their respective standard deviation are observed in Table 2, showing a solvatochromic effect at different solvent concentrations.

Table 2. Results obtained by solvatochromism of Malvidine.

Ethanol concentration	40 %	55 %	70 %
Wavelengthnm>nm	565.2586 ± 3.2784	472.5498 ± 2.5128	457.3589 ± 6.2586

The effect of the solvent produced by these ternary systems (water:ethanol:anthocyanin) is determined by the polarity parameters of the ethanol: water ratio. Solvatochromism was evaluated by the ability of the solvent to produce dipole changes in the malvidine molecule caused by the change of the solvent molar fraction. Mayerhöfer and Popp (2020) describe that the polar changes produced by the solvent present spectral modifications of the absorption bands. Polar variations produced by the solvent are evidenced by the maximum wavelength alterations in Table 2. Therefore, when developing a quantification method with molecules with similar structure to Malvidine-3-glycoside hydrochloride, it is advisable to maintain the molar fraction of

the solvent in order to avoid solvatochromic behaviors of the analyte.

3.2 Evaluation of solvatochromic behavior of natural dye of *Vaccinium floribundum* Kunth

3.2.1 Extraction

The standardization process of dry extract of moritño started from a dry and ground sample of the fruit with the following specifications: particle size 595 μ m, moisture 3.05 \pm 0.05% and total fat content of 2.5 \pm 0.2%. The extraction method of natural dye and its stabilization in pectin described in materials and methods allowed to obtain a yield as dry extract of 53.1 \pm 4.4% and stabilized dye of 91.8 \pm 4.6%.

Table 3. Two-factor ANCOVA.

pH (X_2)	Coloring Type(X_1)	
	Wavelength [nm]	Wavelength [nm]
	Stabilized dye	Natural dye
4	511.5092 \pm 1.4934	517.0453 \pm 4.3861
6	511.9522 \pm 1.2678	512.5500 \pm 1.3859

Table 3 presents maximum wavelengths of malvidine in natural dye and stabilized in pectin when there is a pH variation in water. The ANCOVA analysis determined that the pH of the natural dye is significant (p Value < 0,05), with a 95% confidence interval. The pigment when exposed to a pH variation of 4 and 6 exhibits changes in the maximum wavelength. These displacements are produced by Malvidine -3- glycoside present in the dry extract.

In an environment between 2 and 4 it leaves the anthocyanin form to be present as the quinoidal species, while at pH 6 it is found as chalcone (Enaru et al., 2021). When comparing the maximum wavelengths of the natural dye with stabilizer, the two-factor ANCOVA analysis determined that there is a significant difference (p -Value < 0,05) with a 95% confidence interval, which can be observed in Figure 2. The binding of Malvidine -3- glycoside with pectin by hydrogen bridging with oxygen of the anthocyanin methoxyl group shows a change in maximum wavelengths (Koh et al., 2020).

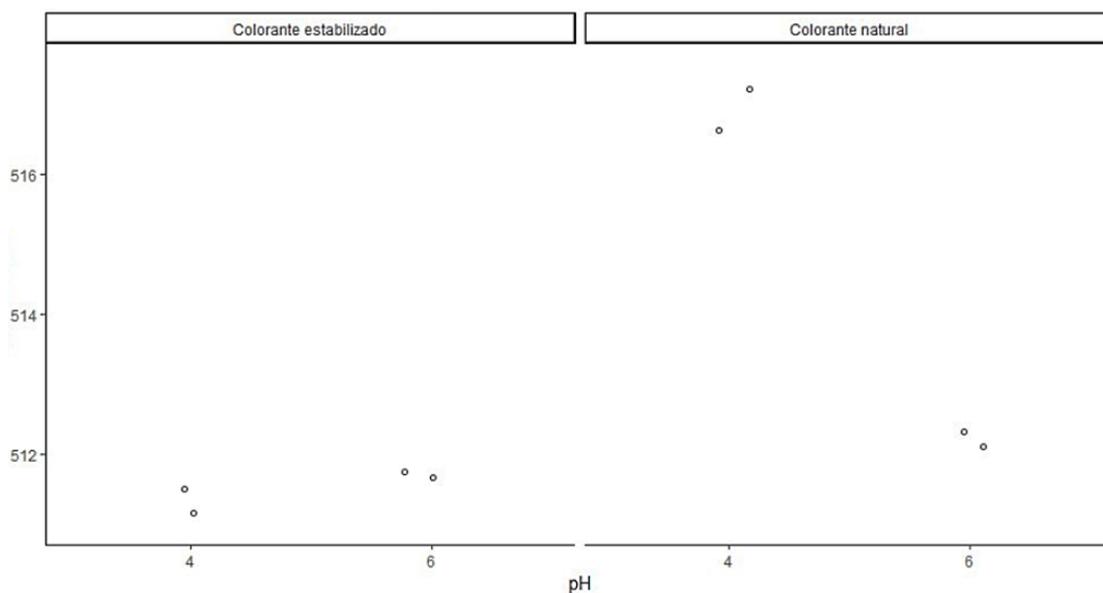


Figure 2. Maximum Wavelength Variation in Natural Dye and Stabilized Dye.

Natural dyes when used for pharmaceutical, cosmetic or food purposes should be stable against changes in pH during manufacturing processes. The study determined that there is no significant difference ($p\text{-Value} > 0,05$), when varying the pH of the solvent when the natural dye is stabilized. The stabilizing matrix pectin prevents the Malvidine -3-glycoside from interacting with the OH^- , H^+ ions of the solvent, preventing solvatochromic shifts.

Analyzes were performed at specific pH values of 4 and 6. However, a food study found that industrialized foods have slightly more acidic pH values than natural foods. This is probably related to the preservation methods used and the addition of vitamins. Cereals showed pH values ranging from 7.95 -y 5.4 [p. 91]Casaubon2018. Therefore, it is necessary to determine solvatochromic changes in a higher pH range.

4 Conclusions

The present research evaluated the change in polarity of malvidine -3- glycoside hydrochloride when using ethanol as solvent in three different proportions. The study determined that there are solvatochromic displacements by the variation of the ma-

ximum wavelengths. The results indicate that the intermolecular hydrogen bonds between the solute and the solvent are responsible for the solvatochromic changes in the analyzed binary mixtures. The study in primary standard was not based on determining significance in solvatochromic changes. The analysis was the reproducibility evaluation of each wavelength in different days.

The dry extract, when exposed to a variation of pH of 4 and 6, presents changes in the maximum wavelength. These displacements are produced by Malvidine -3- glycoside present in the pigment. The ANCOVA analysis determined that the pH variation in the natural dye produces significant variations ($p\text{-Value} < 0,05$), with a 95% confidence interval.

The stabilization of the pigment in a natural matrix determined that there is no significant difference ($p\text{-Value} > 0,05$), when varying the pH of the solvent. Solvatochromic displacements produced by the presence of hydrogen and hydroxyl ions at pH 4 and 6 were eliminated by the polysaccharide-polyphenol binding.

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