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# Usage of Two Extraction Methods for Natural Dyes (Anthocyanin) from Blackberries of Castilla (*Rubus Glaucus* Benth) and Its Application in Yogurt

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**Abstract** The advances in chemistry have led to use synthetic additives to impart color to dairy products, this due to its high performance. However, the excessive use of additives such as dyes of chemical origin have been causing health problems in consumers, which has contributed to the dairy industry finding a way to reuse pigments that are naturally found in some fruits and vegetables, especially the anthocyanin pigments of blackberry, due to its great industrial and therapeutic importance. During this investigation, two blackberry accessions were used; accession 1: blackberry without thorns, and accession 2: blackberry with thorns, and submitted to two extractions methods for 10 and 12 hours of dehydration. The physicochemical analysis of the raw material was under the control policies. Through sensory analysis, it was determined as the best treatment was the T7 combination (Blackberry without thorns, maceration, dehydrated 10 hours-65°C), able to be applied in fermented beverages (yogurt). In each treatment, the pH and °Brix analysis was under current regulations. In the best treatment, microbiological analyzes such as coliforms, fungi and yeasts were also under the allowed regulations. The extractions of three different solvents (methanol, ethanol and water) were compared with standards to determine the presence of polyphenols, concluding that the chromatographic peaks of the analyzed spectrum belong to polyphenols, from the anthocyanin group cyanidation-3-glucoside and confirmed by a paper chromatography test.

***Keywords:*** *extraction, natural dyes, Rubus glaucus, polyphenols, anthocyanin, yogurt*

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## 1. Introduction

Ecuador has a great diversity of genetic phytopic ecosystems and resources that must be used in a sustainable manner for the food security of the population [1]. For this reason, it is important to support agricultural research, which is the basis for innovation and development of technologies that improve production and productivity, not only of raw materials, but also of agro industrial products with benefit to consumers.

The blackberry of Castilla (*Rubus glaucus* Benth), native of the Andean region, is a fruit traditionally cultivated by small and medium farmers, contribute in the creation of employment, for its industrial and pharmaceutical importance and multiple benefits to human health by the contributions of vitamins, minerals and antioxidants [2]. In Ecuador, approximately 5000 cultivation ha of blackberry is reported, with average yields of 5 t ha/year [3].

The Castilla-blackberry, belong to the taxonomic-order of "Rosales", family "Rosaceae," *Rubus* genus consisting in around 700 species, distributed worldwide, which are grouped into 12 subgenres [4,5]. In Ecuador, 21 species of the *Rubus* genus are reported, being the *Rubus glaucus* of Castilla (blackberry), commercially grown from 2200 to 4000 masl [6].

*Rubus glaucus*, was discovered by Hartw and described by Benth [7], the scientific name comes from the words *Rubus*, rubies, red, after the fruits color and *Glaucus*, glaucous, light green, after the stems color. In the molecular characterization developed by the INIAP (local agro-industrial organisation), it was determined that in Ecuador there are two well-formed groups of Castilla-Blackberry, the first constituted group includes with thorns varieties (traditional blackberry) and the second group corresponds to without thorns ones. [8,9].

The dyes are substances that may being the color have a natural or artificial origin and are used to improve the product visual appearance and to respond to consumer expectations, given that food usually suffers loss of color during the industrial treatment.

Dyes in the food, play a very important role, given that studies have established that 80% of food choices is determined by color, then taste and texture become ones of the most important sensory qualities for processed food to be accepted or rejected [10,11]. Natural additives are currently booming, since artificial dyes such as red 40 and yellow 5 have had negative effects in terms of consumers health [12]. Natural pigments such as anthocyanins, besides recovering or improving the color of processed foods have shown to have therapeutic and positive effects on health such as: anti-carcinogenic, anti-diabetes and anti-rust, since these traps or neutralizes free radicals that are dangerous for human beings [13].

Anthocyanins, are used as a natural additive in the production of beverages such as yoghurts, flavored milks and jams, structure of anthocyanins is showed in the Figure 1. Polyphenols, being natural compounds with antioxidant characteristics, their application is normal in functional foods [14].

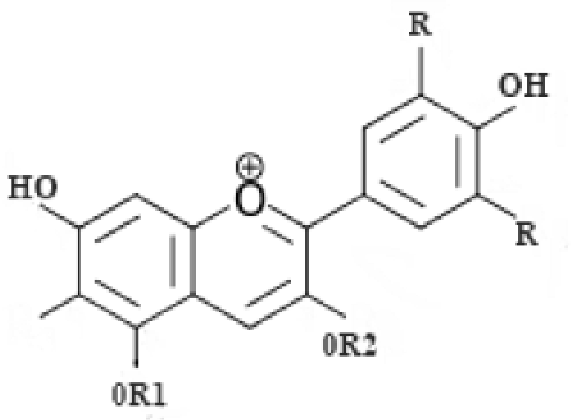


**Figure 1.** Structure of anthocyanins pigments; R1 and R2 can be H or sugars and R can be OH or H

Yogurt is a dairy product that has been consumed for centuries, is an excellent food product of high biological value, it is rich in vitamins like those of the B complex. In addition, due to its presence of lactic acid, the daily intake of yogurt increases the availability of micro-elements such as: Ca and P. According to the above mentioned background and given the great acceptance and high consumption rates among population, the application of natural dyes in yogurt becomes a very fitting resource to promote this product. In other words, dyeing process in yogurt helps to modify its organoleptic features, to improve acceptance among consumers [15].

Since the additives that are added to yogurt are mostly artificial, the main goal of this scientific research has been to study two methods to extract natural dyes (anthocyanins) from two varieties Castilla-blackberry and their application in yogurt.

## 2. Ease of Use

To carry out this investigation, 1000 g of blackberry with and without thorns was used as raw material to obtain the natural dyes. For this purpose, clean berries were selected and of firm consistency with an optimum maturity state between 5-6 °Brix, according to the Standard of the Ecuadorian Normalization Institute [16], for the matrix of fruit color scale, obtained in the cantons of Chillanes and Ambato (Ecuador).

### 2.1. Obtaining the Natural Dyes

To obtain anthocyanins from blackberry, two methods were used: dehydration and maceration.

#### 2.1.1. Through Dehydration

The pulp of the blackberry was extracted, using a pulper (Vulcano, DFV 19-40 I/C, Peru), after of a filtered, the concentration of the pulp was carried out, subjecting it at 65°C, eliminating 20% of water for the conservation of aroma of the fruit. Finally, the dehydration was carried out in a tray dryer (Zhengzhou, PHG6, China) at 65°C for 11 ±1 hours, until obtaining a dry paste, then was pulverized to obtain the dye powder.

#### 2.1.2. Through Maceration

In this case, the fruit was cut into four parts in order to obtain a greater contact surface with the alcohol for the extraction of the dye. The chopped blackberry was placed in 500 mL of ethanol at 96 GL acidified with 1% hydrochloric acid for 72 h, then the samples were filtered on canvases to separate the chopped fruit from the liquid phase (this contains dye and ethanol). The alcohol-dye separation was carried out in a rotary evaporator (Butchi, R-220, Switzerland) at 50°C. The obtained concentrate was transferred to expanded polystyrene plates and taken to the tray dryer (M-Cotopaxi, Ecuador) at 65°C for 11±1 hour. The dried product was crushed to obtain the dye, subsequently; the powdered dyes (anthocyanins) were packed.

### 2.2. Yogurt Elaboration

To elaborate yogurt, we started with fresh whole bovine milk, the quality analysis of the raw milk and elaborated products was carried out, according to the Ecuadorian Technical Norm of the Ecuadorian Standardization Institute [17]. "Fermented Milk Drink Requirements".

#### 2.2.1. Preparation of Natural Yogurt Incorporating Anthocyanins

The flow diagram for the preparation of natural yogurt it is shown in the Figure 2.

After obtaining natural yogurt, the formula was mixed with the powdery dye (anthocyanins) at 2% concentration, according to the levels of typical use for dairy products such as yogurt (0.03-3%), established by FARBE, [18], then the product was packed and stored at 4ºC.

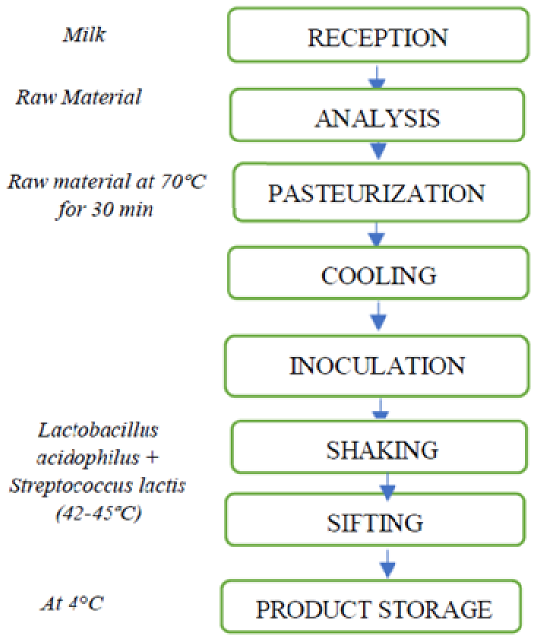


**Figure 2.** Flowchart for natural yogurt obtantion

## 2.3. Statistical Analysis

A tri-factorial completely randomized block design (CRBD) 2x2x2 was applied, with 3 repetitions, to determine the best treatment (Table 1).

**Table 1. Study factors combination t+o obtain dye**

| Study factors | Study levels |
|---|---|
| A: Blackberries variety | A1=Castilla-Blackberry with thorns<br>A2=Castilla-Blackberry without thorns |
| B: Extraction methods | B1= Dehydration<br>B2= Maceration |
| C: Dehydration times | C1= 10 hours<br>C2= 12 hours |

Eight treatments were studied.

## 2.4. Analysis in the Finished Product

The analysis executed in the dye obtained were: pH analysis (NTE INEN 389-783) [19], weight (NTE INEN 2074) [20], moisture (NTC 409 (5-6,5) [21]. Through a sensory analysis and with the organoleptic standards: color, odor, texture and acceptability, allowed to determine the best treatment. Besides, aditional analysis were carry auth to the best treatments as microorganisms count following standards: Total coliforms, colony forming unit CFU/g (NTE INEN 1529-7) [22], *Escherichia coli*, CFU/g (AOAC 991.14) [23], and mold and yeast, CFU/g (NTE INEN 1529-10) [24]; paper chromatography and spectrophotometry.

### 2.4.1. Anthocyanin Content Spectrophotometry Evaluation from the Obtained Dye

A watery dissolution from the obtained extract was made (1 g in 100mL of distilled water), absorbance capacity was determined in a spectrophotometer at the maximum absorption for anthocyanin, it was expressed in grams of cyaniding-3-glucoside/Kg of blackberry pulp (*Rubus glaucus* Benth) [25]. Which were studied with the anthocyanin extract, in a GENESYS 10 spectrophotometer, at a wave longitude of 500, 510, 520, 530, 540, 550, 560, 570, 580 nm [26]. The results were compared with anthocyanin informatics standards.

### 2.4.2. Anthocyanin Determination through High-Resolution Liquid Chromatography

HPLC is the most common method to perform an anthocyanin analysis. The samples were semi-purified using a C-18 cartridge and the phenolic fraction (anthocyanin content) was eluted with methanol acidifying with HCl at 1%. It was evaporated with methanol in a Buchi steam routing machine, then acidified water with HCl at 0.01% used to achieve a known volume and using a polypropylene filter (Whatman 0.45 mm) it was strained before chromatograph injection. Separation was realized in a C-18 Waters Symmetry column (4.6mm x 150mm y 3.5m) using the HPLC and following the established standards from the laboratory analyst in Central University of Ecuador. Anthocyanin peaks identification was made comparing chromatograms and retention times from the blackberry anthocyanin concentrate extracts.

# 3. Results and Discussion

## 3.1. Raw Material Variable Analysis

The raw material analysis (Castilla-Blackberry with thorns and without thorns), is presented in Table 2.

**Table 2. Raw material initial analysis**

| Raw material | Fruit weight (g) | Pulp weight (g) | pH | °Brix |
|---|---|---|---|---|
| Castilla-Blackberry with thorns | 1000 | 820 | 3.35 | 8.50 |
| Castilla-Blackberry without thorns | 1000 | 826 | 3.50 | 11.50 |

In the physical-chemical parameters, it was observed that there is a directly proportional difference between the pH and the °Brix (soluble solids) in blackberry of Castilla with and without thorns, the factors that can affect the pH values can be the clime and growth conditions of the plant. [27].

Besides, the concentration of sugars of the raw material used was determined, where blackberry with thorns showed a lower concentration with 8.50 °Brix, while for the blackberry without thorns a value of 11.50 °Brix was obtained. This range of soluble solids shows that the berries are in a state of appropriate physiological maturity. In an investigation, development by Alves da Cunha et al. [28] a value of 14±6 °Brix was found.

Anthocyanin extracted from Castilla-Blackberries were added as a natural dye to fermented dairy product (yogurt), and the organoleptic features were measured by a semi-trained tasters panel.

Similar research managed by Salinas et al. [29], they used corn grain anthocyanins in yogurt at a concentration of 1 mg of dye/100 g of yogurt. In another investigation managed by Aguilera et al. [30], they added 1.2g/g of yogurt of anthocyanins from fig skin, both obtained a color similar to that of a commercial strawberry yogurt.

### 3.2. Sensory Analysis

In general, sensory analysis is a good tool to assess the impact on consumer acceptability, in such a way that, in this work it was determined if the types of default accessions, the extraction methods of the dye and the time of extraction, influence the acceptability of the yogurt obtained.

By applying the Tukey test at 95% confidence level, to organoleptic characters, it was determined that there is a significant statistical difference between each of the treatments of the color parameter. However, in the following features: odor, texture and acceptability, there was no significant statistical difference.

The intense red color of the dye in the treatments is given by the low pH value of the blackberry (at lower pH, higher anthocyanins concentration) [31]. Numerically, treatment T3, (A1B2C1) had the highest value (3.80), with a rating scale from red to bright red.

In the odor, the treatments with higher valuation were the T6, (A2B1C2), T7, (A2B2C1) and T8, (A2B2C2) with a score of 3.90 each. In texture the treatment T1, (A1B1C1) presented a higher score with respect to the other treatments, this being 4.00 which corresponds to a fine granularity.

About the acceptability (response variable), the T7 treatment, (A2B2C1) (Castilla blackberry without thorns+Maceration+Dehydrated 10h, 65°C), presented a higher score with 4.20 (Figure 3).

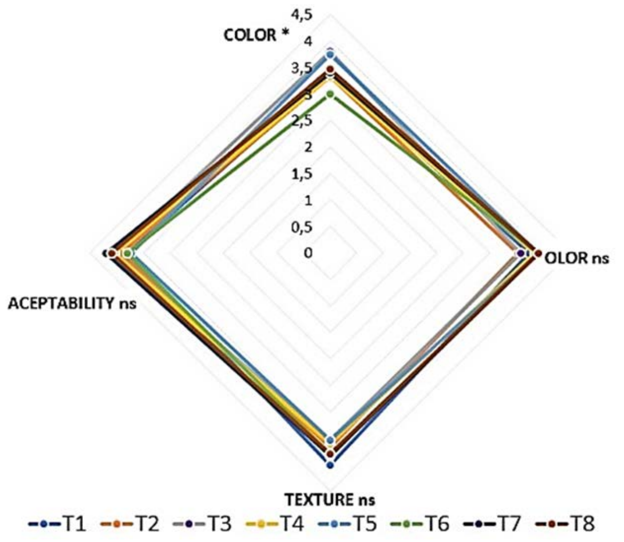


**Figure 3.** Sensory analysis radar of yogurt after natural dye application for each treatment (*, *Significant statistic difference; ns, Non-significant statistic difference)*

### 3.3. Physical Chemical Analysis

In the Table 3, shows the results of physical chemical analysis.

pH analysis results in order to (NTE INEN 789) regulation, are in the ranges that NTE INEN 389 [19] regulation allows, which is between 3.3 and 3.5 for anthocyanin dye, same happens if pH increases over the dye maximum value, this will turn lightly into blue [10]. In a weight analys of treatments, T2 treatment (A1B1C2) showed the best performance, with 48 grams. The treatments T1 (A1B1C1) and T3, (A1B2C1) have higher moisture values, due to low dehydration submission processes, compared with the NTC 409 [21] allowed regulation, they showed between 3% and 6%, which means that the results aggred with the regulation.

**Table 3. Treatments physicochemical analysis**

| Treatments | pH | Weights (g) | Moisture (%) |
|---|---|---|---|
| A1B1C1 | 3.41 | 47.50 | 6.43 |
| A1B1C2 | 3.49 | 48.00 | 6.12 |
| A1B2C1 | 3.43 | 44.50 | 6.43 |
| A1B2C2 | 3.42 | 43.50 | 5.95 |
| A2B1C1 | 3.47 | 46.00 | 6.35 |
| A2B1C2 | 3.45 | 46.50 | 5.79 |
| A2B2C1 | 3.43 | 44.30 | 5.80 |
| A2B2C2 | 3.43 | 43.00 | 5.56 |
| Allowed regulation | NTE INEN 389-783 (3.3 y 3.5) [19] | NTE INEN 2074 [20] | NTC 409 (5-6.5) [21] |

### 3.4. Microbiological Analysis in the Best Treatment

In Table 4, microbiological exams results applied to T7 treatment (Castilla-Blackberry without thorns+Maceration +Dehydration at 65°C for 10 hours), accomplished the microbiological requirements, in that way they are placed on a top range established by NTC ICONTEC 409 [32] for natural dye (anthocyanin).

**Table 4. Microbiological analysis of dye obtained**

| Sample | Total, coliforms | Molds | Yeasts |
|---|---|---|---|
| T7 (A2B2C1) | Absence | 110 CFU/g | Absence |
| Method | ISO 7954 Routine method NF V08-050 | Molds recount NF V 08-059, ISO 7954 | Yeasts recount NF V 08-059, ISO 7402 |

### 3.5. Anthocyanins Content Spectrophotometric Exam from the Obtained Dye

It was found that a methanolic extract of Castilla blackberries had a 1.578 g/kg pulp with anthocyanin content; reported as cyanidin-3-glucoside (Image 2) the anthocyanin are majority in blackberries [27]. Within a research managed by Moreno et al. [33] he has obtained a value of1.100g/kg. The anthocyanin content variations in blackberries are due to environmental conditions, production area, and blackberries.

In addition, the final results within this research allow us to infer that, the extract has an important dye with antioxidant capacity and can be used in foods [2]. In Figure 4, anthocyanin absorption chromatography spectrum peaks present in blackberries are shown; there, two maximum absorption lengths can be seen, one at 500 nm and the other at 570 nm. These two maximum absorption values showed as cyanidin-3-glucoside characteristic aspects [34]. Individual anthocyanin proportion was calculated starting from the peaks area contributions until the total anthocyanin standard (Figure 4).

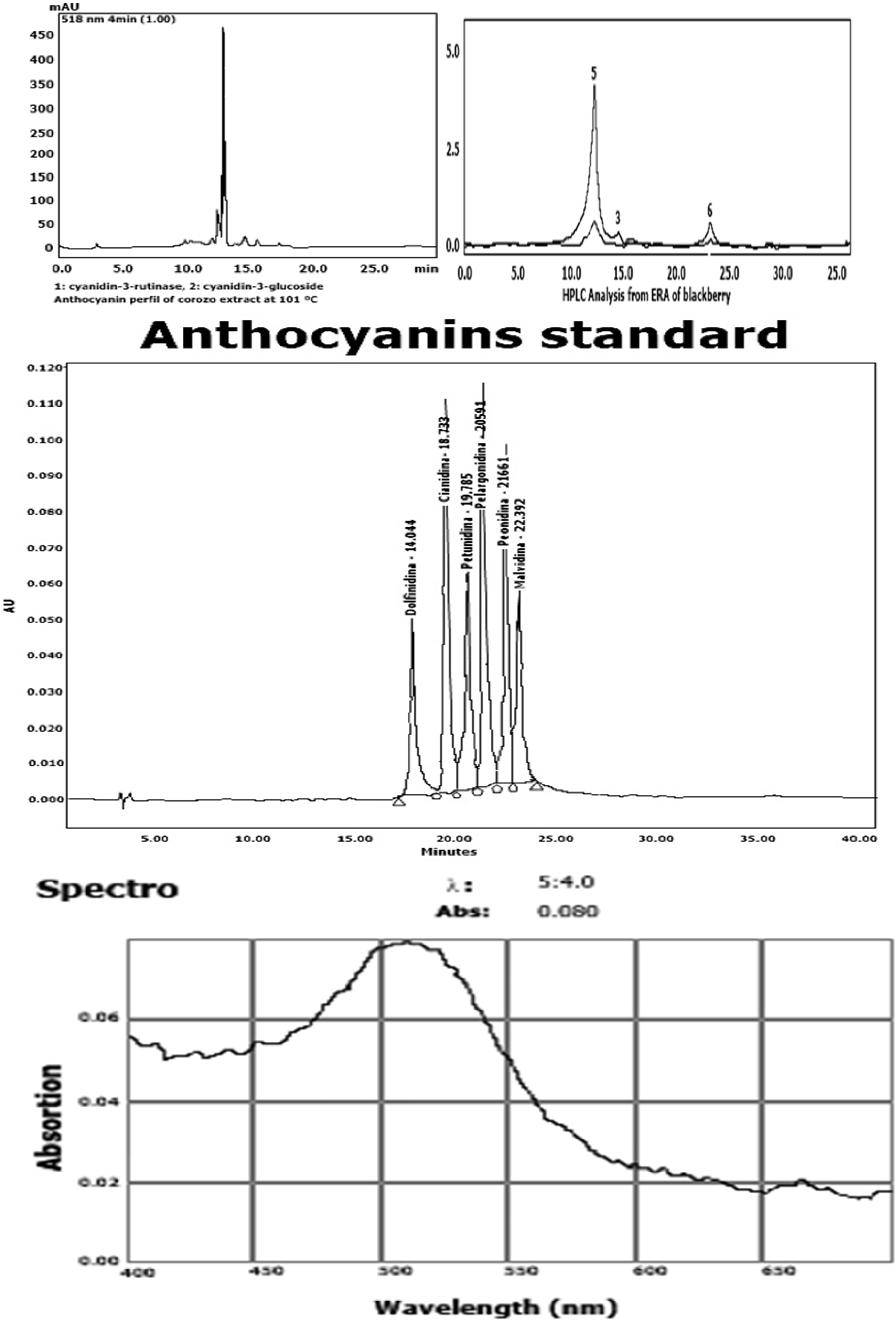


**Figure 4.** Anthocyanin absorption chromatogram peaks spectrum presents in Castilla blackberries second accession

The results obtained in this investigation, using different methodologies for the evaluation of anthocyanins from default, showed that there were no marked differences between the techniques applied; thus, there were similar concentrations of cyanidin-3-glucoside in the peaks 514, 518, 520 (nm), both in the spectrophotometry and in the HPLC, respectively.

### 3.6. Paper Chromatography

The obtained chromatogram reveals the colored compounds existence in the extract, evidencing themselves for the red superior mark appearance (Figure 5), which could be easily observed; among color classification rates, this chromatographic mark belongs to anthocyanin (cyanidin-3-glucoside), flavonoids responsible color of blackberries fruit, [30]. In the paper chromatography, the red fraction composition can be appreciated (anthocyanin, cyanidin-3-glucoside) which is the natural blackberry pigment with more proportion found, accordingly the anthocyanin quantity can be determined by the parameter of the retention factor (Rf) as the dye quantity as the solvent (Figure 5).

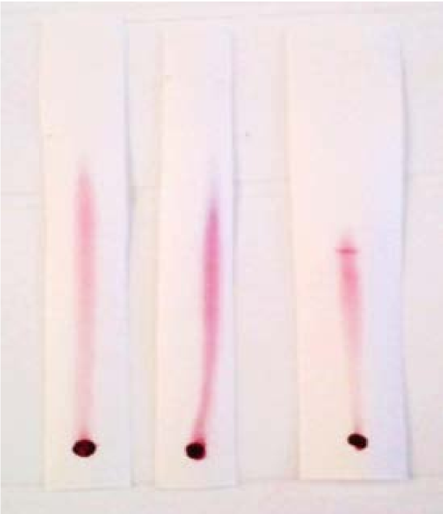

**Figure 5.** Paper chromatography (*Whatman N° 1*), from the best dye using an elution solvent (hydrochloric acid at 0,01% "w/v") of acid as solvent for the absorption spectrums

Determination of the parameter of the retention factor (Rf = a/b), defined as the distance traveled by the anthocyanin, cyanidin-3-glucoside (a) between the distance traveled by the solvent (b). It is shown in the Table 5.

**Table 5. Rf compound length/Eluent length**

| Sample | Eluent sample's traveled distance | Sample's traveled distance | Sample's Rf |
|---|---|---|---|
| Ethanol | 13.00 cm | 8.50 cm | 0.653 |
| Methanol | 13.00 cm | 6.00 cm | 0.461 |
| Water | 8.00 cm | 3.00 cm | 0.375 |

The distribution of anthocyanins based on their composition frequently occurs in about 50% with cyanidin, 12% for pelargonidin and delphinidin and 7% for petunidin and malvidin. For glycosides, the 3-glycosides have an occurrence 2.5 times greater than the 3.5-di glycosides, the most common being cyanidin-3-glucoside.

## 4. Conclusion

The results of this research have allowed to obtain natural dyes (polyphenols) from blackberry without thorns, the dye presented conditions to be applied in foods such as yogurt with organoleptic characteristics acceptable to the consumer.

## Conflicts of Interest

All author declares no conflict of interest.

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## References


[1] Ministerio del Ambiente del Ecuador. Estrategia Nacional de Biodiversidad Ecuador, 2016. http://www.ambiente.gob.ec/ecuador-presenta-la-estrategia-nacional-de-biodiversidad/.

[2] Astrid G. Las antocianinas como colorantes naturales y compuestos bioactivos: revisión (Eng: The antocianinas like coloring natural and bioactives compound: revision). Acta Biol Colombian 2008; 13: 27-36.

[3] INIAP. Instituto Nacional de Investigaciones Agropecuarias. El cultivo de mora en el Ecuador. Estación Experimental Santa Catalina, Programa Nacional de Fruticultura. INIAP Press 2016; 1: 11-15.

[4] Oureck D.K. Zarzas. *In* Avances en la Genotecnia de frutales. Moore y Janick ed. México DF. México. AGT Editor1993; 1: 124-157.

[5] Romoleroux K. Diversidad de las moras (*Rubus* spp.) en el Ecuador: Un recurso filogenético poco explotado. *In* R. Castillo; C. Tapia; J. Estrella. Memorias de la II Reunión Nacional sobre recursos filogenéticos. Quito, *Escuela Politécnica Nacional*. Facultad de Ingeniería Química y Agroindustrias 1991; pp. 195.

[6] Romoleroux K. Flora of Ecuador. 1 ed. University of Goteborg. Estocolmo, Noruega, Department of Systematic Botany 1996; pp. 169.

[7] Jennings D. Raspberries and Blackberries: Their breeding, disease and growth. New York, USA. Academic Pres 1998; pp. 230.

[8] Martínez A, Vásquez W, Viteri P, Jácome R, Ayala G. Ficha Técnica de la variedad de mora de espinas sin espinas (*Rubus glaucus* B.) *INIAP*-Andimora. Programa Nacional de Fruticultura. Quito-Ecuador. INIAP Press 2013; pp. 14.

[9] Montalvo D. Evaluación de la calidad pos-cosecha de las accesiones seleccionadas de mora de Castilla (*Rubus glaucus* B.) provenientes de las provincias de Tungurahua y Bolívar. Tesis de Ing. Agroindustrial. Quito, *Escuela Politécnica Nacional*. Facultad de Ingeniería Química y Agroindustrias 2010; pp. 195.

[10] Ortiz J, Mertín-Arroyo M, Noriega M.J, Navarro M, Arozarena I. Color, phenolics, and antioxidant activity of blackberry (*Rubus glaucus* Benth.), blueberry (Vaccinium floribundum Kunth.), and apple wines from Ecuador. C Food Chem 2013; 78: 985-993.

[11] Poiana M, Munteanu M, Bordean D, Gligor R, Alexa E. Assessing the effects of different pectins addition on color quality and

antioxidant properties of blackberry jam. Chem Central Journal 2013; 7: 121.

[12] Lock O. Colorantes Naturales, Pontificia Universidad Católica del Perú. PUCP 1era edit 1997; pp. 274.

[13] Skrovankova S, Sumczynski D, Micek J, Jurikova T, Sochor J. Bioactive compounds and Antioxidant activity in different types of berries. Int J Mol Sciences 2015; 16: 24673-24706.

[14] Lecker Argentina SA. Colorantes Naturales, Bebidas 2011. Available in: (http://leckerargentina.com.ar/colorantes.html). [Cited: 13th May 2017].

[15] Routray W and Mishra HN. Scientific and technical aspects of yogurt aroma and taste: A review. Comprehensive Reviews in Food Science and Food Safety 2011*;* 10: 208-220.

[16] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 2427 (2011). Madurez Optima del Fruto. [Cited: 13th May 2017].

[17] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 2608 (2012). Bebida de Leche Fermentada. [Cited: 13th May 2017].

[18] FARBE. Tasting the color of nature. Farbe Mexico 2016 [Cited: 25th Feb 2018].

[19] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 389-783 (2011). Análisis Físicoquímicos (pH). [Cited: 13th May 2017].

[20] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 2074 (2011). Análisis Físicoquímicos. Peso (g). [Cited: 13th May 2017].

[21] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 409 (2011). Análisis Físicoquímicos. Humedad (%). [Cited: 13th May 2017].

[22] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 1529-7 (2011). Control microbiológico de los alimentos. Determinación de microorganismos coliformes por la técnica de recuento [Cited: 02th Jun 2017].

[23] AOAC. Association of Official Analytical Chemists, Official Method 991.14 (1994). Coliform and *Escherichia coli* Counts in Foods [Cited: 02th Jun 2017].

[24] Norma Técnica Ecuatoriana (NTE). Instituto Ecuatoriano de Normalización (INEN) 1529-10 (2011). Control Microbiológico de los Alimentos. Mohos y levaduras viables. Recuento en placa por siembra en profundidad [Cited: 02th Jun 2017].

[25] Johnson M.H, González de Mejía E, Fan J, Ann M, Jousef G. Anthocyanins and proanthocyanidins from blueberry-blackberry fermented beverages inhibit markers of inflammation in macrophages and carbohidrate-utilizing enzymes in vitro 2013; Mol Nutr Food Res; 57: 1182-1197.

[26] Gomara F, Sato M, Pontarolo L. Desarrollo y validación de un método espectrofotométrico para cuantificación de ácido kójico. Ars Pharm 2004; 45: 145-153.

[27] Verma R, Gangrade T, Punasiya R, Ghulaxe C. *Rubus fruticosus* (blackberry) use as an herbal medicine. Pharmacognosy Reviews 2014; 8: 101-104.

[28] Alves da Cunha M.A, de Lima K, Queiroz V.A, Heinz O.L, Pizarro C.A. Blackberry vinegar produced by successive acetification cycles: Production, characterization and bioactivity parameters. Braz Arch Biol Technol 2016; 59: 1-12.

[29] Salinas Y, Rubio D, Díaz A. Extracción y uso de Pigmentos del grano de Maíz (Zea mays L.) como colorantes en yogur "Engl: Extraction and use of pigments from maize grains (zea mays L.) as colorants in yogur" Arch Latin Nutr 2005; 55: 293-298.

[30] Aguilera-Ortiz M, Reza M.C, Chew R, Aguilar J, Ramírez P. Fig anthocyanins as plain yogurt colorants. Rev Biotecnia 2012; 14: 18-24.

[31] Khoo HE, Azlan A, Tang S, Lim SM. Anthocyanidins and anthocianins: colored pigmentsas food, pharmaceutical ingredients, and the potencial health benefits. Food Nutr Res 2017; 61: 1361779.

[32] NTC. Norma Técnica Colombiana. Instituto Colombiano de Normas Técnicas y Certificación (ICONTEC). (2001). Industrias Alimentarias. Colorantes Aditivos para Alimentos. [Cited: 25th Feb 2018].

[33] Moreno MJ, Viloria MA, López E, Douglas BC. Estabilidad de antocianinas en jugos pasteurizados de mora (*Rubus glaucus* Benth). Archivos Latinoamericanos de Nutrición 2002*;* 52: 181-186.

[34] García G, Quintero R, López M. Biotecnología alimentaria. Noriega Editores, Editorial Limusa S.A de C.V 2005; pp. 36.