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Effect of Drying and Maturity on the Antioxidant Properties of the Blueberry (*Vaccinium Floribundum Kurth*) from the Ecuadorian Moorland and Sensory Evaluation of its Infusion

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Abstract

The mortiño (Vaccinium floribundum) is characterized by its high content of polyphenols and anthocyanins, as well as high antioxidant activity. This research analyzed the effect of drying (convection drying and lyophilization) together with the degree of ripeness (50% and 100%) on the fruit's antioxidant properties. Additionally, a sensory evaluation of an infusion prepared with the dried product was conducted. The treatment consisting of lyophilization and 100% ripeness had the highest values for polyphenols and anthocyanins, 4733.50 mg of gallic acid/100 g DW and 778.70 mg of cyanidin 3-glucoside chloride / 100 g DW, respectively. The ascorbic acid content was highest in the treatment with lyophilization and 50% ripeness, with 69.50 mg / 100 g DW. The antioxidant activity for the treatments with 100% ripeness had similar results for convection drying and lyophilization, 87.28 and 88.62 mmol TE / kg DW, respectively. An infusion was made from the dried mortiño product and subjected to a sensory evaluation from a panel of tasters. The samples with 100% ripeness, regardless of the drying method, received a "Like very much" qualification for color, aroma, and taste attributes. Testing for antioxidant activity, it was found that between 2.5 to 3.5 % of the original antioxidant content is transferred to the infusion. The findings suggest that mortiño can be used in the preparation of an infusion with functional properties, creating an opportunity for the local communities of venturing into this crop for industrial purposes.

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Introduction

The mortiño is a plant endemic to the Ecuadorian moorlands. It is widespread in the wild. The fruit has been used by locals since ancient times to prepare colada morada, a traditional drink consumed on Day of the Dead. There are three species of mortiño in Ecuador: *Vaccinium floribundum*, *Vaccinium crenatum*, and *Vaccinium distichum*. *Vaccinium floribundum*, native to the Andes, is the most common species and is cultivated between 3400-3800 m above sea level. In the province of Bolivar, it is mainly found in the higher elevation communities within the parish of Salinas.[1,2]

There is epidemiological evidence correlating fruit and vegetable consumption to a decrease in the occurrence of a variety of diseases, due to the antioxidant effect of their polyphenolic compounds. The blueberry, as with other Vaccinium, is characterized by a high content of phenols, anthocyanins, and antioxidants, giving it a reputation as the "king of antioxidant fruit".[3] In Colombia, the mortiño (also known as agraz) is consumed as fresh fruit or turned into artisanal liquors, marmalades, and desserts. It is an important source of polyphenolic compounds linked to positive effects on human health.[4] In addition to their antioxidant activity and their high content of polyphenolic compounds, blueberries are rich in anthocyanins, which are the most important pigment in the vascular system of plants and are beneficial to health due to their anti-inflammatory, cardioprotective, anticancer and neuroprotective, anti-aging properties, eye and kidney protectors, they also act against type 2 diabetes reducing obesity.[5,6,7,8,9,10]

Koca and Karadeniz[11] found high levels of antioxidant activity in blueberries and blackberries, the antioxidant activity levels were higher in the wild berries as opposed to their cultivated version, they also found that antioxidant activity is highly correlated to anthocyanin and polyphenol content. A study conducted by Lohachoompol *et al.,*[12] did not find significant differences in antioxidant activity between fresh, dried, and frozen mortiño.

In recent years, new food products have been developed with plant compounds such as anthocyanin as active compounds in the food matrix of processed foods. It is important to use raw materials rich in antioxidants, such as Andean blueberries, in the formulation of functional foods and beverages.[2] Blueberries are considered functional foods since anthocyanins can represent up to 60% of the total phenolic compounds in mature blueberries.[13]

Kharadze *et al.*[14] found anthocyanins and antioxidant activity in wines from different varieties of grapes endemic to Georgia, reporting values between 327.1 – 871.7 mg/L and 36.4 – 59.6% for anthocyanin content and antioxidant activity, respectively. Da Silva *et al.,*[15] developed an infusion from the skin of the jaboticaba fruit, their findings suggest that functional substances can be extracted by means of water infusion while retaining high anti-radical capacity. Goldmeyer *et al.,*[16] indicate that industrializing the mortiño fruit would keep its functional properties while making it widely available, however, the fruit does not exist yet as an industrialized product.

Drying processes are one of the main options for creating aggregated value in agricultural products, allowing for longer periods of conservation, the reduction in water content inhibits the microorganisms that cause damage reactions, which stabilizes the properties of the product and the composition of its active ingredients, the most common drying process uses the natural or forced convection of air. Other more sophisticated industrial processes, such as lyophilization, exist as well. When comparing food products dried by means of lyophilization and convection, lyophilization appears to have more benefits as a drying process.[17]

Tea is one of the most popular drinks worldwide and contains large quantities of polyphenols and antioxidants, Afroz *et al.,*[18] evaluated the phenolic content and antioxidant potential of a variety of commercially available black teas and found values between 13.58 – 36.23 GAE mg /g DW. Amadou *et al.,*[19] found 3.06 GAE mg/g DW and compounds like narcissin, hirsutrin, quercetrin, ilixantrin, rutin, isorhamnetin and diverse flavone were identified, in the infusion prepared from flowers (Balanites aegyptiaca Del).

The increase in recent years in degenerative diseases such as cancer, osteoporosis, cardiovascular disease, and diabetes, calls for an urgent change in eating habits, towards foods that bring health benefits in addition to their nutritional value.[20]

There is great potential in the use of wild native plant species with high antioxidant activity such as the mortiño fruit, however, this climacteric fruit is highly perishable, quickly losing commercial quality as well as nutritional and antioxidant properties. Drying the fruit is a suitable way to preserve its antioxidant properties. The goal of this research was to determine the effect of types of drying, convection and lyophilization, on the antioxidant activity and polyphenolic, anthocyanin, and vitamin C content of mortiño fruit at different degrees of ripeness. This, in order to make a tea from dried mortiño and evaluate the transfer of these properties to the resulting infusion, as well as test the consumer acceptance of the product.

Materials and Methods

Selection of the Fruits

The mortiño fruits were collected through a random sampling of the plantations located in Las Mercedes (Salinas Parish, Guaranda Canton, Bolivar Province, Ecuador), conducted during the months of August, September and October, can be seen in Figure 1. The fruits were washed with generous amounts of water and impurities were removed by flotation. Subsequently, they were visually classified according to the fruits maturity: Degree of ripeness 1 (DR1) where 50% of the epicarp is black - 50% of the epicarp is pink, and Degree of ripeness 2 (DR2) where 100% of the epicarp is back.

Fig. 1: Two degrees of ripeness of the mortiño fruits used in the experiment (A= DR1, B= DR2)

Samples from the DR1 and DR2 sets were analyzed for humidity, ash, °Brix, pH, and water activity (wa) in the Bromatology laboratory of the Universidad Estatal de Bolívar, according to the methods shown in Table 1.

Experimental Design

A 2^2 experimental design that considers the two degrees of ripeness of the fruit and the two types of drying is presented in Table 2.

Table 1: Analysis and methods used for the characterization of mortiño fruits

Parameter	Norm and equipment
pH	Technical norm INEN 1842, Hanna digital pH meter
°Brix	Technical norm INEN 2173, REF-113 ATC refractometer
Humidity	Methods of AOAC (2001), muffle and scale
Ash	Methods of AOAC (2005), muffle and scale
Water Activity (wa)	Digital method, Testo 645 AG Germany

Table 2: Experimental design

Treatment/level of the variables	Type of drying	Degree of ripeness
T1	D1: Convection drying	DR1: 50% mature
T2	D1: Convection drying	DR2: 100% mature
T3	D2: Lyophilization drying	DR1: 50% mature
T4	D2: Lyophilization drying	DR2: 100% mature

Treatment of the Extracts

The washed fruits were crushed, then separated into two batches. The first batch underwent forced convection drying in a nine-tray Excalibur dehydrator, calibrated to 40 °C, for 15 hours. The second batch was lyophilized. To achieve this, it first went through a deep-freeze process at -80 °C in a deep freezer (Panasonic, MDF-U76VA-PA). Then it was moved to a lyophilizer (MARTIN CHRIST GEFRIERTROCKNUNGSANLAGEN, BETA 1-8 LSCPLUS), where it stayed at -52 °C and 0.03 mbar of pressure for 48 hours. The obtained samples were subsequently pulverized and stored in 250 mL amber glass jars with screw-on caps.

To make the extracts, 2 g of each sample were mixed with 50 mL of distilled water in separate amber jars. Each jar was stirred constantly for 30 minutes at room temperature in a Thermal stirrer (YVIMEN TR100-G) at 200 rpm, then stored in a place out of direct light for 24 hours. The extracts were placed in 50 mL Eppendorf tubes (Eppendorf 5804R) and centrifuged at 6000 rpm at 10 °C, for 12 minutes. The centrifuged product was filtered through Whatman No. 4 paper, discarding the residue and refrigerating the supernatant for posterior use.

Determination of Antioxidant Properties

The quantity of anthocyanins, total polyphenols, ascorbic acid, and antioxidant activity was measured in the extracts of the four treatments, three times each. The same analysis was conducted on the two dried mortiño infusions evaluated in the sensory analysis. The studies were conducted in the research laboratory of the Universidad Estatal de Bolívar, according to the procedure detailed in the following sections.

Quantification of the Anthocyanins

The quantification of the anthocyanin content was conducted according to the differential pH method.[21] Two aliquots of 2 mL were taken from the supernatant. One aliquot was mixed with a buffer solution with a pH of 1.0 (potassium chloride 0.2M adjusted with hydrochloric acid). The other aliquot was mixed with a buffer solution with a pH of 4.5 (sodium acetate 1M adjusted with acetic acid). The registered absorbance was 510 nm. Equation 1 was used to calculate the anthocyanin concentration.

$$C mg/L = (Abs\ pH\ 1.0 - Abs\ pH\ 4.5) \times MW \times 1000 / 24825\ DF \quad ...(1)$$

Where; MW is the molecular weight of cyanidin 3-glucoside chloride, with a value of 484.83; 24825 is the molar absorptivity at 510 nm; Abs with a pH of 1.0 and Abs with a pH of 4.5 is the correction from the formation of degradation products, DF is the dilution factor. The results were expressed in mg of cyanidin 3-glucoside chloride / 100 g dry weight (mg CYE/ 100 g DW).

Determination of Total Polyphenols

The determination of total polyphenols was conducted using the Folin-Ciocalteu colorimetric method.[22] A calibration curve was built using a standard solution of gallic acid (0.1 mg/mL), which yielded a total of 9 dilutions (including white). To each standard and previously prepared samples, were added 250 µL of Folin-Ciocalteu 1N reactive. They were then sonicated for 5 minutes. Subsequently, 250 µL of Na_2CO_3 at 7.5 % were added to the solutions and left to rest for 1 hour. The concentration of total polyphenols in the extracts was measured with a spectrophotometer (NANO DROP), measuring the absorbance at 750 nm. The results were expressed in mg of gallic acid / 100 g dry weight (mg GAE/ 100 g DW).

Determination of Ascorbic Acid

The quantification of ascorbic acid was carried out using the method by Obregon *et al.*.[23] An amount of 100 µL of the aqueous extracts was reacted with 900 µL of 2.6-dichlorophenolindophenol. Equation 2 was used to calculate the absorbance, obtaining a value of 515nm.

Abs 515 nm = Abs control – Abs sample ...(2)

The control absorbance was calculated from the reaction of 100 µL of 0.4% oxalic acid with 900 µL of 2.6-dichlorophenolindophenol. The ascorbic acid content was expressed as mg/ 100g dry weight (mg/100 g DW). All measurements were done in triplicate.

Determination of Antioxidant Activity

The antioxidant capacity of the dried fruits was measured by the oxygen radical absorbance capacity (ORAC) using the procedure by Lin *et al.*[24] Their method measures a compound's capacity to eliminate a 2,2'-azobis(2-methylpropionamidine) dihydrochloride (ABAP) free radical, as compared to Trolox. It is expressed in Trolox equivalent millimoles per kilogram of dry weight (mmol TE / kg DW).

Preparation of the Infusion

To make the dried mortiño teabags, 5 g of each of the treatments T1, T2, T3, and T4 were placed in standard teabags, made with disposable filter paper, and sealed with a packer (SAMEK). A total of 15 units per treatment were made, immediately storing them in a dry and cool place in 7 x 7 x 13 cm cardboard boxes. The teabags were steeped in 200 mL of water between 90 to 95 °C for 8 minutes to prepare the infusion.

Ethics and Safety Management

The experimental protocol was approved by the Ethics Committee for Research on Human Beings of the Technical University of Babahoyo, Los Rios-Ecuador, and conformed to the ethical principles set forth in the Declaration of Helsinki. Voluntary written informed consent was obtained from all participants panelists. The experimental protocol is registered as CEISH-UTB-0052021.

Sensory Analysis

A sensory analysis was conducted on the infusions using a 5-point hedonic scale25, where 1 is "Dislike very much", 2 is "Dislike slightly", 3 is "Neither like nor dislike", 4 is "Like slightly", and 5 is "Like very much". The attributes measured in this analysis were color, aroma, and taste. The samples were codified according to treatment and presented in disposable cups to 24 semi-trained panelists. Each panelist was requested to drink purified water to clean their palate before proceeding to taste the samples.

Statistical Analysis

The Info Stat statistical software was used to measure the difference in the treatments, using analysis of variance. Tukey's test at the 5 % probability was used to find the difference between the means of the treatments.

Results and Discussion

The physical properties of the mortiño fruit are shown in Table 3, based on the average of three tests. Uncertainty is expressed as the standard deviation. As seen in the table, the mortiño in DR1 has a lower pH compared to that in DR2. This is due to the concentration of acids that occurs during fruit maturation. The pH is within the reported optimal range for a variety of blueberry cultivars, between 2.5 - 4.0.[26]

Table 3: Physical characterization of the mortiño fruit according to degree of ripeness

Property	DR1	DR2
pH	2.7±0.04	3.2±0.30
°Brix (%)	7±0.47	10.6±1.12
Humidity (%)	78±3.14	83.35±2.8
Ash (%)	1.6±0.21	1.8±0.23
wa	0.997±0.004	0.999±0.0001

The concentration of soluble solids (°Brix) is higher in the fruits in DR2. This was expected since the more mature fruits have a higher content of sugars, salts, organic acids, and other water-soluble compounds, as compared to the fruits in DR1. The obtained values are similar to the optimal levels reported for blueberries, between 10.6 to 13.2 °Brix.[26]

The humidity and ashes are higher in the DR2 group. As for water activity, the value is similar for both groups and enough to render the fruits susceptible to microbial contamination. These values coincide with the ones reported by Reque *et al.*[27]

Table 4 shows the results of the analysis conducted on the dehydrated samples. As the table shows, there is a significant difference ($p>0.05$) in the polyphenol, anthocyanin, and ascorbic acid content. The average values for polyphenols and anthocyanins are higher in treatment T4 (lyophilization and fruits in DM2), followed by T2 (convection drying and fruits in DM2). Lyophilization preserves the majority of bioactive compounds, while convection drying leads to a loss of the compounds due to the increase in temperature.[17] The polyphenol and anthocyanin values are lower for the fruits in DR1 (T1 and T3), regardless of the drying method employed.

Gaviria *et al.,*[28] determined 4804 mg GAE/100 g DW in the mature fruits and Kim *et al.,*[29] determined 3120 mg GAE/100 g DW of Blueberry. Similar values to those obtained in this research.

Zapata *et al.,*[30] found a total anthocyanin value of 879 mg CYE / 100 g DW and Kim et al,[29] determined 1190 mg CYE / 100 g in DW of Blueberry, which are higher than the values found in this research, this may be due to the method used for the analysis.

Table 4: Polyphenol, anthocyanin, ascorbic acid and antioxidant activity for each treatment of the dehydrated mortiño samples

Treatment	Polyphenols (mg GAE/ 100 g DW)	Antocyanins (mg CYE/ 100 g DW)	Ascorbic acid (mg/100 g DW)	Antioxidant Activity (mmol TE/kg DW)
T1	2893.83 ± 2.16d	573.40 ± 2.16d	49.47 ± 3.49b	73.33 ± 1.26c
T2	3409.30 ± 5.39b	649.54 ± 3.48b	34.67 ± 3.15d	87.28 ± 2.59a
T3	3026.49 ± 1.04c	612.92 ± 2.04c	69.50 ± 3.98a	80.14 ± 1.99b
T4	4733.50 ± 1.99a	778.70 ± 2.88a	39.69 ± 2.15c	88.62 ± 2.07a

GAE= Gallic acid; CYE= Cyanidin 3-glucoside chloride; TE=Trolox equivalent; DW= Dry Weight.

As for ascorbic acid, the highest value corresponded to the T3 treatment. Similar to that reported by Zia *et al.,*[31] who report values of 40.29 ± 0.012 mg/100 g for fresh and ripe blueberries. This treatment had a higher concentration of organic acids, regardless of the drying method.

As for antioxidant activity, the results show that the fruits in DR2 are statistically equal, independent of the drying process. The fruits in DR1 had exhibited lower antioxidant activity. The values obtained are within the range reported by Lin *et al.,*[24] between 21.77 to 101.07 mmol TE/ kg DW. Their study found a relation between antioxidant activity and anthocyanin content, reporting an increase of antioxidant activity with higher levels of anthocyanins. Similarly, this study found an increase in antioxidant activity with higher levels of anthocyanins and polyphenols.

The treatments with lyophilization had higher values for polyphenols, anthocyanins, ascorbic acid, and antioxidant activity, in comparison to the treatments with convection drying. However, the freeze-drying process takes longer and is more expensive, increasing the value of the final product. Ramirez *et al.,*[17], reached a similar conclusion when comparing both drying methods for tarragon.

The results of the sensory evaluation were high acceptance ("Like very much") for the attributes of color, aroma, and taste in the treatments with fruit in DR2, for both drying methods (TR2 and TR4). The results of the sensory evaluation are shown in Figure 2. Saftner *et al.,*[32] conducted sensory evaluations of different blueberry varieties and found variation in the sensory intensity and acceptability scores.

The results of the quantification of the polyphenols, anthocyanins, ascorbic acid, and antioxidant activity in the infusions with the highest acceptance from the sensory evaluation (T2 and T4) are shown in Table 5.

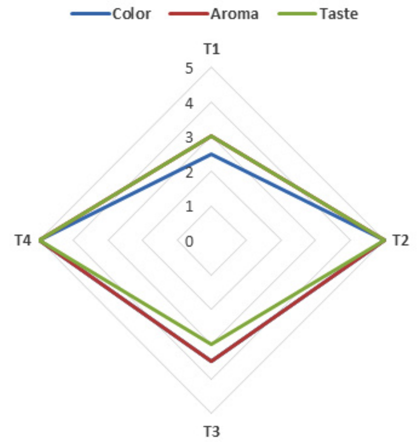

Fig. 2: Results from the sensory evaluation

Table 5: Polyphenol, anthocyanin, ascorbic acid and antioxidant activity in 200 mL of infusions prepared from T2 and T4

Treatment	Polyphenols (mg GAE/ 100 g DW)	Antocyanins (mg CYE/ 100 g DW)	Ascorbic acid (mg/100 g DW)	Antioxidant Activity (mmol TE/kg DW)
T2	128.06 ± 2.42b	16.41 ± 0.73b	0.86 ± 0.26a	2.19 ± 0.05a
T4	136.40± 3.00a	20.13 ± 0.08a	1.00 ± 0.06a	2.25 ± 0.03a

GAE= Gallic acid; CYE= Cyanidin 3-glucoside chloride; TE=Trolox equivalent; DW= Dry Weight

The antioxidants in the infusions prepared with fruit in DR2 were higher in the lyophilization treatment (T4) by 6.11 and 18.48 % for the polyphenols and anthocyanins, respectively. The ascorbic acid and antioxidant activity are statistically similar for both drying methods. According to the results, between 2.5 and 3.5 % of the original antioxidants content is passed on to the infusion. Da Silva *et al.,*[15] prepared an infusion with the peel of the jaboticaba fruit and found a lower phenolic content than this research, while Valerga *et al.,*[33] found similar values for yerba mate. Additionally, important sources of bioactive compounds such as anthocyanins and ascorbic acid were identified.

According to Maya-Cano *et al.,*[34] *mortiño, Vaccinium spp*, has been shown in vitro and in vivo to contain bioactive compounds. These compounds have protective effects for skin cells by inhibiting proliferation and interrupting the cellular cycle of cancerous cells, diminishing macromolecular oxidation, regulating inflammation and mitigating oxidative stress. The inclusion of these bioactive compounds in daily diet is a chemopreventative option against skin cancer.

Conclusion

The mortiño, like other Vaccinium, is characterized by its high levels of antioxidants. This fruit grows in the wilderness of the Ecuadorian moorlands but has yet to be used for agro-industrial ends. This research determined the impact of the degree of ripeness of the fruit and drying method on the total content of polyphenols, anthocyanins, ascorbic acid, and antioxidant activity. The results show the fruits at 100% maturity have the highest levels of polyphenols, anthocyanin, and antioxidant activity, while the fruits at 50% have the highest level of ascorbic acid. As for the drying methods, lyophilization proved to preserve the largest amounts of polyphenols and anthocyanins. Convection drying yields slightly lower values for these two compounds but preserves ascorbic acid just as well. There was minimal variation for antioxidant activity between the two drying methods. Infusions prepared with mortiño in DR2, regardless of the dehydration process, had between 2.5 to 3.5 % of the original antioxidant content. The sensory attributes of the infusions were qualified as very pleasant by the panel of tasters.

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Conflict of Interest

All authors declare no conflict of interest.

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