Study of extractive processes of bioactive components of mortiño (*Vaccinium floribundum* K.) by conglomerative analysis

Estudio de los procesos extractivos de los componentes bioactivos del mortiño (*Vaccinium floribundum* K.) mediante un análisis de conglomerado

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ABSTRACT

Mortiño (*Vaccinium floribundum* K.) is a fruit native to the Andean region of South America, a berry rich in polyphenolic compounds and with a high antioxidant capacity beneficial to health, therefore the extraction of functional compounds from this fruit is a good option for the development of functional foods. The aim of this work was to study and classify the different conditions for extracting bioactive compounds from mortiño using statistical cluster analysis. For this purpose, combinations of the following conditions were used: fruit condition (entire-E and ground-G), extraction process (agitation-A and maceration-M) and extraction medium (acidified water-W and acidified methanol-Me), and in each of these conditions the content of total anthocyanins (TAC), total polyphenols (TPC) and antioxidant capacity (ORAC and DPPH) were determined. The results of the cluster analysis showed the formation of three groups. Moreover, the samples of cluster 1 showed the highest values of AT 696.34 mg cyanidin-3-glucoside $100g^{-1}$ and PT 132.39 mg EAG $100g^{-1}$. In terms of ORAC antioxidant capacity, cluster 1 showed a higher antioxidant capacity (31422.40 µmol TE $100mL^{-1}$), while for DPPH the highest value was for cluster 2 (2625.14 µmol TE $100mL^{-1}$). It was concluded that the best conditions for the extraction of bioactive compounds from mortiño were crushing the fruit and using acidified methanol as the extraction medium.

RESUMEN

El mortiño (*Vaccinium floribundum* K.) es un fruto originario de la región andina de Sudamérica, es una baya rica en compuestos polifenólicos y con alta capacidad antioxidante que es beneficiosa para la salud, por lo tanto; la extracción de compuestos funcionales a partir de este fruto es una buena opción para el desarrollo de alimentos funcionales. El objetivo de este trabajo fue estudiar y clasificar las diferentes condiciones de extracción de compuestos bioactivos del mortiño mediante un análisis estadístico de conglomerados. Para ello se utilizaron combinaciones de las siguientes condiciones: estado del fruto (entero-E y molido-G), proceso de extracción (agitación-A y maceración-M) y medio de extracción (agua acidificada-W y metanol acidificado-Me), y en cada una de estas condiciones se determinó el contenido de antocianinas totales (TAC), polifenoles totales (TPC) y capacidad antioxidante (ORAC y DPPH). Los resultados del análisis de conglomerados mostraron la formación de tres grupos. Además, las muestras del grupo 1 mostraron los valores más altos de AT 696,34 mg de cianidina-3-glucósido/100g y PT 132,39 mg de EAG/100g. En términos de capacidad antioxidante ORAC, el clúster 1 mostró una mayor capacidad antioxidante (31422,40 µmol TE/100mL), mientras que para DPPH el valor más alto fue para el clúster 2 (2625,14 µmol TE/100mL). Se concluyó que las mejores condiciones para la extracción de compuestos bioactivos del mortiño fueron la trituración del fruto y el uso de metanol acidificado como medio de extracción.

Falta Palabras clave: compuestos bioactivos, capacidad antioxidante, mortiño, bayas, análisis de conglomerado.

INTRODUCTION

Natural antioxidants are bioactive compounds that play a crucial role in protecting cells from oxidative stress, a condition associated with various chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer (Muscolo *et al*., 2024). Oxidative stress results from an imbalance between reactive oxygen species (ROS) and the capacity of body to neutralise them through endogenous and exogenous antioxidant mechanisms mechanisms (Demirci-Çekiç *et al*., 2022). Among the richest natural sources of an-

tioxidants are fruits, which contain diverse compounds such as polyphenols, flavonoids, and anthocyanins that contribute significantly to their health-promoting properties (Shahidi & Ambigaipalan, 2015).

Mortiño (*Vaccinium floribundum* K.), commonly known as the Andean blueberry, is a wild berry native to the high-altitude regions of the Andes, particularly in Ecuador (Caranqui-Aldaz *et al*., 2022). This fruit has attracted considerable scientific interest due to its high content of bioactive compounds, especially anthocyanins, which are responsible for its deep blue colour and contribute to its strong antioxidant activity (Vasco *et al*., 2009). The increasing focus on natural antioxidants, driven by their potential health benefits and applicability in the food and pharmaceutical industries, has positioned mortiño as a promising candidate for further research and development (Llivisaca-Contreras *et al*., 2022). The antioxidant capacity of mortiño is primarily attributed to its abundant polyphenolic compounds, including anthocyanins, flavonols, and hydroxycinnamic acids (Vasco *et al*., 2009). These compounds are known for their ability to scavenge free radicals, thereby mitigating oxidative stress and reducing the risk of chronic conditions such as cardiovascular diseases and certain types of cancer (Topal *et al*., 2017). Studies have demonstrated that mortiño exhibits a substantial antioxidant potential, often comparable to or exceeding that of other well-known antioxidant-rich fruits. For example, its antioxidant capacity has been reported to be like that of blackberries and superior to that of guava, ranking it among the most antioxidant-rich fruits in Ecuador (Caranqui-Aldaz *et al*., 2022).

Efficient extraction of these antioxidant compounds from mortiño is essential for their potential application in nutraceuticals, functional foods, and pharmaceuticals (López Hernández *et al*., 2022). Various extraction methodologies have been studied to optimise the yield and preserve the bioactivity of these compounds. One study assessed on the extraction of anthocyanins from mortiño using different solvents (ethanol and methanol) at varying concentrations (20% and 60%) and temperatures (30 °C and 60 °C). The findings revealed that a 20% ethanol solution at 60 °C was most effective, yielding an antioxidant capacity of 19,653.3 ± 256.62 µmol TE per 100 g of fresh weight, as determined by the Ferric Reducing Antioxidant Power (FRAP) assay. Similarly, the ABTS assay revealed high antioxidant capacities for both methanol and ethanol (20%) at 60 °C. These findings underscore the importance of selecting appropriate extraction conditions to maximize the maximise the recovery of bioactive compounds from mortiño (Pérez *et al*., 2021).

Beyond the extraction techniques, the analysis and interpretation of data related to antioxidant capacities require robust statistical tools. Cluster analysis, a multivariate statistical method, has proven useful for classifying and interpreting complex datasets by grouping samples with similar attributes (Jaeger & Banks, 2023). In antioxidant research, cluster analysis enables the identification of patterns and relationships among different samples or treatments. For instance, Yun *et al*., (2016), employed factor analysis and k-means clustering to classify vinegars based on total phenolic content, flavonoid levels, and antioxidant activity. This method allowed the identification of distinct clusters linked to specific antioxidant profiles, offering deeper insights into the underlying factors influencing antioxidant potential.

Similarly, Shahinuzzaman *et al*., (2020) applied hierarchical cluster analysis to classify *Ficus carica* cultivars based on their phenolic content and antioxidant activities. This approach effectively grouped cultivars with comparable antioxidant characteristics, highlighting its value in distinguishing between varying levels of bioactive compounds. Applying similar statistical techniques to mortiño could help elucidate differences in antioxidant content and activity, thereby guiding breeding strategies, cultivation practices, and processing techniques aimed at enhancing its functional properties.

In summary, mortiño (*Vaccinium floribundum* Kunth) stands out as a fruit rich in potent antioxidant compounds, particularly anthocyanins, which offer notable health benefits and industrial potential. Optimising extraction conditions is vital to maximise the yield and effectiveness of these bioactive compounds. Furthermore, the use of cluster analysis provides a powerful tool for interpreting antioxidant-related data and informing decision-making in food, health, and agricultural contexts. Thus, the objective of this study is to determine the optimal extraction conditions for bioactive compounds from mortiño using cluster analysis. By evaluating different extraction parameters, this research aims to enhance the efficiency of obtaining antioxidant-rich extracts suitable for applications in functional foods, nutraceuticals, and pharmaceuticals. The findings will contribute to the growing body of knowledge surrounding natural antioxidants and their relevance in health-oriented industries.

MATERIALS AND METHODS

Raw materials and reagents

Mortiño (*Vaccinium floribundum* K.), were from Machachi, Pichincha Province. Methanol (MeOH), hydrochloric acid (HCl), Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), galic ácid, potassium chloride (KCl), acetic acid (CH_3COOH), cyanidin-3-glucoside (C3GC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic

acid), 2,2′-azobis(2-amidinopropane) (AAPH), and fluorescein were purchased from Merck (Merck, Darmstadt, Germany).

Conditions of the process of obtaining fruit extracts

The process of obtaining fruit extracts was carried out under two conditions: agitating and maceration and each of these conditions was evaluated according to: (i) fruit condition: entire-E or ground-G; and (ii) medium used for extraction: acidified water-W and acidified methanol-Me. The extraction of berry components was carried out by using entire o mixing ground fruit with the corresponding acidified medium (0.1% HCl v/v) at a 3:5 (w/w) volumetric ratio of fruit. The mixture underwent maceration or shaking for 60 minutes at 3000 rpm (for agitated condition). It was then centrifuged at 5000 rpm for 5 minutes, allowing the separation of the upper layer, referred to as the 'supernatant. This supernatant is the fruit extract and was subsequently stored at 4 °C until analysis.

Characterization of the extract

After obtaining each of the extracts by both agitation and maceration, analysis was performed to quantify the contents of total anthocyanin, total polyphenol and antioxidant capacity (DPPH and ORAC). The methods are described below:

Determination of total polyphenol content (TPC)

To determine the total phenolic content (TPC) of berry extracts, the Folin-Ciocalteu method was employed with slight modifications. The procedure involved adding the following components to a test tube: 40 µL of the sample, 3.16 mL of distilled water, 200 µL of Folin-Ciocalteu reagent, and 600 µL of Na_2CO_3 (20%). The mixture was then shaken and incubated in darkness at ambient temperature (20 °C) for 120 minutes. Absorbance was subsequently measured at 765 nm using a Spectronic® 20 Genesys™131 spectrophotometer (Illinois, USA). A calibration curve was generated using gallic acid solutions ranging from 0 to 1000 µL g^{-1}, and the results were expressed as gallic acid equivalents (mg GAE 100 mL^{-1}). Each measurement was conducted in triplicate.

Determination of total anthocyanin content (TAC)

The total anthocyanin content (TAC) was determined using the pH differential method, following the procedure described by Yang *et al*. (2019). Two buffer solutions were prepared: KCl (0.025 M, pH 1.0) and CH_3COOH (0.4 M, pH 4.5). The sample was diluted in each buffer solution at a 1:9 ratio (sample:buffer), vortexed, and allowed to stand in the dark for 5 minutes. Absorbance was measured at 510 and 700 nm using a Spectronic® 20 Genesys™131 spectrophotometer (Illinois, USA), applying the molar extinction coefficient of cyanidin-3-glucoside. The total absorbance of each sample was calculated using Equation 1, and the results were expressed as cyanidin-3-glucoside equivalents (mg C3GC 100 mL^{-1}). All measurements were performed in triplicate.

$$A = \begin{bmatrix} (A_{510\,nm} - A_{700\,nm})_{pH=1} \\ -\,(A_{510\,nm} - A_{700\,nm})_{pH=4.5} \end{bmatrix} \quad \textbf{Equation 1}$$

Antioxidant capacity by DPPH assay

The antioxidant capacity was assessed using the DPPH spectrophotometric method, following the procedure described by Yang *et al*. (2019), with slight modifications. Extracts were diluted to various concentrations, and 1 mL of the sample extract was mixed with 2 mL of a 0.1 mM DPPH solution in methanol. The mixture was vortexed for 30 seconds and then incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 515 nm using a Spectronic® 20 Genesys™ spectrophotometer (Illinois, USA), with pure methanol serving as the blank. Calibration curves were generated for each assay using Trolox, and the results were expressed as micromoles of Trolox equivalents (TE) per 100 grams of sample.

Antioxidant capacity by ORAC assay

The ORAC assay was conducted following the procedure outlined by Normah & Hanapi (2019). A fluorescein stock solution (100 µM) was prepared in phosphate buffer (75 mM, pH 7.4) and stored at 4 °C in the dark. A fresh working fluorescein solution (100 nM) was prepared daily by diluting the stock solution in phosphate buffer. For the assay, 200 µL of the working fluorescein solution was added to each sample (40 µL) and to Trolox standards (6, 12, 18, and 24 µM), all prepared in phosphate buffer, using a black 96-well plate. The plate was then incubated at 37 °C for 30 minutes. The reaction was initiated by adding a peroxyl radical generator in phosphate buffer. Specifically, 50 µL of AAPH (108 mM) was added, and fluorescence was recorded every minute (Ex = 485 nm, Em = 535 nm) using a double-scan microplate spectrofluorometer (MAX Gemini Plate Reader, Thermo Fisher Scientific Inc., Waltham, USA) maintained at 37 °C. Measurements continued until fluorescence decreased to less than 5% of the initial value. The ORAC results were calculated using a regression equation correlating Trolox concentrations with the net area under the fluorescein

kinetic decay curve. Final values were expressed as micromoles of Trolox equivalents per 100 grams of sample (µmol TE/100 g).

Statical analysis

An analysis of variance (ANOVA) was performed. Homogeneous groups were analysed using Tukey's test at 95% confidence level. A cluster analysis was performed to identify, within a set of data, those subsets characterised by certain similarities between the different samples. The variables used were total anthocyanin content, total polyphenol content and antioxidant capacity (ORAC and DPPH). The method used was Ward's method with 3 clusters. All experiments were performed in triplicate. Statistical analyses were performed using the Statgraphics Centurion XV.II® software package.

RESULTS AND DISCUSSION

Characterization of fruit extract

Figure 1 presents the characterisation of the antioxidant capacity and the presence of bioactive compounds in mortiño extracts obtained under various extraction conditions. To improve the clarify of the results, both macerated and agitated fruits were categorised into two groups, each including two specific conditions fruit from (entire fruit-E and ground fruit-G) and extraction medium (water-W and methanol-Me).

In general, significant differences ($p<0.05$) can be observed between macerated and agitated fruits samples. The antioxidant capacity was assessed using two commonly applied methods: DPPH and ORAC, shown in Figure 1A and 1B respectively. These assays are widely used for evaluating the antioxidant potential of bioactive compounds, though they differ in their mechanisms, sensitivity, and physiological relevance. The ORAC method is considered more biologically representative, as it measures antioxidant activity against peroxyl radicals—prevalent in biological systems—and allows the assessment of both hydrophilic and lipophilic antioxidants (Carvalho et al., 2023). In contrast, DPPH is simpler, faster, and more cost-effective but less reflective of in vivo antioxidant mechanisms, as it only measures electron-donating capacity (Gulcin & Alwasel, 2023). Despite their methodological differences, both assays exhibited similar trends across the tested extraction conditions.

Among all samples, the E/W (whole fruit extracted with water) condition exhibited significantly lower antioxidant capacity ($p<0.05$) than the others. This

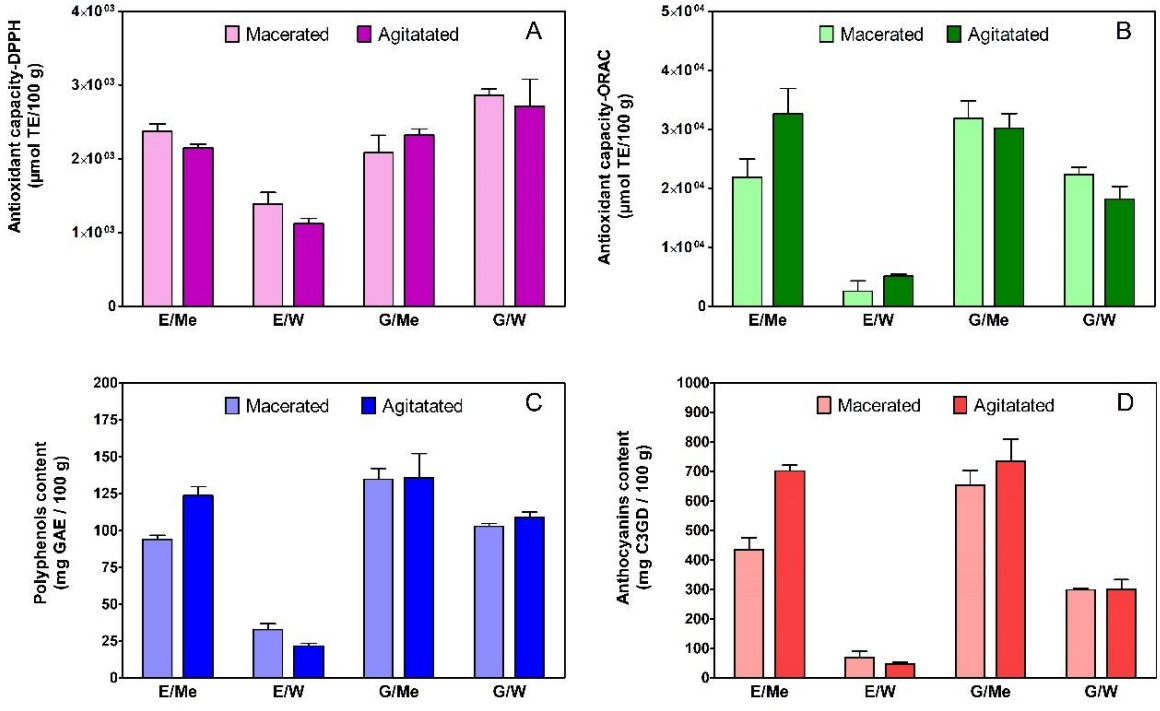

Figure 1. Antioxidant activity measured by DPPH method (A) and ORAC method (B), total polyphenol content (C) and total anthocyanin content (D) of different extraction states.

Figura 1. Actividad antioxidante medida a través de los métodos DPPH (A) y ORAC (B), contenido total de polifenoles (C) y contenido total de antocianinas (D) de los diferentes estados de extracción.

suggests that whole fruit, whether macerated or agitated in water, is suboptimal for extracting bioactive compounds such as polyphenols and anthocyanins. Figures 1C and 1D further support this, showing a lower concentration of total polyphenols and anthocyanins in the E/W samples. These findings imply that efficient extraction of such compounds requires the disruption of the food matrix to facilitate the release of intracellular components into the solvent. It is well-established that these antioxidants are concentrated in the skin of red fruits such as berries; thus, mechanical disruption (e.g., grinding) enhances release, whereas using the whole fruit limits extraction efficiency. Furthermore, the extraction medium plays a critical role. As shown in Figure 1, the E/Me (whole fruit in methanol) sample exhibited a higher antioxidant capacity than its aqueous counterpart (E/W). This can be attributed to the superior solvent properties of methanol compared to water, even when acidified. It is important to note that the stability of polyphenols, particularly anthocyanins, is influenced by the pH of the extraction medium, with these compounds being more stable under acidic conditions (Muñoz-Fariña et al., 2023). Consequently, the extraction media used in this study were acidified to pH 3.0.

Acidified methanol is recognised as being more effective than acidified water for extracting phenolic compounds due to several key factors. Methanol, an organic solvent with intermediate polarity, offers greater solubility for both polar and some less polar compounds, thereby enhancing overall extraction efficiency (Tena & Asuero, 2022). Additionally, methanol interacts less strongly with proteins and polysaccharides, whereas water tends to form strong hydrogen bonds with such macromolecules in plant tissues, which can impede the release of polyphenols (Alara et al., 2021). Methanol also penetrates plant cell walls and membranes more effectively, facilitating the release of phenolic compounds (Brglez Mojzer et al., 2016). While anthocyanins exhibit greater stability in acidic aqueous environments, the presence of methanol reduces enzymatic and oxidative degradation, producing more concentrated and stable extracts (Muñoz-Fariña et al., 2023). For these reasons, acidified methanol is widely used in protocols for the extraction of polyphenols and anthocyanins, although it is often combined with other solvents (e.g., ethanol-water or methanol-water) to optimise both yield and safety.

Supporting this, a study by Rahimi et al., (2024), extraction methods were compared in Vitis vinifera L. In this study, four methods for extraction of phenolic compounds from seeds and berry skins of the cultivar Carménère (Vitis vinifera L.) were evaluated. The extractions were performed by shaking at room temperature using different solvent mixtures, including 80:20% v/v methanol/water. The results showed that the successive use of methanol/water and acetone/water gave the highest concentrations of phenolic compounds compared to other solvents. In another study by (Quiroz-Reyes et al., 2013), a comparison was made between ultrasound and maceration techniques in cocoa (Theobroma cacao L.). This study compared the efficiency of ultrasound and maceration techniques for the extraction of phenols from cocoa beans. It was found that, by using ultrasonic radiation, it was possible to extract a higher content of polyphenols compared to traditional maceration. Although the study focused on the ultrasound technique, the efficacy of different solvents was also evaluated, highlighting the efficiency of acidified methanol in the extraction of phenolic compounds. Similar results were also reported by (Vázquez, 2019), who carried out an optimisation of the extraction of phenolic compounds in Syrah grape pomace: In this work, the use of organic solvents of protic and aprotic nature was studied for the extraction of phenolic compounds and their antioxidant activity from Syrah (Vitis vinifera) grape pomace. A mixture design was implemented to evaluate the interaction between the solvents and their influence on the extraction. The results showed that acidified methanol was one of the most effective solvents for the extraction of phenolic compounds by maceration techniques. Currently, other methods have been studied for the extraction of this type of compounds, such as ultrasonic, microwave and cold plasma assisted extraction (Pogorzelska-Nowicka et al., 2024), as well as the use of more benign solvents known as eutectic solvents (Aktaş & Kurek, 2024); however, this work is a first study on the extraction of bioactive compounds from mortiño.

Regarding the total polyphenol content (TPC) and total anthocyanin content (TAC), shown in Figures 1C and 1D, the results reflect the trends already discussed. Both TPC and TAC were highest in samples subjected to agitation in methanol, whether extracted from whole or ground fruit (E/Me and G/Me). These findings support the conclusion that a more effective release of bioactive compounds from a food matrix requires mechanical disruption of the fruit structure, enabling greater interaction between the solvent and the target compounds.

Cluster analysis

The results of the cluster analysis are shown below. The purpose of cluster analysis is to group observations or variables into clusters based on their similarity; thereby identifying patterns or structures within the data. In this study, observations for each variable were grouped into three clusters using Ward's method, which aims to minimise within-cluster variance as a criterion of statistical homogeneity. The clusters generated according to these criteria are shown in Table 1.

The study considered three main extraction parameters: the application of force (agitated or macerated), fruit condition (whole or ground), and extraction medium (acidified methanol or acidified water).

Table 1. Clusters formed and their description.

Tabla 1. Conglomerados formados y sus descripciones.

Cluster	Samples	Condition of extraction
1	AEMe	Agitated and entire fruit in methanol
	AGMe	Agitated and ground fruit in methanol
2	AEW	Agitated and entire fruit in water
	MEW	Macerated and entire fruit in water
3	AGW	Agitated and ground fruit in water
	MEMe	Macerated and entire fruit in methanol
	MGMe	Macerated and ground fruit in methanol
	MGW	Macerated and ground fruit in water

Figure 2A illustrates the clustering results based on total anthocyanin content and total polyphenol content. Regarding the distribution of the groups, Cluster 1 appears in the upper right quadrant of the plot, comprising samples with the highest concentrations of both anthocyanins (approximately 700-800 mg C3DG 100 g^{-1}) and polyphenols (approximately 120-150 mg GAE 100 g^{-1}). These samples are characterised by a high content of bioactive compounds. Cluster 2 is in the lower left quadrant, with markedly lower levels of anthocyanins (ca. 100-200 mg C3DG 100 g^{-1}) and polyphenols (ca. 20-50 mg GAE 100 g^{-1}), representing samples with minimal bioactive content. Cluster 3 occupies the central region of the graph located in the middle of the graph, with intermediate values of anthocyanins (300-600 mg C3DG 100 g^{-1}) and polyphenols (70-120 mg GAE 100 g^{-1}). This group shows a large variability within its data but is located between the extreme clusters. A positive trend is observed between anthocyanin and polyphenol levels: samples with higher polyphenol content tend to have more anthocyanins, as expected. Cluster 1 is associated with samples richer in bioactive compounds, while cluster 2 corresponds to samples with lower concentrations.

Regarding the antioxidant activity (ORAC) of each of the bioactive compounds (polyphenols and anthocyanins), the results of the cluster analysis can also be seen in Figure 2B and 2C respectively. Cluster 1 is in the highest range of polyphenols and ORAC, cluster 3 is between the two extremes, with intermediate values, and cluster 2 is grouped in the lower left part, with lower content of polyphenols and ORAC. In this sense, it can be said that there is a clear differentiation between the groups according to their polyphenol content and antioxidant capacity. Regarding anthocyanin content, the trend is similar and perfectly logical and consistent with the presence of these components in the extracts and their consequent antioxidant capacity. On the basis of this analysis, it can be said that cluster 1 represents the samples with the best results, which means that the best conditions for the extraction of bioactive compounds are the use of stirring, either with whole or ground fruit and acidified methanol as extraction medium, which is in line with the results previously discussed; on the other hand, the least favourable condition is the use of whole fruit and acidified water as extraction medium.

In this context, cluster analysis proves to be a valuable statistical tool for identifying patterns and classifying samples according to their chemical and functional properties. When applied to the extraction of bioactive compounds such as anthocyanins and polyphenols, it enables the classification of samples based on compound concentration and antioxidant capacity (ORAC), thereby helping to determine optimal extraction conditions. By identifying clusters with the highest levels of polyphenols and anthocyanins in conjunction with strong antioxidant activity, this method supports the selection of extraction protocols that maximise efficiency. Consequently, data-driven decisions can be made to improve process performance. In conclusion, cluster analysis serves as a key tool for the optimisation of bioactive compound extraction, enhancing process efficiency and ensuring the recovery of extracts with high antioxidant potential.

Conclusions

Mortiño is a berry rich in bioactive compounds such as polyphenols and anthocyanins, which confer notable antioxidant properties to the fruit. One way to harness the benefits of such fruits is through the preparation of extracts. In this regard, there are various methods for extracting bioactive compounds, and according to the protocols evaluated in this study, the most favourable conditions involved the use of ground fruit and acidified methanol as the extraction medium. Thus, it is important to emphasise that both the physical state of the fruit and the choice of extraction solvent are key parameters to consider when isolating these compounds. Methanol, due to its chemical characteristics, offers greater solubility in such food matrices, resulting in superior extraction efficiency compared to water. Moreover, grinding the fruit enhances the release of active compounds into the solvent by disrupting the cellular structure, which is not achieved when using whole fruit. However, a critical limitation of this study lies in the use of methanol, which—despite its high extraction efficiency—is not suitable for food applications due to its toxicity. Thus, future research should focus on eva-

luating alternative food-grade solvents, such as ethanol or eutectic mixtures, which can offer similar efficiency while complying with food safety standards. Additionally, the antioxidant activity was assessed only through in vitro methods (DPPH and ORAC), which, although widely used, do not necessarily reflect the bioavailability or physiological effects of the compounds in vivo. Further studies should include bioaccessibility assays

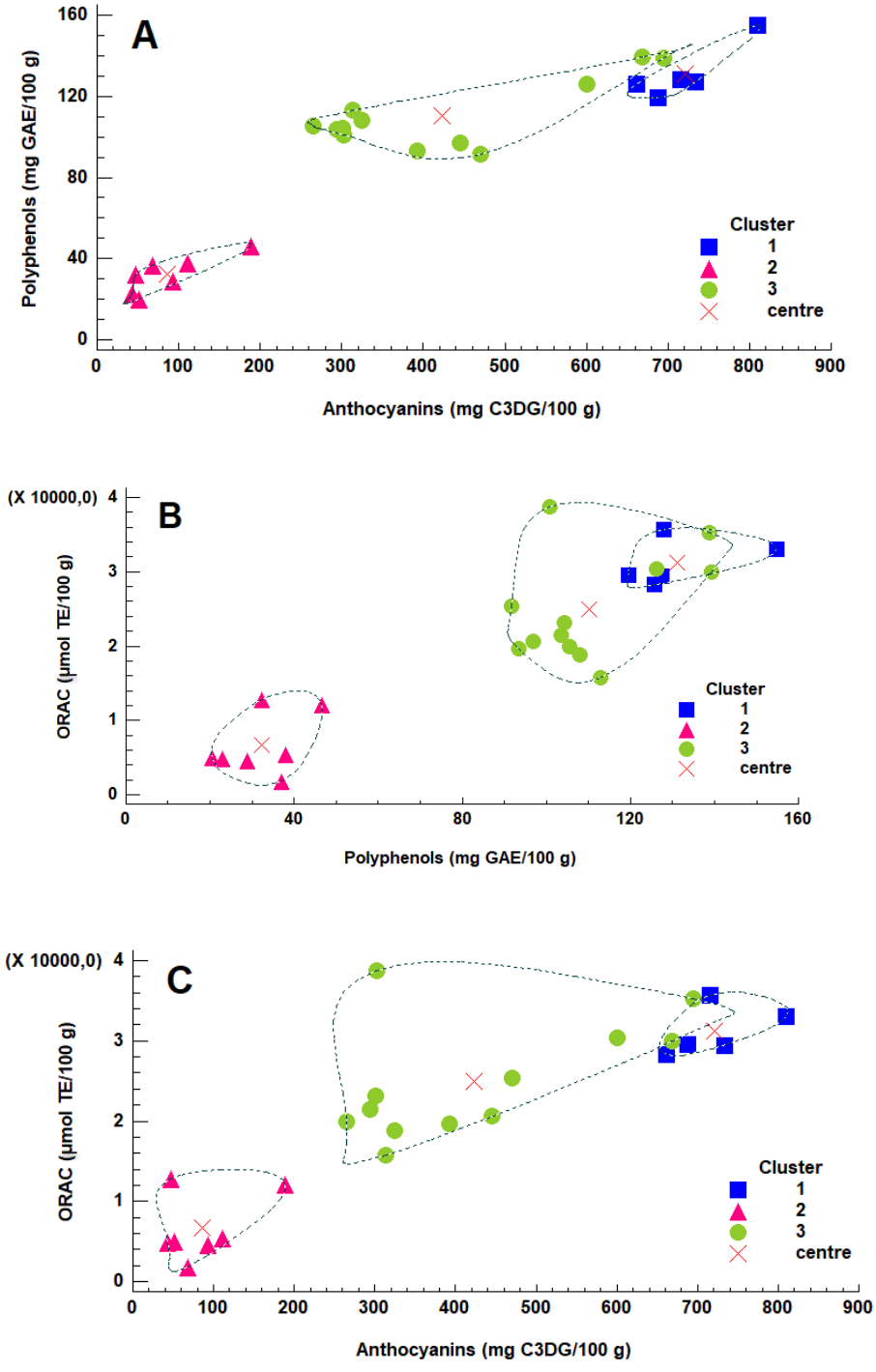

Figure 2. Cluster analysis for the different study variables: (A) Anthocyanin and polyphenol content, (B) Polyphenol content and antioxidant capacity-ORAC and (C) Anthocyanin content and antioxidant capacity-ORAC.

Figura 2. Análisis de conglomerados para las diferentes variables de estudio: (A) Contenido de antocianinas y polifenoles, (B) Contenido de polifenoles y capacidad antioxidante ORAC, (C) Contenido de antocianinas y capacidad antioxidante ORAC.

and model digestion systems to better estimate the potential health benefits of these extracts.

From a technological standpoint, the findings of this work may serve as a basis for developing value-added products such as nutraceutical supplements, natural food colourants, or functional ingredients for the formulation of beverages and dairy alternatives enriched with antioxidant compounds. Moreover, the application of statistical tools such as cluster analysis can be expanded to process optimisation in industrial extraction settings, allowing for data-driven decisions that maximise yield and minimise resource use. In summary, while this study identifies optimal conditions for extracting bioactive compounds from mortiño, further research is needed to translate these findings into safe, scalable, and consumer-acceptable applications within the food and nutraceutical industries.

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