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# Isotonic Drinks Based on Organic Grape Juice and Naturally Flavored with Herb and Spice Extracts

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**Abstract:** The aim of this study was the elaboration of isotonic drinks rich in bioactive compounds and antioxidant activity using organic ingredients and without synthetic additives. Grape juice was used as a natural source of sugars and phenolic compounds, combined with lemon juice and natural flavors from herb and spice extracts. The ingredients were diluted in two types of water with different mineralization, to which three different determined concentrations of salts (sodium chloride and potassium chloride) were added. The beverages had a sugar content ranging from 72.73 $\pm$ 0.23 to 78.43 $\pm$ 0.06 g/L, total soluble solids between 4.23 $\pm$ 0.06 and 4.83 $\pm$ 0.29 °Brix, and total acids from 1.75 $\pm$ 0.02 to 2.39 $\pm$ 0.08 g/L. Generally, antioxidant activity was higher in the beverages flavored with herb and spice extracts, ranging from 3.28 $\pm$ 0.01 to 4.27 $\pm$ 0.09 µmols Trolox equivalent/mL. Color intensity showed an increase of up to 129.39% in all samples during the storage period, being higher in beverages prepared with high-mineral water and having high pH values. The results of sensory analysis revealed that the flavored beverages had higher values of global perception than the controls. Thus, the functional properties of grape juice have been increased, and these beverages can be alternative natural and healthy products because their formulation is based only on organic and natural ingredients.

**Keywords:** isotonic drink; grape juice; polyphenols; anthocyanins; color; natural flavors

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## 1. Introduction

Adequate hydration is required before, during, and after high-intensity exercise to perform well in training sessions and competitions, control body temperature, and maintain general fitness. To achieve optimal hydration, the frequent intake of a combination of water, carbohydrates, and electrolytes in specific amounts is suggested, considering local temperature and substrate type [1]. Isotonic beverages are associated with physical activity and are intended to replace electrolytes, carbohydrates, and other nutrients lost through sweating during physical exercise [2]. In general, they contain water, carbohydrates in the form of monosaccharides or polysaccharides, electrolyte salts, juices, vitamins, colorants, and flavorings to improve organoleptic properties and have osmolality values close to blood osmotic pressure (270–330 mOsm per kg of water) to ensure rapid absorption and maintain hydration [3]. In sports drinks, sodium and potassium are important for replacing the electrolytes of athletes and helping them be absorbed more quickly during training without causing gastrointestinal problems [4]. In addition, the intake of carbohydrates is necessary as a source of energy in isotonic drinks [5] to offset the loss of carbohydrate stores and improve performance during exercises [6]. Furthermore, physical exercise has been shown to increase the production of free radicals and other reactive oxygen species. Thus, athletes need to improve their antioxidant defense systems to prevent oxidative damage from physical exercise [7]. Different researchers are interested in the antioxidant enrichment of these beverages by incorporating natural sources rich in phenolic compounds to enhance antioxidant activity during physical exercise [4,8–10].

Grape juice is a rich source of phenolic compounds, and its consumption is associated with many human health benefits, mainly antioxidant activity [11], which helps to prevent different diseases associated with oxidative stress, including cancers and cardiovascular and neurodegenerative diseases [12]. The identification of the phenolic composition of grape juices from different varieties and cultivars revealed that the phenolic content in grape juices includes flavonoids (flavanols, flavonols, flavanones, anthocyanins) and non-flavonoids (phenolic acids, stilbenes) [13–16]. The phenolic content in grape juices depends on the grape variety, grape maturity, geographical origin, soil type, and sunlight exposure [12]. A review by Granato et al. [17] analyzed the information reported in the literature concerning the differences between organic, biodynamic, and conventional grapes and mentioned that biodynamic and organic grape juices have very similar quality traits, and there is a trend of organic juices presenting higher contents of bioactive compounds compared to their conventional counterparts. However, in vivo studies using animals (Wistar rats) and clinical trials using healthy individuals have shown that the differences in functional properties, mainly antioxidant effects, between organic and conventional grape juices are negligible from the nutritional and biochemical perspectives.

For functional foods, it is important to pay attention to the favorable nutritional profile in addition to the content of bioactive compounds by limiting sugar content in fruit products and replacing added sugars with low-calorie sweeteners in low-sugar products to give them an acceptable quality [18]. On the other hand, the adoption of sustainable lifestyles by consumers increases their demand and preference for more natural, beneficial, innovative, and tastier products with nutraceutical and sustainable characteristics and minimum amounts of chemical preservatives and processing technologies [19]. With this trend, the search for organic products has increased [17]. Accordingly, our previous work [20] concerned the elaboration of healthy organic beverages by diluting concentrated red grape juice in mineral water to obtain products with low sugar content, avoiding chemical additives, and using herb and spice extracts to improve the sensory profile of the beverages.

Color and flavor are essential sensory characteristics that affect the appearance and acceptance of food products [21]. In addition, color is an indicator consumers use when anticipating the flavor and taste of food or beverages [22]. The most-used natural food pigments are anthocyanins, carotenoids, betalains, and chlorophylls [23]. However, the classification of natural colorants is based on their source, water or oil solubility, and chemical structure, with the latter being the most commonly used to classify natural colorants [24]. Anthocyanins are water-soluble polyphenolic pigments belonging to the flavonoid class and are used as food colorings due to their variable color (red-orange to blue-purple) [25]. In grapes, the color of anthocyanins ranges from brownish-red to purple, with a range of maximum absorbance from 518 nm (cyanidin) to 528 nm (malvidin) [26]. In addition to coloring properties, anthocyanins have important health benefits. In vivo and in vitro studies have demonstrated their antioxidant, anti-cardiovascular, anticancer, anti-allergenic, anti-inflammatory, anti-thrombotic, anti-ulcer, and anti-coagulant effects and their immunomodulatory, vasodilatory, and analgesic activities [27–29]. The application of herbs and spices, fresh and in the form of powders, extracts, and essential oils, in dairy products such as yoghurts, cheeses, butter, gee, and ice creams to improve the nutritional, medicinal, and organoleptic characteristics has been described [30].

The aim of this study was to develop sports beverages based on formulations of the healthy beverages prepared previously [20] by adding determined concentrations of salts to make them isotonic drinks containing a combination of natural sources of bioactive compounds, minerals, colors, and flavors from organic ingredients: grape juice, lemon juice, herbs, and spices (hops, tea, and mint). These beverages can be an alternative for athletes and consumers who are searching for drinks that are less artificial and more beneficial to their health. In addition, physicochemical and sensory analyses were conducted on the beverages to verify their quality and acceptance.

## 2. Materials and Methods

### 2.1. Raw Material

The concentrated grape juice used for the elaboration of isotonic drinks was provided by the company Vinos y Bodegas (Ciudad Real, Spain). This juice is an organic product with a sugar content of 65 °Brix, pH 3.5, and $SO_2$ < 40 ppm. Lemon juice was used in the formulation with the purpose of correcting the acidity of the isotonic drinks. We used a commercial pasteurized squeezed lemon juice from a Spanish supermarket (Hacendado, Mercadona, Spain) containing 40 mg/L of vitamin C and 10 mg/L of sodium (taken from the label of lemon juice bottles). Organic red tea, dried mint (Cafetearte, Madrid, Spain), and hops (Summit, Tienda de la Cerveza, Spain) were used for the extraction of natural flavors to improve the sensory profile of the isotonic drinks. We used the table salts sodium chloride (Sal Costa, Spain) and potassium chloride (Aranca, Spain), without any additives, from the same source (Mercadona, Spain). The ingredients were diluted using two types of mineral water with different mineralization: low-mineral water Bezoya (Calidad Pascual, Spain) with 28 mg/L of dry residues and high-mineral water (Solan de Cabras, Spain) with 278 mg/L of dry residues. All ingredients used for the elaboration of the isotonic drinks were food-grade.

### 2.2. Isotonic Drink Design

The basic composition of the prepared isotonic drinks was the same as that used in the preparation of healthy beverages in our previous experiment [20] except for the addition of a measured amount of salts to these formulations to obtain beverages characterized as isotonic drinks for athletes' consumption. Two groups of isotonic beverages were prepared by diluting concentrated red grape juice (47 mL) with mineral water (Group A with Bezoya and Group B with Solan de Cabras), using a bottle of 500 mL for each sample. Table 1 describes the label and the composition of the 14 formulations. The same amount of lemon juice (7 mL) was added to all samples for pH correction and flavoring. The isotonic drinks were flavored with extracts of hops–tea (hops = 2 mL; tea = 1 mL) or hops–mint (hops = 2 mL; mint = 1 mL). For each flavored beverage, three different concentrations of salt (sodium chloride/potassium chloride) were used: Concentration 1: 0.5 g/L Na; Concentration 2: 0.5 g/L Na + 0.2 g/L K; Concentration 3: 0.3 g/L Na + 0.2 g/L K. All samples were thermally treated with any autoclave at 100 °C for five minutes. Later, they were kept under refrigeration at 4 °C until being subjected to physicochemical and sensory analysis. All samples were prepared and analyzed in triplicate.

**Table 1.** Drink formulation: composition and nomenclature.

| Formulation | Group | Composition |
|---|---|---|
| BAN<br>BBN | A<br>B | Water + Grape juice + Lemon juice (Control) |
| BAHTC1<br>BBHTC1 | A<br>B | Water + Grape juice + Lemon juice + Hops + Tea + Concentration 1 of salt |
| BAHTC2<br>BBHTC2 | A<br>B | Water + Grape juice + Lemon juice + Hops + Tea + Concentration 2 of salt |
| BAHTC3<br>BBHTC3 | A<br>B | Water + Grape juice + Lemon juice + Hops + Tea + Concentration 3 of salt |
| BAHMC1<br>BBHMC1 | A<br>B | Water + Grape juice + Lemon juice + Hops + Mint + Concentration 1 of salt |
| BAHMC2<br>BAHMC2 | A<br>B | Water + Grape juice + Lemon juice + Hops + Mint + Concentration 2 of salt |
| BAHMC3<br>BAHMC3 | A<br>B | Water + Grape juice + Lemon juice + Hops + Mint + Concentration 3 of salt |

Note: The composition of both types of water (taken from the label of the water bottles) and the method of extraction of flavors were described in our previous work [20].

### 2.3. Color Parameters and Total Phenolic Index

During the storage period of four months, red color (RC), color intensity (CI), and tonality (T) were evaluated spectrophotometrically according to the method of Burin et al. [31] using an Agilent 8453 spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) and a 1 mm optical path glass cuvette according to the following formula:

$$CI = A_{420} + A_{520} + A_{620};\ T = A_{420}/A_{520};\ RC = A_{52} \tag{1}$$

The total phenolic index (TPI) of the prepared isotonic drinks was analyzed with the same instruments at 280 nm [32].

### 2.4. pH Measurement

The pH evolution was determined and studied in both groups of beverages during the storage period with a Crison brand pH meter, GLP 21 model (Hach Lange Spain, S.L.U., Madrid, Spain).

### 2.5. Nutritional Composition

Sugar concentration, total acid, malic acid, alpha amino acids, and ammonia were identified with OenoFoss™ equipment (Foss Iberia SA, Barcelona, Spain) using Fourier transform infrared spectroscopy.

### 2.6. Total Soluble Solids

Total soluble solids were measured with an HI 96812 model refractometer (Hanna Instruments, RI, USA).

### 2.7. Determination of Anthocyanins

The identification of anthocyanins was carried out at the beginning and end of the storage period. All samples were filtered through a 0.45 µm membrane, and a volume of 50 µL was injected into a series 1200 high-performance liquid chromatography (HPLC) set (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector using a gradient of solvents: deionized water (Milli-Q)/formic acid (Panreac, Barcelona, Spain), 95:5 $v/v$ (solvent A) and methanol, 99.9% purity (Panreac, Barcelona, Spain)/formic acid, 95:5 $v/v$ (solvent B) in a reverse-phase Poroshell 120 C18 column (Phenomenex, Torrance, CA, USA) (50 × 4.6 mm; particle size 2.7 µm). Concentrations were calculated with a calibration curve of malvidin-3-O-glucoside ($r^2$ = 0.9999, LOD = 0.1 mg/L).

### 2.8. Determination of Antioxidant Capacity

The antioxidant activity of the prepared isotonic drinks was determined by the $ABTS^+$ method according to the procedure described by Re et al. [33], using diammonium salt (ABTS) [2,2′-azino-bi(3-ethylbenzothiazoline-6-sulfonic acid)] (Thermo Fisher, Kandel, Germany), potassium persulfate (Sigma Aldrich, St. Louis, MO, USA), and Trolox (Fisher Scientific, Waltham, MA, USA). First, the $ABTS^+$ radical was produced by mixing ABTS stock solution (7 mM) with 2.45 mM of potassium persulfate in darkness at room temperature for 16 h before use. Subsequently, the absorbance of the formed radical was adjusted to 0.70 (±0.02) at 734 nm by dilution with 95% ethanol. Finally, 30 µL of Trolox or diluted samples was added to 3 mL of $ABTS^+$ radical. After incubation at room temperature in the absence of light for six minutes, the absorbance was measured at 734 nm, and the results were expressed as µmols Trolox equivalents (TE)/mL. Samples were analyzed at four different concentrations in triplicate. The $ABTS^+$ inhibition percentage was calculated using the formula $I = [(AB - AA)/AB] \times 100$, where I = $ABTS^+$ inhibition %, AB = absorbance of the blank, and AA = absorbance of the sample/Trolox.

### *2.9. Sensory Analysis*

The new isotonic drinks were subjected to sensory analysis carried out at the Chemistry and Food Technology Department of the School of Agricultural, Food and Biosystems Engineering (ETSIAAB) at the Universidad Politécnica de Madrid (Spain) following the reference procedure [34]. The test was conducted by eight trained panelists from both genders, aged between 22 and 60, including professionals from the companies involved in the project and personnel from said department. During the sensory evaluation, each panelist was provided with tasting glasses of the prepared beverages (25–30 mL/8 ± 2 °C) with another glass of water to clean the palate between samples. CI, tonality, turbidity, aromatic intensity, aromatic quality, herbaceous, floral, fruity, reduction, oxidation, body, bitterness, sweetness, acidity, and a final general overall note were the sensory attributes chosen to describe the new isotonic drinks on a scale of intensity from low to high (scored from 1 to 5). The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (UPM Ethics Committee) of the Universidad Politécnica de Madrid (AIDLAPPMLB-AMB-HUMANOS-20221026 on 14 November 2022).

### *2.10. Statistical Analysis*

Microsoft Excel 2016 was used to determine means (±standard deviations). Analysis of variance (ANOVA), a least significant difference (LSD) test, and principal component analysis (PCA) were calculated using Statgraphics Centurion 18 software V.18.1.06 (Graphics Software Systems). The LSD test was used to detect significant differences between the means. Significance was set at $p < 0.05$.

## 3. Results

### *3.1. Nutritional Composition*

Table 2 shows the chemical composition of the prepared beverages measured using the FOSS analyzer. Sugar concentrations ranged from 72.73 ± 0.23 to 78.43 ± 0.06 g/L, presenting a significant difference ($p < 0.05$). The total soluble solids in the samples were between 4.23 ± 0.06 in BAN and 4.83 ± 0.29 °Brix, with the highest value in BBHMC3. The values obtained are lower than the values observed (7.83 °Brix) by Porfírio et al. [10] in two formulations of isotonic drinks based on an extract of Myrciaria jabuticaba and close to those of some commercial isotonic drinks found by Gironés-Vilaplana et al. [9]. Regarding total acids, higher values were found in Group A, which ranged between 2.15 ± 0.20 and 2.39 ± 0.08 g of tartaric acid/L, while the content in Group B was between 1.75 ± 0.02 and 1.98 ± 0.07 g of tartaric acid/L. The main values of malic acid differed slightly among samples, being between 1.37 ± 0.15 and 1.63 ± 0.12 g/L. However, the malic acids in these beverages were from the grape juice, because analyses of these acids were performed in both diluted grape juice and lemon juice used for the elaboration to check the source of these acids, and it was observed that there were no malic acids in the lemon juice. The values of ammonia content were higher in Group B, around 20 mg/L, with no significant difference between samples of this group. However, Group A presented lower values ranging from 4.63 ± 0.32 to 7.90 ± 0.17 mg/L. The samples also presented an alpha amino acid content between 14.50 ± 0.69 and 31.97 ± 2.54 mg/L.

### *3.2. Physicochemical Characteristics*

The results of color analysis, phenolic content, and pH of the prepared beverages are reported in Table 3. The data indicate that both groups of isotonic drinks suffered significant changes between the beginning and the end of the storage period, with a significant difference between samples ($p < 0.05$).

**Table 2.** Chemical composition of beverages measured with FTIR parameters.

| | Sugar (g/L) | Total Soluble Solids (°Brix) | Total Acid (g/L) | Malic Acid (g/L) | Alpha Amino Acids (mg/L) | Ammonia (mg/L) |
|---|---|---|---|---|---|---|
| BAN | $73.90 \pm 1.39$ a | $4.23 \pm 0.06$ a | $2.24 \pm 0.12$ c | $1.43 \pm 0.06$ ab | $17.57 \pm 2.00$ b | $4.73 \pm 0.93$ a |
| BAHTC1 | $78.30 \pm 0.17$ d | $4.57 \pm 0.06$ abc | $2.30 \pm 0.07$ c | $1.50 \pm 0.10$ bc | $14.50 \pm 0.69$ a | $4.63 \pm 0.32$ a |
| BAHTC2 | $78.20 \pm 0.17$ cd | $4.67 \pm 0.31$ bc | $2.19 \pm 0.15$ bc | $1.50 \pm 0.10$ bc | $17.17 \pm 2.69$ b | $5.23 \pm 0.78$ a |
| BAHTC3 | $77.90 \pm 0.17$ cd | $4.47 \pm 0.35$ abc | $2.15 \pm 0.20$ bc | $1.53 \pm 0.12$ bc | $17.23 \pm 1.68$ b | $4.67 \pm 0.29$ a |
| BAHMC1 | $78.43 \pm 0.06$ d | $4.70 \pm 0.10$ bc | $2.39 \pm 0.08$ c | $1.50 \pm 0.17$ bc | $18.53 \pm 0.49$ b | $6.83 \pm 0.47$ b |
| BAHMC2 | $72.73 \pm 0.23$ a | $4.53 \pm 0.32$ abc | $2.24 \pm 0.06$ c | $1.50 \pm 0.10$ bc | $18.50 \pm 0.53$ b | $6.53 \pm 0.55$ b |
| BAHMC3 | $74.27 \pm 0.42$ a | $4.67 \pm 0.29$ bc | $2.21 \pm 0.11$ bc | $1.40 \pm 0.10$ ab | $17.00 \pm 1.92$ b | $7.90 \pm 0.17$ c |
| BBN | $77.60 \pm 0.95$ bcd | $4.47 \pm 0.06$ abc | $1.90 \pm 0.18$ a | $1.50 \pm 0.10$ bc | $31.97 \pm 2.54$ d | $20.67 \pm 0.29$ d |
| BBHTC1 | $76.47 \pm 0.40$ bc | $4.47 \pm 0.21$ abc | $1.98 \pm 0.07$ ab | $1.63 \pm 0.12$ c | $17.50 \pm 0.00$ b | $20.57 \pm 0.40$ d |
| BBHTC2 | $73.67 \pm 1.23$ a | $4.33 \pm 0.32$ ab | $1.76 \pm 0.19$ a | $1.37 \pm 0.15$ ab | $18.97 \pm 0.64$ b | $20.47 \pm 0.65$ d |
| BBHTC3 | $76.10 \pm 0.17$ b | $4.47 \pm 0.35$ abc | $1.90 \pm 0.20$ a | $1.50 \pm 0.12$ bc | $22.13 \pm 1.68$ c | $20.33 \pm 0.29$ d |
| BBHMC1 | $76.10 \pm 2.00$ b | $4.33 \pm 0.12$ ab | $1.82 \pm 0.19$ a | $1.43 \pm 0.15$ ab | $22.60 \pm 1.39$ c | $20.87 \pm 0.47$ d |
| BBHMC2 | $74.30 \pm 1.73$ a | $4.20 \pm 0.20$ a | $1.80 \pm 0.21$ a | $1.40 \pm 0.00$ ab | $22.23 \pm 0.58$ c | $20.83 \pm 0.58$ d |
| BBHMC3 | $73.43 \pm 1.07$ a | $4.83 \pm 0.29$ c | $1.75 \pm 0.02$ a | $1.30 \pm 0.10$ a | $18.23 \pm 0.29$ b | $20.10 \pm 0.36$ d |

Note: Values are means with standard deviations, $n = 3$. Values with the same letter in the same parameter are not significantly different ($p < 0.05$). Total acids expressed as g of tartaric acid per liter.

Regarding color parameters, all samples showed an increase in values during storage. At the beginning, samples showed CI values ranging from $0.44 \pm 0.02$ to $0.52 \pm 0.01$, without a significant effect of water mineralization on the color of either group. The CI and RC were higher in BAHMC2 (Group A), followed by BBHMC2 (Group B). However, at the end of the storage, a significant effect of water mineralization was observed. Samples prepared with high-mineral water (B) showed higher values of CI than samples prepared with low-mineral water (A) except for the control (BAN), which presented similar values to the samples of Group B. Comparison with our previous work [20] demonstrated that the color analysis of two groups of healthy beverages that had the same composition as the prepared isotonic drinks in the current work, except for the addition of salts to make them isotonic, showed that the beverages prepared with low-mineral water had the highest CI at the beginning and at the end of the storage period. Consequently, the addition of salts to the samples led to a change in color parameters, giving the samples with high-mineral water high CI values. Additionally, comparing the three different salt concentrations added to samples, the samples in Group B (BBHTC2 and BBHMC2), which had the highest salt concentration (C2 = 0.7 g/L), presented higher CI values at the end of storage. However, in Group A, CI values were higher for the control (without salts) and samples that had low salt concentration (C1/C2 = 0.5 g/L). With regard to the total polyphenol index, values were between 2.44 and 2.66 at the beginning and 2.44 and 2.54 at the end of storage. Most samples showed a slight decrease in values during storage, except for BAN, BBHTC2, BBHTC3, BBHMC2, and BBHMC3, in which TPI increased from 2.47, 2.44, 2.49, 2.51, and 2.45 to 2.50, 2.48, 2.54, 2.53, and 2.47, respectively. However, T values increased slightly during storage, changing from 0.66 to 0.72 at the beginning to 0.77 to 0.90 at the end of the storage period. Regarding the herb extracts used for flavoring, the comparison of the flavored isotonic drinks with the controls and the comparison of the hops–tea beverage values with hops–mint beverage values demonstrated that the extracts did not have a strong effect as there is not a large difference between the samples, and the largest and smallest values were not confined to a specific herb extract. Nevertheless, even if there were slight differences, they were sensorily perceived. On the other hand, the results of pH analysis (Table 3) demonstrated that although the same amount of lemon juice was added to all samples, they were divided into two groups depending on the type of water. pH values were lower in the samples prepared with low-mineral water, ranging from 3.24 to 3.30 at the beginning of storage and from 3.26 to 3.29 at the end. The pH values of the beverages prepared with high-mineral water increased slightly during storage, changing from 3.43 to 3.47 to 3.45 to 3.50. The mineralization of water also influenced the CI. Thus, samples that presented high pH values (3.45 to 3.50) in Group B and BAN, which presented a high value in Group A (3.29), showed higher CI. These results revealed that the CI of the prepared beverages was influenced by pH, minerals, and salt concentration. However, the pH values of the prepared isotonic drinks are in the same range of pH values evaluated in six commercial isotonic drinks, ranging from 2.58 to 3.75 [35]. Ferreira et al. [4] found a pH value of 3.66 in an elaborated isotonic drink based on whey permeate with carotenoid powder from pequi. Gironés-Vilaplana et al. [36] also evaluated the pH of different prepared isotonic drinks enriched with lemon and berries and found lower pH values that were between 2.35 and 2.88 at the beginning and between 2.46 and 2.97 at the end of the storage period.

**Table 3.** Color characterization, TPI, and pH of the prepared beverages at the beginning and end of the storage period.

| SAMPLES | | RC | CI | T | pH | TPI |
|---|---|---|---|---|---|---|
| BAN | Beginning | 0.24 ± 0.01 $^{b}$ | 0.46 ± 0.01 $^{b}$ | 0.70 ± 0.01 $^{bc}$ | 3.30 ± 0.01 $^{c}$ | 2.47 ± 0.01 $^{ab}$ |
| | End | 0.43 ± 0.01 $^{g}$ | 1.04 ± 0.01 $^{g}$ | 0.88 ± 0.01 $^{ef}$ | 3.29 ± 0.01 $^{c}$ | 2.50 ± 0.03 $^{bcde}$ |
| BAHTC1 | Beginning | 0.25 ± 0.01 $^{bc}$ | 0.49 ± 0.01 $^{def}$ | 0.71 ± 0.02 $^{bcd}$ | 3.29 ± 0.01 $^{c}$ | 2.55 ± 0.01 $^{cd}$ |
| | End | 0.26 ± 0.01 $^{a}$ | 0.56 ± 0.03 $^{a}$ | 0.81 ± 0.02 $^{ab}$ | 3.28 ± 0.01 $^{b}$ | 2.45 ± 0.03 $^{abc}$ |
| BAHTC2 | Beginning | 0.24 ± 0.01 $^{b}$ | 0.47 ± 0.01 $^{cde}$ | 0.72 ± 0.02 $^{d}$ | 3.27 ± 0.01 $^{b}$ | 2.55 ± 0.07 $^{cd}$ |
| | End | 0.29 ± 0.02 $^{ab}$ | 0.67 ± 0.06 $^{ab}$ | 0.89 ± 0.05 $^{ef}$ | 3.27 ± 0.01 $^{a}$ | 2.51 ± 0.02 $^{cde}$ |
| BAHTC3 | Beginning | 0.25 ± 0.01 $^{b}$ | 0.46 ± 0.02 $^{cd}$ | 0.69 ± 0.01 $^{b}$ | 3.27 ± 0.01 $^{b}$ | 2.54 ± 0.04 $^{bcd}$ |
| | End | 0.30 ± 0.01 $^{bc}$ | 0.68 ± 0.03 $^{b}$ | 0.82 ± 0.01 $^{bc}$ | 3.28 ± 0.01 $^{b}$ | 2.47 ± 0.05 $^{abcd}$ |
| BAHMC1 | Beginning | 0.26 ± 0.01 $^{cd}$ | 0.49 ± 0.01 $^{ef}$ | 0.69 ± 0.02 $^{b}$ | 3.25 ± 0.01 $^{a}$ | 2.63 ± 0.04 $^{e}$ |
| | End | 0.33 ± 0.02 $^{bcd}$ | 0.74 ± 0.07 $^{bcd}$ | 0.83 ± 0.02 $^{bcd}$ | 3.26 ± 0.01 $^{a}$ | 2.47 ± 0.04 $^{abcd}$ |
| BAHMC2 | Beginning | 0.28 ± 0.01 $^{e}$ | 0.52 ± 0.01 $^{g}$ | 0.67 ± 0.01 $^{a}$ | 3.25 ± 0.01 $^{a}$ | 2.51 ± 0.07 $^{cd}$ |
| | End | 0.34 ± 0.03 $^{cde}$ | 0.73 ± 0.06 $^{bc}$ | 0.77 ± 0.02 $^{a}$ | 3.26 ± 0.01 $^{a}$ | 2.44 ± 0.04 $^{a}$ |
| BAHMC3 | Beginning | 0.27 ± 0.01 $^{de}$ | 0.51 ± 0.02 $^{fg}$ | 0.69 ± 0.02 $^{b}$ | 3.24 ± 0.01 $^{a}$ | 2.57 ± 0.08 $^{de}$ |
| | End | 0.36 ± 0.02 $^{de}$ | 0.83 ± 0.06 $^{cde}$ | 0.81 ± 0.02 $^{b}$ | 3.26 ± 0.01 $^{a}$ | 2.48 ± 0.02 $^{abcd}$ |
| BBN | Beginning | 0.23 ± 0.01 $^{b}$ | 0.44 ± 0.02 $^{a}$ | 0.72 ± 0.01 $^{cd}$ | 3.47 ± 0.01 $^{g}$ | 2.47 ± 0.01 $^{ab}$ |
| | End | 0.30 ± 0.03 $^{abc}$ | 0.67 ± 0.07 $^{ab}$ | 0.86 ± 0.01 $^{def}$ | 3.49 ± 0.01 $^{f}$ | 2.45 ± 0.04 $^{ab}$ |
| BBHTC1 | Beginning | 0.24 ± 0.01 $^{b}$ | 0.47 ± 0.01 $^{cd}$ | 0.71 ± 0.01 $^{bcd}$ | 3.45 ± 0.01 $^{ef}$ | 2.54 ± 0.04 $^{bcd}$ |
| | End | 0.37 ± 0.03 $^{ef}$ | 0.88 ± 0.09 $^{ef}$ | 0.86 ± 0.02 $^{def}$ | 3.45 ± 0.01 $^{d}$ | 2.46 ± 0.02 $^{abc}$ |
| BBHTC2 | Beginning | 0.24 ± 0.01 $^{b}$ | 0.46 ± 0.01 $^{b}$ | 0.70 ± 0.01 $^{bcd}$ | 3.45 ± 0.01 $^{f}$ | 2.44 ± 0.03 $^{a}$ |
| | End | 0.43 ± 0.04 $^{g}$ | 1.06 ± 0.13 $^{g}$ | 0.87 ± 0.01 $^{ef}$ | 3.47 ± 0.01 $^{e}$ | 2.48 ± 0.01 $^{abcd}$ |
| BBHTC3 | Beginning | 0.24 ± 0.01 $^{b}$ | 0.46 ± 0.02 $^{ab}$ | 0.70 ± 0.02 $^{bc}$ | 3.47 ± 0.01 $^{g}$ | 2.49 ± 0.01 $^{abc}$ |
| | End | 0.36 ± 0.03 $^{de}$ | 0.86 ± 0.09 $^{ef}$ | 0.90 ± 0.02 $^{f}$ | 3.50 ± 0.01 $^{f}$ | 2.54 ± 0.05 $^{e}$ |
| BBHMC1 | Beginning | 0.25 ± 0.01 $^{b}$ | 0.47 ± 0.01 $^{cd}$ | 0.70 ± 0.01 $^{bc}$ | 3.45 ± 0.01 $^{f}$ | 2.54 ± 0.03 $^{bcd}$ |
| | End | 0.36 ± 0.02 $^{de}$ | 0.85 ± 0.04 $^{def}$ | 0.89 ± 0.01 $^{ef}$ | 3.50 ± 0.01 $^{f}$ | 2.48 ± 0.04 $^{abcde}$ |
| BBHMC2 | Beginning | 0.27 ± 0.02 $^{de}$ | 0.51 ± 0.03 $^{fg}$ | 0.66 ± 0.02 $^{a}$ | 3.43 ± 0.01 $^{d}$ | 2.51 ± 0.03 $^{abcd}$ |
| | End | 0.41 ± 0.01 $^{g}$ | 0.97 ± 0.02 $^{fg}$ | 0.86 ± 0.02 $^{cde}$ | 3.47 ± 0.01 $^{e}$ | 2.53 ± 0.04 $^{de}$ |
| BBHMC3 | Beginning | 0.27 ± 0.01 $^{de}$ | 0.51 ± 0.01 $^{fg}$ | 0.66 ± 0.01 $^{a}$ | 3.44 ± 0.01 $^{de}$ | 2.45 ± 0.01 $^{a}$ |
| | End | 0.41 ± 0.04 $^{fg}$ | 0.96 ± 0.12 $^{fg}$ | 0.86 ± 0.03 $^{cde}$ | 3.49 ± 0.01 $^{f}$ | 2.47 ± 0.03 $^{abcd}$ |

Note: Values are means ± SD ($n$ = 3). A different letter for the same parameter means significant differences ($p < 0.05$) between samples in the same period (B and E).

*3.3. Anthocyanin Content*

Anthocyanins were identified in the grape-juice-based isotonic drinks using HPLC at the beginning and at the end of the storage period to evaluate the change in the anthocyanin profile. The results revealed the presence of a wide range of anthocyanins, with a significant difference between samples ($p < 0.05$). Figures 1 and 2 show the anthocyanin content in the beverages classified into two groups: non-acylated and acylated anthocyanins. The non-acylated anthocyanins comprised D3G, C3G, Pt3G, Pn3G, and M3G. The acylated group comprised Pt3G Ac, M3G Ac, C3G Cum, Pt3G Cum, and M3G Cum. In general, all samples showed a higher amount of non-acylated anthocyanins than the acylated ones, with a predominance of malvidin pigments. The BBHMC3 sample had significantly higher concentrations than the rest of the samples, followed by BBHMC2, with contents of $119.46 \pm 0.99$ mg/L and $115.16 \pm 4.45$ mg/L of non-acylated anthocyanins and $17.73 \pm 0.17$ mg/L and $16.79 \pm 0.86$ mg/L of acylated anthocyanins, respectively. The total anthocyanin content tended to decrease in all the samples during storage, and a loss of some pigments was also observed, such as in the case of C3G Cum and Pt3G Cum. At the beginning, non-acylated anthocyanins ranged from $52.95 \pm 5.45$ to $119.46 \pm 0.99$ mg/L, which decreased to $2.86 \pm 0.22$ to $5.37 \pm 0.03$ mg/L at the end. The same pattern was observed for the acylated anthocyanins: samples had a content between $9.92 \pm 0.78$ and $17.73 \pm 0.17$ mg/L at the beginning and a content of $0.33 \pm 0.04$ to $0.58 \pm 0.01$ mg/L at the end of the storage period.

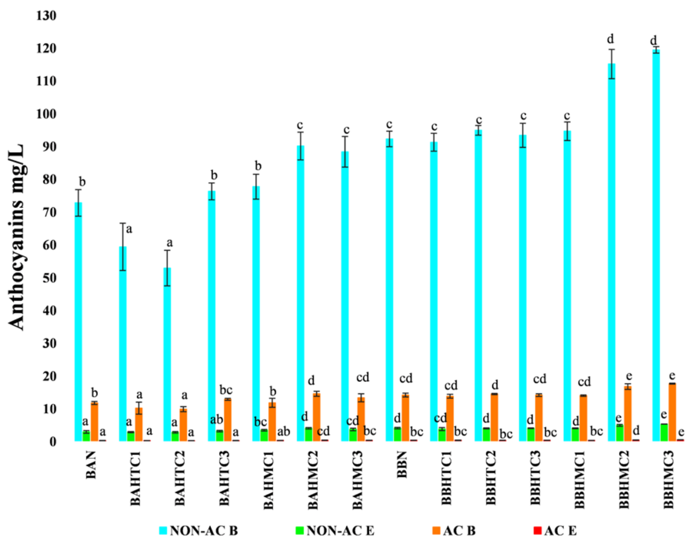


**Figure 1.** Changes in the anthocyanin content of samples determined with HPLC–DAD and grouped by anthocyanin families at the beginning and the end of the storage period. Different letters indicate a significant difference between means ($p < 0.05$). Non-AC = Non-acylated anthocyanins; AC = acylated; B = beginning; E = end.

PCA was carried out for the anthocyanin content at the beginning and the end (Figure 2A,B). The distribution is explained by the first two principal components. PC1 is positively contributed to by the identified anthocyanins. Two different clusters can be identified. The first represents samples prepared with the low-mineral water, characterized by lower content of anthocyanins, and the second group consists of samples presenting higher content of anthocyanins, mainly in samples BBHMC2 and BBHMC3. These results showed that samples can be classified according to their mineral composition and their acidity. Beverages prepared with high-mineral water and with high pH values presented a higher content of anthocyanins. However, in the first group, BAHMC2 and BAHMC3 had a high content of anthocyanins that was closer to the samples of the second group. Thus, this can be an indication that mint extracts influence anthocyanin content.

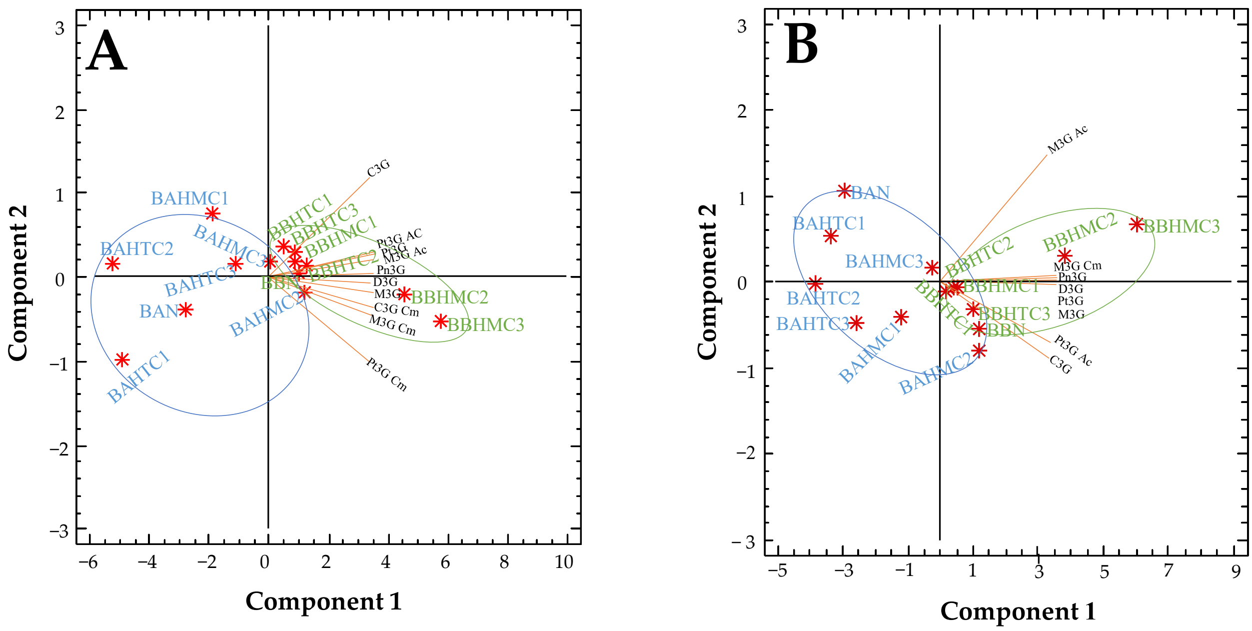


**Figure 2.** PCA of anthocyanin pigments present in the beverages at the beginning (**A**) and at the end (**B**). Abbreviations: D3G: Delphinidin-3-O-glucoside; C3G: Cyanidin-3-O-glucoside; Pt3G: Petunidin-3-O-glucoside; Pn3G: Peonidin-3-O-glucoside; M3G: Malvidin-3-O-glucoside; Pt3G Ac: Petunidin-3-(6′′-acetylglucoside); M3G Ac: Malvidin-3-(6′′-acetylglucoside); C3G Cum: Cyanidin-3-(6′′-*p* coumaroylglucoside; Pt3G Cum: Petunidin-3-(6′′-*p* coumaroylglucoside); M3G Cum: Malvidin-(6′′-*p* coumaroylglucoside).

### 3.4. Antioxidant Activity

The results of antioxidant activity measured using an ABTS assay are shown in Figure 3. The values ranged from 3.28 ± 0.01 to 4.27 ± 0.09 µmols TE/mL. The higher values were reached in the beverage containing the mixture of hops and tea (BAHTC3, followed by BBHTC1 and BBHTC3). However, the lowest value was observed in the control Group A. In general, samples that contained spice extracts showed good antioxidant capacity. Numerous studies based on natural sources of bioactive compounds in the preparation of isotonic drinks measured their antioxidant capacities using different analytical methods. Gironés-Vilaplana et al. [9] designed new isotonic beverages based on berries (maqui, açaí, and blackthorn) and lemon juice and compared their antioxidant activity with some commercial isotonic drinks (Aquarius, Gatorade, Powerade, Isostar, Hacendado, and Ev2o light). The results revealed that the prepared isotonic drinks had higher antioxidant activity compared to the commercial ones due to the presence of the bioactive compounds in berries. The antioxidant activity measured in model isotonic drinks colored with anthocyanin powder of Andean berries prepared by Estupiñan et al. [37] ranged from 0.58 ± 0.01 to 0.99 ± 0.05 µmols TE/mL. Ferreira et al. [4] prepared an isotonic beverage based on whey permeate with carotenoid extract powder from pequi and evaluated its antioxidant capacity using ABTS and DPPH, yielding results of 10.79 µmols TE/100 mL and 73.38 µmols TE/100 mL, respectively.

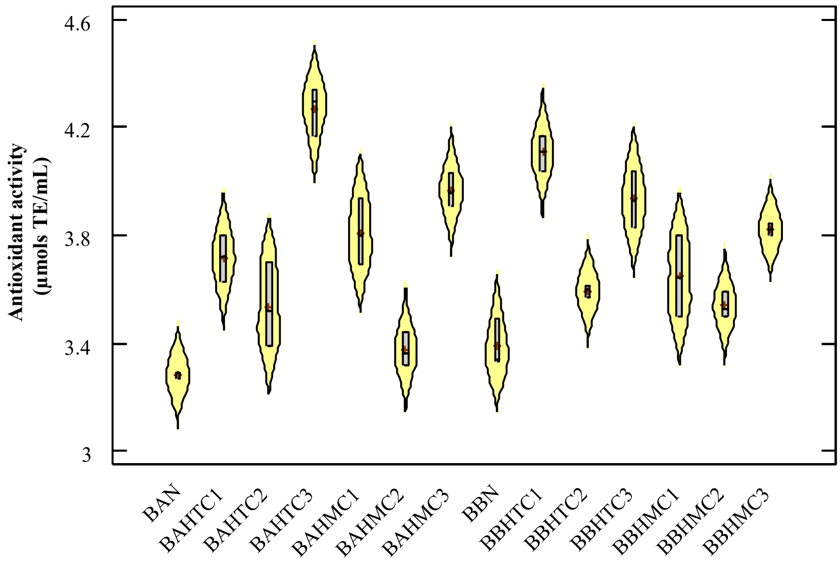


**Figure 3.** Violin chart for the antioxidant activity measured in the prepared beverages.

*3.5. Sensory Evaluation*

The results of the sensory analysis are presented in Table 4. In general, the panelists preferred the flavored isotonic drinks to the control drinks. As shown in Table 4, the controls BAN and BBN presented lower average scores of global perception. However, BBHMC1 and BBHMC3 had the best global perception. Concerning color intensity, BAHMC2 was slightly higher than the rest of the samples, with a maximum value of 3.9 out of 5. This result agrees with spectrophotometric color measurements (Table 3), as the initial sensory test was carried out at the beginning. The tonality values were slightly lower in samples of Group B, although without statistical differences between the two groups. The turbidity was lower in the elaborated isotonic drinks, with no significant differences. In terms of aromatic intensity, the tasters gave both samples BAHTC1 and BBHMC1 the highest values and gave the lowest value to the control BBN. However, BBHMC1 was the best in terms of aromatic quality. Herbaceous flavors were higher in BBHTC3 and BBHMC2. Regarding floral and fruity attributes, the tasters described BBHMC2 beverages as more floral, and BAHMC3 as fruitier, without significant difference between samples. All beverages obtained the lowest scores for the reduction and oxidation attributes as none were detected. Regarding body, BBHMC1 had the highest score (3), and the controls had the lowest scores. On the other hand, the tasters identified from no to scarcely any bitterness in all beverages and a medium level of sweetness, with BAN having the highest score (2.5). Salinity was found to be lower in the two controls as they were without salt. However, BBHMC2 was described as having the most salinity, with a maximum value of 3 due to the high concentration of salt added to this beverage (C2 = 0.7 g/L). There were no significant differences in the acidity of the samples, and BAHMC3 and BBHTC2 presented the highest scores for this parameter.

**Table 4.** Sensorial properties of the prepared isotonic beverages evaluated by the trained panelists.

| | BAN | BAHTC1 | BAHTC2 | BAHTC3 | BAHMC1 | BAHMC2 | BAHMC3 | BBN | BBHTC1 | BBHTC2 | BBHTC3 | BBHMC1 | BBHMC2 | BBHMC3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Color I | 3.3 $^{a}$ | 3.5 $^{ab}$ | 3.5 $^{ab}$ | 3.6 $^{ab}$ | 3.6 $^{ab}$ | 3.9 $^{b}$ | 3.6 $^{ab}$ | 3.5 $^{ab}$ | 3.6 $^{ab}$ | 3.8 $^{ab}$ | 3.3 $^{a}$ | 3.8 $^{ab}$ | 3.8 $^{ab}$ | 3.8 $^{ab}$ |
| Tonality | 2.4 $^{a}$ | 2.8 $^{a}$ | 2.8 $^{a}$ | 2.8 $^{a}$ | 2.3 $^{a}$ | 2.4 $^{a}$ | 2.5 $^{a}$ | 2.4 $^{a}$ | 2.1 $^{a}$ | 2.3 $^{a}$ | 2.0 $^{a}$ | 1.8 $^{a}$ | 2.0 $^{a}$ | 1.8 $^{a}$ |
| Turbidity | 1.4 $^{a}$ | 1.6 $^{a}$ | 1.5 $^{a}$ | 1.6 $^{a}$ | 1.4 $^{a}$ | 1.3 $^{a}$ | 1.6 $^{a}$ | 2.0 $^{a}$ | 1.6 $^{a}$ | 1.5 $^{a}$ | 1.4 $^{a}$ | 1.5 $^{a}$ | 1.5 $^{a}$ | 1.4 $^{a}$ |
| Aromatic I | 3.1 $^{abc}$ | 3.5 $^{c}$ | 3.0 $^{abc}$ | 3.1 $^{abc}$ | 3.1 $^{abc}$ | 3.3 $^{abc}$ | 3.4 $^{abc}$ | 2.6 $^{a}$ | 3.0 $^{abc}$ | 2.8 $^{ab}$ | 3.0 $^{abc}$ | 3.5 $^{c}$ | 3.0 $^{abc}$ | 3.4 $^{bc}$ |
| Aromatic Q | 2.6 $^{ab}$ | 3.1 $^{abc}$ | 3.0 $^{abc}$ | 3.3 $^{abc}$ | 3.1 $^{abc}$ | 3.3 $^{abc}$ | 3.4 $^{abc}$ | 2.6 $^{ab}$ | 3.1 $^{abc}$ | 2.5 $^{a}$ | 3.0 $^{abc}$ | 3.8 $^{c}$ | 3.6 $^{bc}$ | 3.4 $^{abc}$ |
| Herbaceous | 1.3 $^{a}$ | 2.0 $^{abc}$ | 1.9 $^{abc}$ | 1.8 $^{abc}$ | 1.6 $^{ab}$ | 1.6 $^{ab}$ | 1.6 $^{ab}$ | 1.8 $^{abc}$ | 2.4 $^{bc}$ | 2.0 $^{abc}$ | 2.5 $^{c}$ | 2.4 $^{bc}$ | 2.5 $^{c}$ | 2.1 $^{bc}$ |
| Floral | 1.8 $^{a}$ | 2.3 $^{a}$ | 2.1 $^{a}$ | 2.4 $^{a}$ | 2.6 $^{a}$ | 2.3 $^{a}$ | 2.6 $^{a}$ | 2.1 $^{a}$ | 2.3 $^{a}$ | 2.1 $^{a}$ | 2.3 $^{a}$ | 2.8 $^{a}$ | 2.9 $^{a}$ | 2.8 $^{a}$ |
| Fruity | 2.3 $^{a}$ | 2.1 $^{a}$ | 2.0 $^{a}$ | 2.1 $^{a}$ | 2.4 $^{a}$ | 2.4 $^{a}$ | 2.5 $^{a}$ | 1.8 $^{a}$ | 2.0 $^{a}$ | 2.1 $^{a}$ | 2.1 $^{a}$ | 1.9 $^{a}$ | 2.0 $^{a}$ | 2.1 $^{a}$ |
| Reduction | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.1 $^{a}$ | 1.1 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.1 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ | 1.0 $^{a}$ |
| Oxidation | 1.3 $^{ab}$ | 1.5 $^{b}$ | 1.5 $^{b}$ | 1.5 $^{b}$ | 1.3 $^{ab}$ | 1.4 $^{ab}$ | 1.3 $^{ab}$ | 1.0 $^{a}$ | 1.1 $^{ab}$ | 1.1 $^{ab}$ | 1.1 $^{ab}$ | 1.1 $^{ab}$ | 1.1 $^{ab}$ | 1.1 $^{ab}$ |
| Body | 2.1 $^{a}$ | 2.8 $^{a}$ | 2.8 $^{a}$ | 2.5 $^{a}$ | 2.6 $^{a}$ | 2.8 $^{a}$ | 2.8 $^{a}$ | 2.3 $^{a}$ | 2.6 $^{a}$ | 2.5 $^{a}$ | 2.8 $^{a}$ | 3.0 $^{a}$ | 2.4 $^{a}$ | 2.8 $^{a}$ |
| Bitterness | 1.4 $^{a}$ | 1.4 $^{a}$ | 1.4 $^{a}$ | 1.6 $^{a}$ | 1.8 $^{a}$ | 1.5 $^{a}$ | 1.5 $^{a}$ | 1.4 $^{a}$ | 1.8 $^{a}$ | 1.8 $^{a}$ | 1.8 $^{a}$ | 1.8 $^{a}$ | 1.9 $^{a}$ | 1.6 $^{a}$ |
| Sweetness | 2.5 $^{a}$ | 2.1 $^{a}$ | 2.0 $^{a}$ | 2.3 $^{a}$ | 2.1 $^{a}$ | 2.3 $^{a}$ | 2.3 $^{a}$ | 2.3 $^{a}$ | 2.1 $^{a}$ | 2.4 $^{a}$ | 2.3 $^{a}$ | 2.3 $^{a}$ | 2.1 $^{a}$ | 2.0 $^{a}$ |
| Salinity | 1.0 $^{a}$ | 2.0 $^{b}$ | 2.9 $^{bc}$ | 2.3 $^{bc}$ | 2.5 $^{bc}$ | 2.8 $^{bc}$ | 2.5 $^{bc}$ | 1.0 $^{a}$ | 2.6 $^{bc}$ | 2.5 $^{bc}$ | 2.4 $^{bc}$ | 2.4 $^{bc}$ | 3.0 $^{c}$ | 2.6 $^{bc}$ |
| Acidity | 2.5 $^{a}$ | 2.3 $^{a}$ | 2.4 $^{a}$ | 2.4 $^{a}$ | 2.5 $^{a}$ | 2.5 $^{a}$ | 2.8 $^{a}$ | 2.5 $^{a}$ | 2.5 $^{a}$ | 2.8 $^{a}$ | 2.6 $^{a}$ | 2.5 $^{a}$ | 2.4 $^{a}$ | 2.6 $^{a}$ |
| Global P | 2.9 $^{a}$ | 3.6 $^{bc}$ | 3.3 $^{abc}$ | 3.5 $^{abc}$ | 3.0 $^{ab}$ | 3.5 $^{abc}$ | 3.8 $^{c}$ | 2.9 $^{a}$ | 2.9 $^{a}$ | 3.0 $^{ab}$ | 3.4 $^{abc}$ | 3.9 $^{c}$ | 3.3 $^{abc}$ | 3.9 $^{c}$ |

The values are the averages from eight tasters. The same attributes with the same letter are not significantly different ($p < 0.05$).

## 4. Discussion

Highlighting the contribution of grape juice, the results obtained demonstrated that the newly designed drinks present good nutritional, biological, and sensorial qualities to be considered isotonic drinks. Concerning sugar and salt concentrations, the beverages are within the normal and acceptable range for these kinds of beverages [4,38]. For sports nutritionists, the optimization of carbohydrates and salt concentration is important for improving athlete performance [39]. Studies have demonstrated that the level of carbohydrates in isotonic drinks is important to provide the optimal quantity of carbohydrates required for oxidation to improve performance, intestinal absorption, rapid gastric emptying, and palatability. However, the high concentrations of carbohydrates and salts lead to a reduction in the rate of gastric emptying and thereby delay the rate of delivery of fluid [40,41]. Isotonic drinks are characterized by an acidic taste and a pH of around 3 [8]. During the storage period, all samples showed low pH values (3.25–3.50) (Table 3). These pH values were correlated with total acid values, which were higher in the beverages of Group A, which presented the lowest pH values, compared with Group B (Table 2), with the presence of malic acid, which is one of the main organic acids of grape juice in addition to other acids such as tartaric and citric acids [17]. Acidic conditions (pH $< 4$) are also used as indicators of beverage quality, which enhances microbiological stability and consumer safety [42]. The color of grape juice is an important characteristic observed by consumers as an indicator of grape juice quality [12,43]. The results of the color analysis (Table 3) demonstrated that the newly elaborated isotonic beverages, despite slight differences between the two groups, can present an attractive red color from grape juice. The evaluation of color parameters indicates that there was an increase in these parameters in all samples during the storage period. Anthocyanins are the pigments responsible for grape color [44]. The identification of anthocyanins in the prepared isotonic beverages showed the presence of acylated and non-acylated glycoside forms of malvidin, petunidin, peonidin, cyanidin, and delphinidin (Figures 1 and 2), with malvidin pigments predominating. In this study, pH and water mineralization influenced the anthocyanin content and the intensity of color. This difference in pH values affected the CI and anthocyanin content, resulting in beverages with pH $\geq 3.30$, higher CI, and higher anthocyanin content. Another factor observed is that the addition of salts (Na and K) to the beverages led to a change in color parameters compared with our previous work [20]. On the other hand, the results for anthocyanins demonstrate a decrease in anthocyanin content in all samples during the storage period, which presents a negative correlation between anthocyanin content and CI. González-Molina et al. [45] attributed the decrease in anthocyanin concentration in blends of lemon juice with elderberry concentrate and lemon juice with grape concentrate to the high vitamin C content in lemon juice, which affects the stability of the anthocyanins, either via their degradation through condensation at the fourth position or a free radical mechanism, or via the degradation of ascorbic acid to dehydroascorbic acid, furfurals, and hydrogen peroxide, which attack anthocyanins. Bingöl et al. [46] also mentioned that high ascorbic acid content affects the stability of anthocyanins in strawberries by accelerating their degradation and increasing the rate of degradation during heating. An increase in CI despite the loss of anthocyanin content was reported for other beverages rich in fruit anthocyanins. The loss of anthocyanins in the blends of lemon juice with berries was due to the presence of lemon juice, which influences their degradation. The RC was stable over time despite the anthocyanin degradation, which could be because of the formation of other colored polymers or the copigmentation of anthocyanins with flavanols, which could lead to a change in the color expression [36,47]. In addition, anthocyanin stability during storage is influenced by multiple factors such as pH, copigments, chemical structure, temperature, ascorbic acid, sugars, metals, oxygen, light, and enzymes [48]. Generally, anthocyanins are more stable under acidic conditions [49] and unstable at neutral and alkaline pH [50]. Therefore, copigmentation is considered a mechanism of anthocyanin stabilization via the interaction with colorless or yellowish copigments through intramolecular copigmentation, intermolecular copigmentation, metal complexation, or self-association [28,51]. This results in a stable structure that preserves the flavylium chromophore from nucleophilic attack by water, leading to a reduction in colorless hemiketal and chalcone formation [29,52]. Thus, researchers have used copigmentation as a natural way to improve the stability of anthocyanins based mainly on phenolic acids (hydroxycinnamic and hydroxybenzoic acids) [49]. Moreover, Vidana Gamage et al. [50] noted that most studies demonstrated that acylated anthocyanins are more stable than non-acylated anthocyanins, mainly in aqueous solutions, because the acylation gives anthocyanins more stability, helping in the intramolecular copigmentation, protecting the flavylium form, and making an acidic medium that preserves color stability.

On the other hand, the purpose of using grape juice, lemon juice, and spices (hops, tea, and mint) is to obtain a natural isotonic drink characterized by a rich content of bioactive compounds so that its consumption can increase the antioxidant capacity in addition to its principal role, which is hydration and providing energy. The results of TPI (Table 3) and antioxidant activity evaluation (Figure 3) demonstrate that the prepared beverages presented a good source of phenolic content and antioxidant capacity. Furthermore, we previously demonstrated [53] the contribution of grape juice to isotonic beverages, citing different studies that demonstrated the positive effect of grape juice consumption during physical activities, which improves antioxidant activity and performance, protects against oxidative damage, and reduces inflammation [14,54–60]. Accordingly, it is known that herbs and spices have excellent antioxidant activity. Thus, they have been used whole, crushed, as extracts or emulsions, or encapsulated as sources of antioxidants [61]. Herbal supplements have long been used to treat problems related to stress, inflammation, and sleep, but their effects on post-exercise recovery have not been measured directly. However, the results of studies and investigations at the cellular level have suggested that some of these compounds have the potential to improve post-exercise recovery [62]. In addition to their positive health effects, it is important to note that hop, tea, and mint extracts provide an interesting natural organoleptic profile for elaborated beverages, mainly when they are incorporated with grape juice and lemon juice. In addition to color, grape juice provides the beverage with a pleasant taste due to its high concentration of sugars and organic acids [12]. The sensory analysis (Table 4) shows that the addition of natural flavor from hop, tea, and mint extracts makes the beverages more acceptable to panelists compared to the control beverages. For this kind of beverage, the distinct palatability (taste, acidity, sweetness, and beverage temperature) is the main characteristic that makes them more consumable than water during sports [42]. This palatability depends on the balance of sugars, acids, and salt [40]. Likewise, hop extract is characterized by its bitterness, flavor, and aroma due to its essential oil fraction, which contains a complex group of volatile compounds [63]. In addition, it has interesting antioxidant, antimicrobial, and antifungal activities [64] and can be used as a natural preservative to extend the shelf life of food products [65]. Mint acts as a breath freshener and a stomach soother. Its smell and taste are due to the presence of a cyclic terpene alcohol compound (menthol) [66]. Tea contains several chemical components responsible for color (theaflavins, thearubigins, pheophorbide), flavor (phenylacetaldehyde, nerolidol, benzaldehyde), and taste (amino acids, polyphenols, caffeine), and exhibits antioxidant and medicinal properties [67]. However, blends of hop and mint extracts in the beverages were preferred because tasters gave BBHMC1 and BBHMC3 higher scores for global perception. Finally, this study presents new natural alternative beverages for athletes that help to replace the loss of minerals during physical activities, provide carbohydrates necessary for energy and performance, and are a rich source of phenolic compounds, which are important for oxidative recovery. These beverages have also been shown to be an alternative for synthetic dyes and flavor additives, as they are naturally colored and flavored with grape juice anthocyanins and herb extracts.

## 5. Conclusions

The newly designed isotonic drinks have shown good nutritional, biological, and sensory profiles. They are useful for the replacement of minerals and glycogen lost during physical activity because they contain minerals, mainly sodium and potassium, and carbohydrates from grape juice that also make them naturally sweet. In addition, they are a rich source of bioactive compounds from the grape juice, lemon juice, herbs, and spices used in their formulation, which is important owing to their antioxidant effect against oxidative stress. The elaborated isotonic drinks had anthocyanins from grape juice in their formulation that contribute to an attractive RC and avoid the use of synthetic coloring. However, their stability was influenced by pH, water mineralization, and salt concentrations, giving more CI for beverages elaborated with high-mineral water, which had higher pH values. In this study, the new isotonic drinks flavored naturally with herb and spice extracts were evaluated better by the panelists. These beverages based on organic and natural ingredients present a novel product in the field of nutrition, sports, and healthy beverages.

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