UNIVERSIDAD POLITÉCNICA DE MADRID
ESCUELA TÉCNICA SUPERIOR DE INGENIERÍA AGRONÓMICA,
ALIMENTARIA Y DE BIOSISTEMAS

HEALTHY BEVERAGES MADE FROM GRAPE JUICE. ANTIOXIDANT, ENERGETIC, AND ISOTONIC COMPONENTS.

TESIS DOCTORAL

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Master in Agri-food and quality control

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DEPARTAMENTO DE QUÍMICA Y TECNOLOGÍA DE LOS ALIMENTOS
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2023

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RESUMEN

Los alimentos y bebidas saludables son cada vez más populares en estos días. La gente ha cambiado sus hábitos alimentarios expresando su preocupación por los aditivos sintéticos y prefiriendo productos de fuentes naturales con atractivo sensorial. El zumo de uva es uno de los zumos de frutas más populares y que ha ganado especial interés por su efecto beneficioso para la salud y su atractivo perfil sensorial. El objetivo de esta tesis es ampliar el conocimiento sobre el jugo de uva y estudiar el potencial de desarrollar bebidas saludables y funcionales utilizando jugo de uva orgánico enriquecido con extractos de hierbas y especias, y evaluar sus propiedades fisicoquímicas y organolépticas. Esta tesis es un compendio de artículos. En él se realizó la primera investigación para investigar cómo el jugo de uva puede contribuir al desarrollo de bebidas funcionales, particularmente bebidas isotónicas, aportando a una bebida deportiva antioxidantes y actividades biológicas. El estudio también destaca las interesantes propiedades sensoriales que se pueden impartir a las bebidas. En el primer ensayo, el objetivo era desarrollar bebidas naturales a base de zumo de uva sin aditivos artificiales. Se utilizaron dos tipos de agua para diluir el jugo de uva concentrado; la corrección del pH se realizó con jugo de limón y luego las bebidas se aromatizaron con extracciones de hierbas, excepto las bebidas de control. Se observó que el pH de las bebidas estaba influenciado por la mineralización del agua. Las muestras con baja mineralización exhibieron mayor acidez que aquellas con alta mineralización. En consecuencia, el pH de las bebidas influyó en el color, lo que resultó en una mayor intensidad del color para las muestras con valores de pH más bajos. La medición de la evolución del color durante 20 días mostró que los valores de color aumentaron en todas las muestras (126%).

Dado que el color depende del contenido de antocianinas, se determinaron estos pigmentos. Los resultados mostraron que las bebidas contienen antocianinas tanto aciladas como no aciladas. La malvidina fue el pigmento predominante (47.54%). En términos de evaluación sensorial, los catadores prefirieron las bebidas aromatizadas con extractos de hierbas más que las bebidas de control, siendo la mezcla de lúpulo y té la más preferida. Los catadores también prefirieron las bebidas elaboradas con baja mineralización. En el segundo ensayo, el objetivo era desarrollar nuevas bebidas isotónicas utilizando zumo de uva como fuente de energía y antioxidantes. Se

utilizaron las mismas formulaciones anteriores, pero cada muestra aromatizada en ambos grupos, excepto las muestras de control, recibió adiciones de sal en tres concentraciones diferentes. Dentro del rango aceptable para bebidas isotónicas, las concentraciones de sal oscilaron entre 0.50 y 0.70 g/L y las concentraciones de azúcar oscilaron entre 72.73 y 78.43 g/L. La actividad antioxidante de las bebidas alcanzó 4.27 moles Trolox eq/mL. Estas bebidas también contienen antocianinas aciladas y no aciladas, que mostraron una disminución durante el período de almacenamiento que se correlacionó inversamente con el aumento en la intensidad del color. Se observó que las sales añadidas a las bebidas tenían un impacto en el color, lo que provocaba diferencias entre las muestras según la concentración de sal y el tipo de agua.

Considerando los diferentes resultados reportados en los artículos, se revela que las bebidas a base de jugo de uva potenciadas con extractos de hierbas son una alternativa a las bebidas naturales y más saludables, lo que incentiva el desarrollo de productos innovadores que incluyan ingredientes clave que ayuden a mejorar su funcionalidad y a mejorar su estabilidad y seguridad a través de métodos innovadores como tecnologías no térmicas.

ABSTRACT

Healthy foods and drinks are becoming more popular these days. People have changed their eating habits expressing concerns about synthetic additives and preferring products from natural sources with sensory appeal. Grape juice is one of the most popular fruit juices that gained specific interest due to its beneficial effect on health and its attractive sensory profile. The aim of this thesis is to expand the knowledge about grape juice and study the potential of developing healthy and functional beverages using organic grape juice enhanced by herb and spice extracts, and to evaluate their physicochemical and organoleptic properties.

This thesis is a compendium of articles. In it, the first research was carried out to investigate how grape juice can contribute to the development of functional beverages, particularly isotonic drinks, providing a sports drink with antioxidants and biological activities. The study also highlights the interesting sensory properties that can be imparted to the beverages. In the first trial, the aim was to develop natural grape juice-based beverages free of artificial additives. Two types of water were used to dilute concentrate grape juice; the pH correction was done with lemon juice then the beverages were flavored with herb extractions except the control beverages. It was observed that the pH of beverages was influenced by water mineralization. Samples with low mineralization exhibited higher acidity than those with high mineralization. Consequently, the pH of the beverages influenced the color, resulting in higher color intensity for samples with lower pH values. The measurement of color evolution for 20 days showed that the color values increased in all samples (126%). Since the color depends on the anthocyanin content, these pigments were determined. The results showed that the beverages contain both acylated and non-acylated anthocyanins. Malvidin was the predominant pigment (47.54%). In terms of sensory evaluation, tasters preferred flavored beverages with herb extracts more than control beverages, with the mixture hop-tea being the most favored. Tasters also favored the beverages made with low mineralization. In the second trial, the goal was to develop novel isotonic drinks using grape juice as an energy and antioxidant source. The same previous formulations were used, but each flavored sample in both groups, except the control samples, received salt additions at three different concentrations. Within the acceptable range for isotonic beverages, the salt concentrations ranged from 0.50 to 0.70 g/L and the sugar concentrations ranged from 72.73 to 78.43 g/L. The antioxidant

activity of the beverages reached 4.27 mols Trolox eq/mL. These beverages also contain both acylated and non-acylated anthocyanins, which showed a decrease over the storage period which was inversely correlated with the increase in color intensity. The salts added to the beverages were observed to have an impact on the color, leading to differences among samples depending on salt concentration and the type of water.

Considering the different results reported in the articles, it is revealed that grape juice-based beverages enhanced by herb extracts are an alternative to natural and healthier drinks which encourages the development of innovative products including key ingredients that help to improve their functionality and to improve their stability and safety through innovative methods like non-thermal technologies.

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CHAPTER 1. INTRODUCTION

1. General Contextualization

Food and dietary choices play an essential role in both maintaining good health and influencing the development of various diseases (Duttaroy, 2019). Recently, the demand for healthy foods and beverages has increased worldwide (Corbo et al., 2014). The belief of consumers that food directly contributes to their health has led to considerable changes in their demands. Thus, food no longer needs to just satisfy hunger and provide needed nutrients, but above all, prevent nutrition-related illnesses and improve physical and mental health (Bigliardi & Galati, 2013). However, food scientists should keep in mind the key and emerging trends in the food industry to develop formulations and technologies fitting the consumer needs (Arenas-Jal et al., 2020). Therefore, the global food industry is facing various changes and challenges (Rosenthal et al., 2021). Accordingly, the demand for eco-sustainable, fresh, and natural products that offer attractive sensory attributes and high nutritional value is rising significantly, reflecting the expanding market for these types of products (Nazhand et al., 2020). The production of healthy and sustainable foods using only biological and ecological methos is the main objective of organic production and different studies have demonstrated people's belief that organically grown foods are healthier because of eco-friendly farming (Suciu et al., 2019). The European Union's organic farming regulations prohibit the application of pesticides and impose severe restrictions on the use of chemical substances, such as fertilizers and allopathic treatments. These regulations also prohibit the use of genetically modified organisms, support enhanced grazing practices and animal well-being, and encourage the utilization and preservation of local breeds. Accordingly, the demand for organic food has increased worldwide, with growth rates of 48% and 28% in the European Union and North America, respectively in the last years (Rodríguez-Bermúdez et al., 2020). However, the global demand for organic food is driven by a variety of economic, psychological, and sociological factors that vary by country, product type, and food group (Nathan et al., 2021). Furthermore, the consumer's trend toward natural and organic ingredients and the growing concern about artificial ingredients like sweeteners and colorings are the main shaping factors of functional product markets. Therefore, health is the important purchasing reason for consumers to purchase functional and organic products (Goetzke & Spiller, 2014). In this perspective, the beverage sector is experiencing diversification and innovation, with a notable focus on creating beverages that provide benefits beyond fundamental hydration and nutrition (Pereira & Coelho, 2019).

2. Beverages

Beverages are all consumable liquids that are intentionally consumed to provide nourishment and satisfy thirst (Aadil et al., 2019). Water is an important element in the body, involving in many important functions, satisfying thirst, and keeping the electrolyte balance. However, for different reasons, a variety of beverages are consumed (Gallo et al., 2019) mainly for their nutritional benefits (milk, fruit juices), ability to quench thirst (non-alcoholic drinks), stimulating properties (coffee, tea), and recreational purposes (alcoholic beverages). Although there is no uniform regulation that prescribes a universal classification system for beverages, they can be classified into several categories depending on different aspects (Table 1), they can be natural or synthetic, carbonated or non-carbonated, stimulating or non-stimulating, alcoholic or non-alcoholic, and cold or hot beverages (Vinci & Maddaloni, 2020). Moreover, the global beverage market can be divided into four main sectors including hot drinks, milk drinks, alcoholic drinks, and soft drinks (Renfrew, 2016)

Table 1. Different beverage classes and aspects of classification.

Aspect	Class	Properties	Example
Formulation	Natural	Natural ingredient Natural transformation process	Milk and fruit juice
	Synthetic	Artificial compound mixing	Aroma, coloring, and sugar syrup
Ethanol content	Alcoholic	The presence of ethanol produced by fermentation processes	Fermented, distilled, and macerated
	Non-alcoholic	Free of ethanol	Fruit juice, soft drinks, and milk
Carbonization	Carbonated	Presence of carbon dioxide: by addition or natural production through fermentation	Cola drinks and carbonated water wine
	Non-carbonated	Absence of carbon dioxide	Fruit juice, sport drinks
Physiological effect	Stimulating	Act on the nervous and circulatory systems (caffeine, theobromine)	Coffee, tea infusion, and energy drinks
	Non-stimulating	No physiological effect	Fruit juices, soft drinks
Temperature	Cold	Served cold	Soft drinks and iced tea
	Hot	Served hot	Tea, coffee, and hot chocolate

2.1. Soft drinks

Soft beverages are water-based drinks, although water is the main component, it is often ignored or maligned. They are typically sweetened and often have a balancing level of acidity. These drinks are flavored and colored, and they may also have added natural ingredients, fruit juice or fruit pulp. However, the primary purpose of soft drinks is to provide hydration. While the sweetness and other attributes are somewhat secondary, they play a significant role in supplying energy and certain minor essential nutrients necessary to meet daily needs (Ashurst, 2005). The term soft is used to differentiate soft drinks from alcoholic drinks, however, milk, coffee, tea, and cocoa are typically not categorized as soft drinks (Walton & Wittekind, 2023). Soft drinks appeared in 1884 with the creation of "Moxie" by a chemist owner in Lisbon Falls, USA. Coca-Cola® and Pepsi-Cola® soon followed. Throughout the past century, soft drinks have undergone a significant transformation, transitioning from a local pharmacy item to a worldwide industry generating $60 billion in revenue and producing 1 billion liters annually. These changes can be attributed to advancements in manufacturing technology and innovative marketing strategies (Tahmassebi & Banihani, 2020).

Figure 1. Different categories of soft drinks.

Modern soft drinks represent a diverse product category and can be classified in several ways, such as based on their sugar and fruit juice content, flavorings, carbonation level, primary non-water ingredients, and functional attributes. They comprise a variety of beverage categories, including bottled waters, carbonated and non-carbonated drinks, fruit and vegetables juices, juice-based beverages, as well sports drinks and energy drinks (Figure 1) (Kregiel, 2015). Soft drink consumption has greatly increased, the estimated market size for non-carbonated soft drinks worldwide is projected to reach USD 208.51 billion by 2028, with an expected compound annual growth rate (CAGR) of 6.9% from 2022 to 2028. This growth is attributed to the expanding soft drinks industry and the increasing per capita consumption of these products, which are anticipated to drive market evolution (Research and Markets, 2022). However, in the past few decades, the consumption of sugar-containing beverages has seen a significant and notable increase, especially among adolescents, and this consumption primarily provides empty calories without significant nutritional value, contributing to the obesity problem and negative health effect. Numerous national health organizations have launched public health campaigns to reduce sugar consumption, and some countries have introduced legal measures such as taxes on sugary drinks. These legal restrictions and growing awareness have led manufacturers to offer alternative beverages with lower sugar content (Hafner et al., 2021). Additionally, packaged non-alcoholic beverages have a substantial impact on the global economy, generating a revenue of 1,038,054 million US dollars in 2020, with an average consumption volume of 109.5 liters per person. The market volume is projected to continue growing at an annual rate of 6.8%, with an anticipated volume of 935,758.3 million liters by the year 2025 (Tireki, 2021). On the other hand, natural drinks are extremely valuable for human health because they contain an abundance of nutrients, minerals, vitamins, and organic compounds. Because of their nutrient richness and potential to reduce the risk of conditions such as type 2 diabetes, heart disease, and chronic illness, they are very popular around the world. In today's health-conscious environment, these beverages play a critical role in promoting wellness (Koner et al., 2019).

2.2. Functional beverages

The concept of "functional foods" originated in Japan during the 1980s, and their popularity has grown significantly in numerous countries worldwide since then (Kolonas et al., 2023). The idea that "food itself is a medicine" sparked interest, and the term "nutraceutical" became popular. The functional food market is growing rapidly due to the increasing prevalence of lifestyle-related diseases and growing public awareness of the importance of a healthy lifestyle (Gayathry & John, 2021). Furthermore, the deterioration of health resulting from busy lifestyles, excessive consumption of convenient and processed foods, and insufficient physical activity are significant contributors to the prevalence of functional foods (Gupta et al., 2023). It is estimated that consuming functional foods could potentially lead to a 20% reduction in annual healthcare expenses (Tireki, 2021). There is no official definition of functional foods accepted by all countries. Nevertheless, there is a consensus in the literature regarding four key aspects that contribute to defining functional foods. These aspects include the health benefits, the nature of the food itself, its functionality, and the consumption pattern associated with it. The health benefit should aim to improve a particular function or prevent the occurrence of disease. The nature of the food should be consistent with that of traditional foods. The level of functionality should go beyond basic nutritional value, and the consumption pattern should be compatible with a normal routine diet (Nazir et al., 2019). Functional foods can be comprised of either a single ingredient or a combination of ingredients that are not typically found in conventional foods. They often include beneficial components such as probiotics, prebiotics, phenolic compounds, dietary fibers, vitamins, minerals, phospholipids, proteins, and amino acids. These ingredients contribute to the unique health-enhancing properties of functional foods (Kolonas et al., 2023).

2.2.1. Properties of functional beverages

A functional beverage is a drink that can positively impact one or more specific bodily functions, going beyond basic nutritional effects, with the goal of either enhancing overall health and well-being or reducing the risk of disease (Gayathry & John, 2021) as they offer a rich supply of nutrients and bioactive compounds such as vitamins, minerals, polyphenols, plant pigments, phytosterols, probiotic fatty acids, and dietary fiber (Maleš et al., 2022). They are typically created by incorporating bioactive elements and, if necessary, eliminating or decreasing unwanted components like sugar and fats. Additionally, supplementary functional elements can be introduced to provide the intended texture and stability characteristics to the final product (Nazir et al., 2019). Beverages are the leading category in functional foods because they are convenient, easy to distribute, and have the potential to include beneficial nutrients and bioactive compounds. They offer flexibility in meeting consumer demand for containers and have the advantage of being both refrigerated and shelf-stable. Thus, beverages are an ideal medium for delivering health-promoting components to consumers (Corbo et al., 2014; Orrù et al., 2018). The global market for functional beverages experienced significant growth over the span of a decade, increasing from approximately $55 billion in 2010 to an estimated $93.7 billion by 2019 (Statista, 2020). It is expected to reach $208.13 billion by 2024, with an anticipated Compound Annual Growth Rate (CAGR) of 7.5% between 2022 and 2027 (Gupta et al., 2023). To create a successful functional beverage for the commercial market, it is vital to consider various factors such as raw materials, processing techniques, taste, texture, flavor, appearance, chemical properties, and functionality. These considerations are crucial for producing a safe, high-quality product with market value. Success also relies on factors such as scientific support, adherence to intake limits, consumer acceptance, commercial aspects, and legal compliance (Tolun & Altintas, 2019).

Functional beverages are divided into several categories that include dairy-based beverages, such as those containing probiotics and fortified with minerals, vegetable- and fruit-based beverages, herbal based beverages, and sports and energy beverages (Nazhand et al., 2020) (Figure 2). These beverages are useful in enhancing heart health, bolstering immune function, help digestion, and providing an energy boost (Kregiel, 2015). Additionally, functional foods and beverages play a crucial role in the sports industry, as an increasing number of athletes incorporate them into their diets to enhance their exercise performance and minimize oxidative stress (Kolonas et al., 2023).

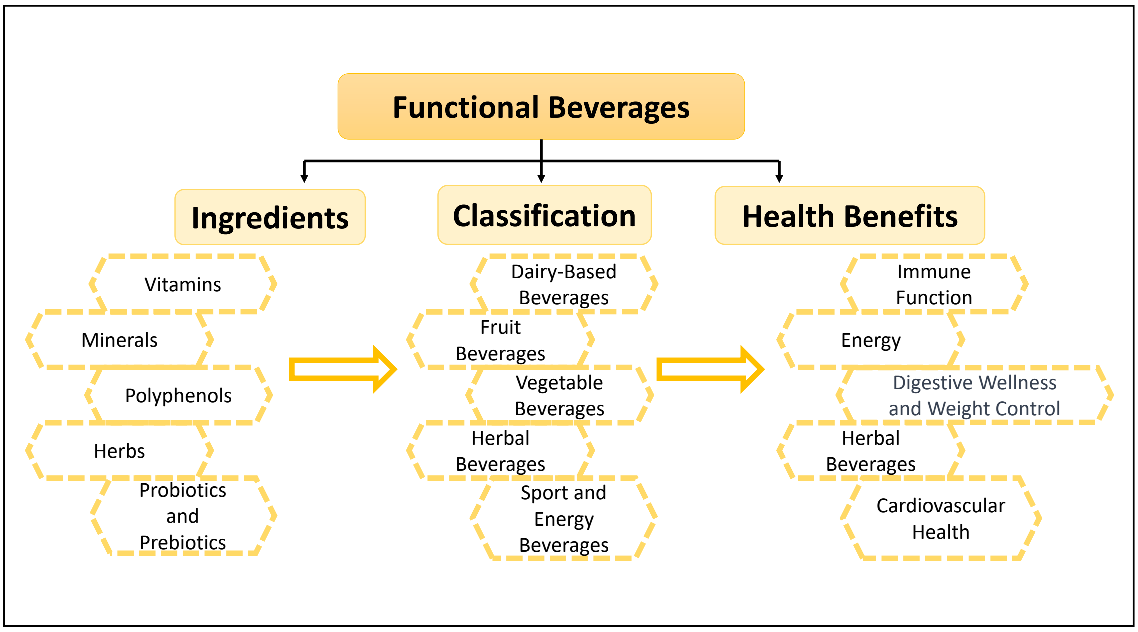


Figure 2. Classification, ingredients, and health benefits of functional drinks.

2.3. Sport drinks

2.3.1. Historic of sports drink

Nutrition and sport are crucial components for sustaining a healthy lifestyle and over the past two decades, there has been a notable increase in the number of studies linking sports and dietary factors (López-Martínez et al., 2022). Nutritional beverages aimed at enhancing athletic performance originated in Sweden during the 1930s. Researchers developed these beverages based on studies of carbohydrate and fat metabolism. Advancements in this field continued until the late 1960s. A Swedish research team conducted a study on the impact of muscle glycogen storage, utilization, and restoration during prolonged exercise to exhaustion. Their findings emphasized the positive effects of carbohydrates on endurance performance and demonstrated that glycogen levels and long-term exercise capacity could be manipulated through different post-glycogen depletion diets. Recognizing the risks of heat-related illnesses and dehydration, researchers at California University created a novel beverage with a balanced blend of carbohydrates and electrolytes. This beverage was designed to replenish vital elements lost through sweat during physical activity. Implementing this beverage with a football team yielded notably positive results. Subsequently, the realm of sports beverages witnessed advancements and variations, giving rise to numerous brands and companies such as Gatorade and PowerAde. This development paved the way for the emergence of a lucrative sports beverage industry, with sales surpassing $2 billion by the late 1990s (Galaz, 2013).

Recently, the global sports drinks market, valued at approximately US$25.9 billion in 2022, is expected to reach US$40.5 billion by 2030 with a 5.8% compound annual growth rate (CAGR) during the period from 2022 to 2030 (Figure 3). The isotonic segment is projected to have a 6% CAGR, reaching US$16.6 billion by the end of the analysis period. Additionally, the hypotonic segment is expected to grow at a 5% CAGR (Research & Markets, 2023). Gatorade® (PepsiCo Inc., U.S.A.), Powerade® (Coca-Cola Co., U.S.A.), and Accelerade® (Pacific Health Laboratories Inc., U.S.A.) are some examples of commercially available products (Corbo et al., 2014).

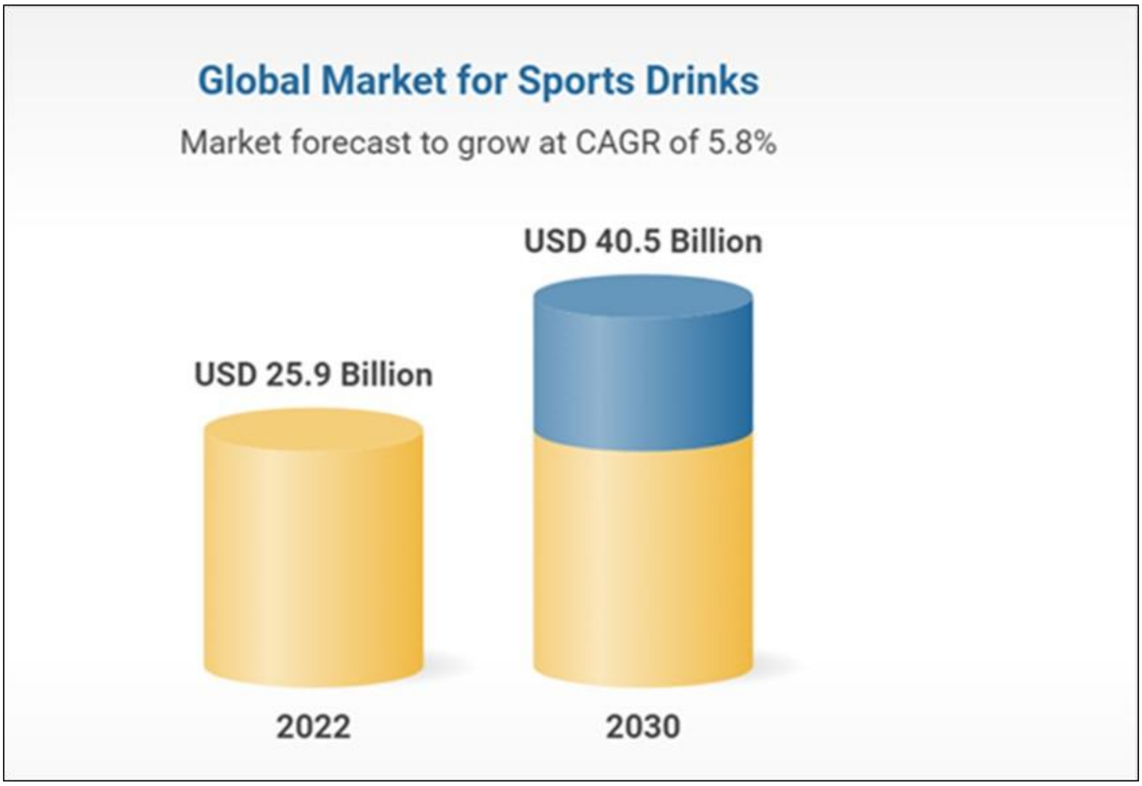


Figure 3. Global sports drinks market growth from 2022 to 2030 (Research and Markets, 2023).

2.3.2. Properties of sports drinks

Sports drinks, a class among the classes that belong to functional beverages, are essential for hydration, improving athletic performance, and addressing various health conditions. They can be tailored to enhance energy, focus, and alleviate joint pain. In sports, their primary function is to rehydrate athletes and replenish vital nutrients lost during exercise (Orrù et al., 2018). Although the composition of sports drinks may have minor differences, they typically consist of carbohydrates such as glucose, fructose, sucrose, and synthetic maltodextrins. These drinks also contain limited quantities of electrolytes like sodium, potassium, and chloride to enhance flavor and support the body's fluid and electrolyte equilibrium (Hamouda et al., 2016) . They have several advantages when consumed with intense exercise, however, excessive consumption of these beverages also comes with notable disadvantages (Table 2) (Chatterjee & Abraham, 2019).

Table 2. Advantages and disadvantages of sports drinks.

Advantages	Disadvantage
• Maintaining proper fluid levels and retaining vital electrolytes, essential for sustaining energy during physical activity and preventing health issues tied to fluid imbalances. • Continuous intake of carbohydrates and electrolytes during exercise provides athletes with ongoing energy and helps maintain plasma levels in their bodies during workouts. • Sports drinks can temporarily increase blood hemoglobin concentration.	• Dental health issues due to their acidic nature. • Potential negative effects from excessive carbohydrates leading to gastrointestinal problems. • The risk of hyponatremia due to excessive sodium loss.

Sports beverages can be categorized into three distinct groups (Figure 4) based on their fluid content, electrolyte levels, and carbohydrate composition, each with specific impacts on the body. Hypotonic drinks, which have a lower osmolarity than body fluids, are intended to boost the body's hydration levels. On the other hand, hypertonic drinks, with a higher osmolarity than body fluids, are primarily designed to reduce the body's hydration levels. Lastly, isotonic drinks, which closely match the osmolarity of body fluids, ensure that the body maintains an appropriate mineral balance. Isotonic drinks are the most widely preferred and account for the majority, being chosen in 99% of all instances when it comes to sports beverages (Sugajski et al., 2023) because they serve as a rapid replenishment for lost fluids, electrolytes, and carbohydrates needed to replenish glycogen stores lost through sweating (Maleš et al., 2022). The European Food Safety Authority (EFSA) has set the optimal osmolality range for isotonic beverages at 270-330 mOsm per kilogram of water. Beverages that fall below this range are hypotonic, while those that exceed it are hypertonic (EFSA, 2011). Isotonic beverages are marketed toward teenagers and young adults, promoted vigorously, and widely available in supermarkets, local shops, kiosks, and gas station convenience stores across nearly all European nations (Leśniewicz et al., 2016).

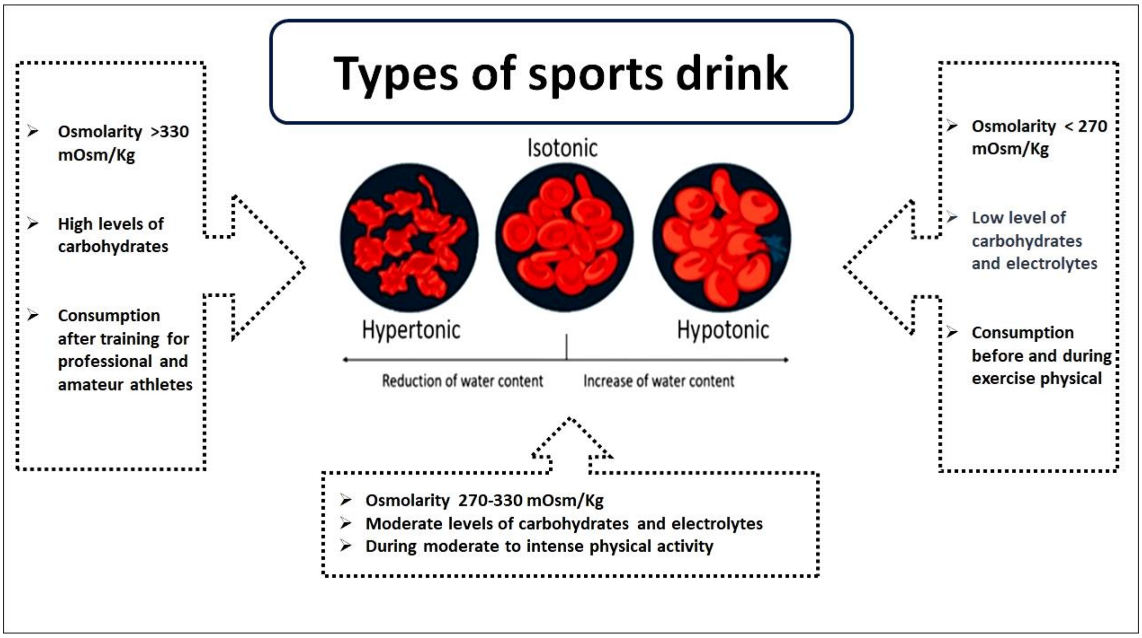


Figure 4. Types of sports drinks and their properties. Adapted from (Sugajski et al., 2023).

3. Fruit juice-based beverages

Free radicals, reactive oxygen species (ROS), and/or reactive nitrogen species (RNS) are necessary for the normal functioning of the human body and its organs. The balance of these radicals is maintained through a redox homeostasis within our body. However, the body may be occasionally affected by an oxidative stress resulting from an off-balance state. This stress can lead to the development of chronic degenerative diseases including coronary heart disease, cancer, and aging (Tena et al., 2020). In addition, regular physical activity is recognized for its ability to enhance the redox equilibrium in the human body and provide positive effects in countering the potential hazards associated with excessive diseases caused by reactive oxygen species. However, engaging in intense exercise could result in oxidative stress (Arazi et al., 2021; Kolonas et al., 2023). The impacts of physical activities on redox equilibrium are complex and depend on factors such as age, gender, training proficiency, alongside the intensity and duration of the exercise (Pingitore et al., 2015). The oxidative stress experienced during intense exercise can lead to inflammation and reduced exercise performance. Hence, the consumption of antioxidants like vitamin C and polyphenols combined with physical activities can reduce the adverse consequences of oxidative stress induced by high-intensity exercise. This combination boosts the body's exercise-related antioxidant defenses and enhances the positive effects of physical activity (Kolonas et al., 2023; Redha et al., 2022). Over the past decade, there has been an increasing focus on incorporating plant-derived antioxidant compounds into a variety of food items to help prevent diseases associated with oxidative stress. These antioxidants are sourced from various plants, especially fruits abundant in phytochemicals and recognized for their health-promoting properties (Todaro et al., 2023). Because fruit is a significant supplier of antioxidant compounds, juice has gained popularity among consumers as a simple and convenient method for fruit consumption (Wu et al., 2023). Several researchers have focused their interest on the innovation in isotonic beverages by developing new isotonic drinks with potential health benefits and biological activities in addition to their principal role which is providing hydration (Świtalski & Rybowska, 2021).

Nowadays, there is an increasing interest in developing fruit juice-based functional beverages in the food industry due to their nutritional value and the potential health benefits of the bioactive compound content which help to prevent the risk of chronic degenerative diseases such as cancer and cardiovascular disorders (Attanzio et al., 2022) due to their abundant vitamins, fibers, minerals, as well phytochemical compounds with antioxidants and anti-inflammatory effects (Martín et al., 2017; Samtiya et al., 2021; Shiekh et al., 2023). Moreover, fruit juices are also known for their palatability and a wide range of sensory attributes including pleasing taste and flavor, sweetness, acidity, and attractive colors. Therefore, when they are added to beverages with a fruit juice base, they can naturally contribute a variety of sensory attributes. On the other hand, blending juices presents an intriguing method for introducing new flavors into the beverage sector, which always seeks novel products. Additionally, the infusion of herbal extracts into fruit juices can enhance not only the nutritional value of the beverage but also balance it in terms of color, acidity, flavor, and palatability to produce consumer-pleasing products. It is widely believed that incorporating herbal extracts into fruit juices does not negatively affect consumer satisfaction with the product (Maleš et al., 2023).

4. Grape juice and herb extract beverages

4.1. Grape juice

Grape juice was chosen as the base of functional beverages, combined with mineral water, lemon juice, herb and spice extracts (Hop, Tea, Mint, and Ginger), and salt (sodium and potassium). Figure 5 illustrates the role of the selected raw materials and their contribution in the elaboration of these beverages. Furthermore, the following sections will highlight the crucial characteristics of each selected ingredient.

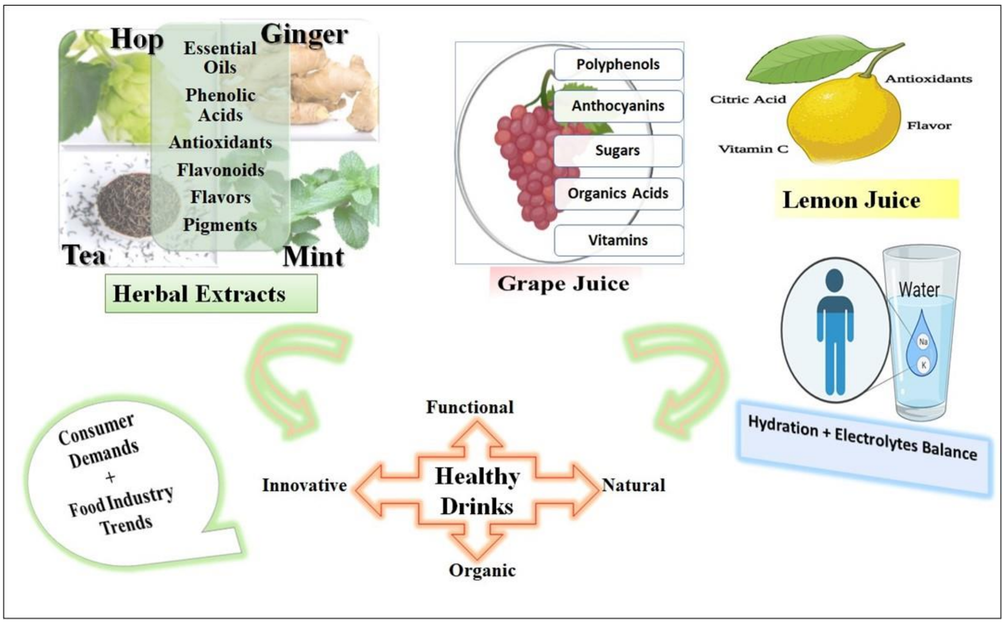


Figure 5. Role of raw materials (grape juice, lemon juice, herb extracts, water, and salts) in the beverage's elaboration.

However, the third chapter of this thesis represents the published mini-review article which includes the properties of grape juice and highlights how it contributes to the development of isotonic sports drinks. By enhancing both the functionality and health advantages of these drinks, in addition to promoting body hydration. Moreover, grape juice contributes to the creation of naturally sweetened and visually appealing beverages, without the need for added sugars or artificial coloring. This is primarily attributed to its abundant sugar, nutrients, and phenolic compound content, mainly anthocyanins which are key components in this study because they played a crucial role by giving a naturally appealing red color for the drinks. Figure 6 provides an overview of the grape juice production process, its composition, and the positive health effects associated with its consumption.

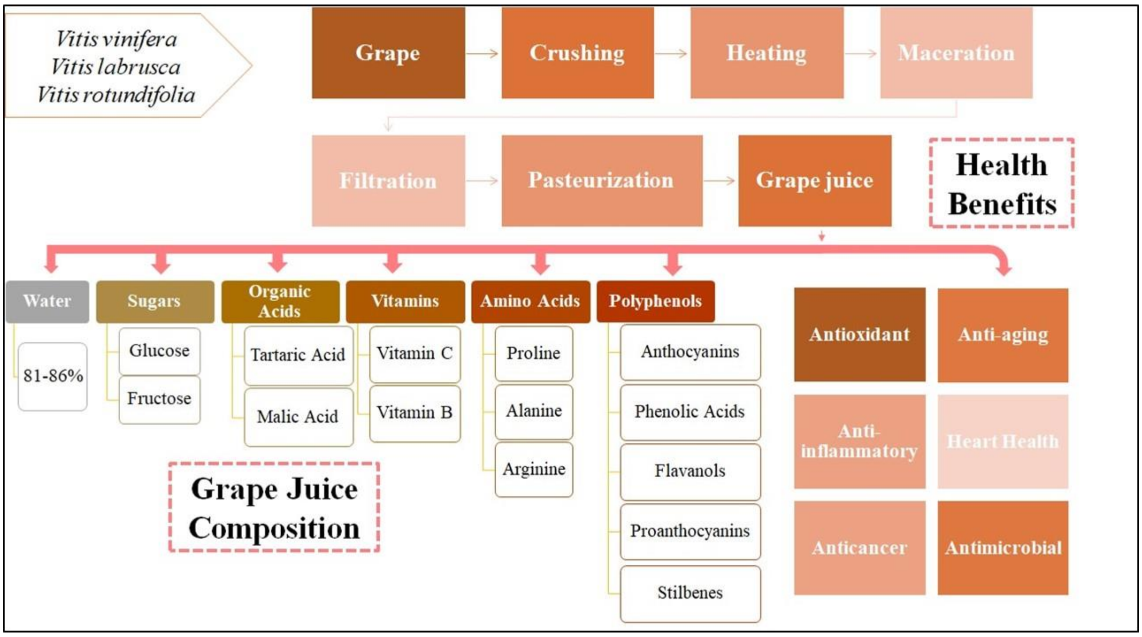
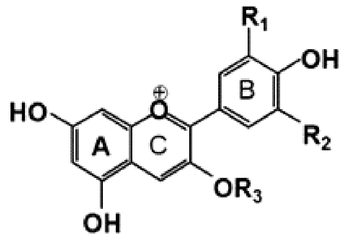


Figure 6. Grape juice production, composition, and positive health effects.

4.1.1. Anthocyanins

The name "anthocyanin" is of Greek origin, where "anthos", meaning "flower", is combined with "kyanos", which refers to the color "blue" (Salehi et al., 2020). Anthocyanins are water-soluble polyphenolic pigments belonging to the class of flavonoids (Matera et al., 2015). Anthocyanins are crucial for the growth of plant organs, pollination, and seed distribution. They also demonstrate the capacity to adjust to challenges like pathogenic attacks, drought, nutrients deficiency, and intense light (Cunha et al., 2023). Nowadays, they are considered potential alternatives to synthetic dyes in the food industry, used as natural colorants and increase the attractiveness of foods, since they are responsible for bright red, purple, and blue colors commonly found in flowers, leaves, fruits, and vegetables, and they are water-soluble and therefore can be incorporated into aqueous food systems (Brenes et al., 2005; Mattioli et al., 2020; Tan et al., 2021). They have a common chemical structure consisting of two aromatic rings (two C-6 benzoyl) attached to a flavylium cation (2-phenylbenzopyrylium) in the center by a heterocyclic C-3 ring (Figure 7) (Chung et al., 2016; Tan et al., 2021). However, the number, nature, and position of sugars bound to the molecule, the nature and number of aromatic acids linked to sugars, and the number of hydroxyl and methoxy groups create the differentiation between anthocyanin types (Morata et al., 2019). In addition, the color of anthocyanins depends on the type of substitution in the B-ring and the acylation ways which influence the electron density and the observed color (Morata et al., 2021). There have been over 600 different anthocyanins identified, and within this group of pigments, the glycoside forms of cyanidin, petunidin, peonidin, delphinidin, pelargonidin, and malvidin are the most frequently found anthocyanins in the nature representing about 90% of all the identified anthocyanins (Chaiyasut et al., 2016; Cunha et al., 2023; Tang et al., 2023). Cyanidin is a reddish-purple pigment (magenta color) found mainly in berries and red vegetables. Peonidin is also abundant in berries and grapes and is magenta-colored methylated anthocyanin. Pelargonidin makes the fruit red and the flowers orange. The color of delphinidin ranges from bluish reddish to purple, giving the plant's flowers their blue color. Petunidin is a dark red or purple methylated anthocyanin which is predominantly found in blackcurrant and purple flowers. Malvidin is an O-methylated anthocyanin with a purple hue that gives certain flowers their blue color (Enaru et al., 2021). The predominant anthocyanins in fruit are cyanidin, pelargonidin, and delphinidin, while peonidin, petunidin, and malvidin are often found in flowers (Morata et al., 2019).



Name	R_1	R_2	R_3
Delphinidin	OH	OH	H
Petunidin	OH	OCH_3	H
Malvidin	OCH_3	OCH_3	H
Cyanidin	OH	H	H
Peonidin	OCH_3	H	H
Pelargonidin	H	H	H

Figure 7. Structure of anthocyanins R3 = sugar and anthocyanidins R3 = H. (Tena & Asuero, 2022).

Nonetheless, the composition and concentration of anthocyanins vary among blue, red, and purple fruits, vegetables, and flowers. Berries, currants, grapes, and select tropical fruits have the highest anthocyanin concentrations among fruits, while among edible vegetables, leafy greens, grains, roots, and tubers exhibit the highest levels of anthocyanins. Additionally, anthocyanins can be found in various plant parts, including stems, leaves, and storage organs (Tena et al., 2020). Each red fruit has a unique anthocyanin profile that contribute to its unique characteristics (Chandra et al., 2021). In grapes, anthocyanins are the predominant flavonoid compounds, and they are predominantly located in the grape skin, although a few grape varieties have them in the pulp (Mirzaee et al., 2016). The color spectrum of these anthocyanins ranges from a brownish-red to purple hue, and their peak absorbance occurs between 518 nm (for cyanidin) and 528 nm (for malvidin) (Morata et al., 2021).

Besides being used to color food products, anthocyanins have the potential to function as nutraceutical components, providing a range of health advantages. These benefits encompass their antioxidant properties, capacity to combat oxidative stress, prevention of cardiovascular and neurodegenerative diseases, management of metabolic disorders, diabetes prevention, obesity control, promotion of visual health, antimicrobial effects, antiviral activities, anti-inflammatory properties, and additional attributes such as anti-ulcer, anti-thrombotic, anti-allergenic, anti-coagulant, anticancer, immunomodulatory, vasodilatory, and analgesic properties (Khoo et al., 2017; Mattioli et al., 2020; Salehi et al., 2020; Serea et al., 2022).

Anthocyanins have low stability and can be degraded during various stages, including extraction, purification, food processing, and storage. To address this issue, it is essential to employ a range of methods, such as physical, chemical, and biological approaches, to enhance the stability and therefore the color of anthocyanins in various applications (Enaru et al., 2021; Morata et al., 2023; Tang et al., 2023).

4.2. Water and minerals

Water is the major constituent of the human body, in adult men and women, it makes up over 60% and 55% of body weight, respectively, and around 75% in children. Water constitutes cells, tissues, and organs, and distributed in intracellular and extracellular compartments (Lorenzo et al., 2019). It maintains hydration and serves many functions, including providing structural support, acting as a solvent and reagent in chemical processes, and is essential for cellular homeostasis. Water is also important for transporting nutrients to cells, maintaining of electrolyte balance, controlling body temperature, removing waste, and allowing proper blood circulation, which is necessary for all body systems to function effectively (Jéquier & Constant, 2010; Palma et al., 2015; Popkin et al., 2010; Zhang et al., 2019). Water is obtained from all the foods and drinks we consume and from all the consumed fluids, including water, juice and fruit drinks, tea and coffee (Benelam & Wyness, 2010). The average daily body water requirement is 2.5 L, however, this amount varies depending on physical activity and temperature (Sugajski et al., 2023). To maintain normal cardiovascular and thermoregulatory function and to lower body temperature, adequate hydration is required. It also supports the appropriate operation of the necessary homeostatic processes and ensures the maintenance of physiological functions during exercise and regular daily activities (Vanderlei et al., 2015). However, it is essential to maintain sufficient hydration by regularly taking fluids while exercising (Péronnet, 2010). Mineral water and isotonic solutions with electrolytes and glucose are both options for fluid administration (Butar-Butar et al., 2022).

Even while water is generally thought to be good for health, consuming large amounts of it to make up for fluid lost during exercise might cause feeling full, increased urination, a decrease in plasma salt levels, and the possibility of water-related health problems. Water intoxication is a problem that develops when a person consumes too much water, diluting vital electrolytes like sodium and potassium, which can cause exercise to end prematurely (Coso et al., 2008; Gujar & Gala, 2014). Sports drinks which are typically consumed in conjunction with sports or exercise, whether in preparation for exercise, during exercise, or as a recovery drink after exercise, are primarily composed of water and supplemented with other key ingredients such as sodium and carbohydrates (Shirreffs, 2009). Sports drinks are therefore highly popular among athletes since they hydrate and prevent dehydration, supply mineral salts, mainly sodium and potassium, provide carbohydrates, and boost the absorption of water by the combination of these mineral salts and carbohydrates, which can increase the performance of the body (Gujar & Gala, 2014; Ruiz & García, 2022; Sadowska et al., 2017; Urdampilleta & Gómez-Zorita, 2014).

4.3. Lemon juice

Lemons (*Citrus limon* (L.) Burm. f.), the third-most important *Citrus* crop species, are among the most significant horticultural crops (Gironés-Vilaplana et al., 2012). Lemon juices are characterized by their nutritious value and unique flavor (Buvaneshwari et al., 2020). Lemon juice is known for its high content of vitamin C and citric acid which contribute to the sour taste feature. It is also rich in various phenolic compounds, including flavonoids such as quercetin, naringin, and luteolin, as well as various phenolic acids. Lemon juice also contains a wide range of essential nutrients, including minerals, essential oils, aminoacids and fiber (Klimek-szczykutowicz et al., 2020; Tsai et al., 2022). These substances have a number of anti-oxidative, anti-inflammatory, anticancer, and antibacterial properties that protect against a variety of illnesses, including obesity, diabetes, blood lipid disorders, cardiovascular diseases, and some types of (Buvaneshwari et al., 2020; González-Molina et al., 2010). Due to these characteristics and properties, lemon juice was included as one of the main ingredients in the raw materials for the formulation of the beverages. The aim was to take advantage of the natural acidic properties of lemon, avoiding the use of chemical acidulants, which help to enhance the flavor, provide the necessary acidity, and enhance the stability. Moreover, to achieve the desired red color and stability of anthocyanins, it is crucial to maintain an acidic pH level (Hani et al., 2019).

4.4. Herbs and spices

To enhance the flavor of the beverage and enrich the sensory qualities of the beverage while retaining their natural desired properties, we selected herbs and spices for the extraction of natural flavors and evaluated their impact on the sensory properties. Moreover, plant extracts have gained recognition for their dual role in the food industry, offering both flavor enhancement and the presence of bioactive compounds, thus adding nutritional value to food products (Salehi et al., 2018). The main components of the herbs used for the flavors extraction and their chemical structures are summarized in Table 3.

Table 3. The main chemical components of herbs used for the extraction of flavors.

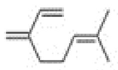
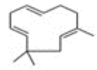
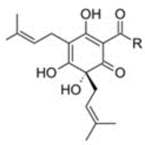
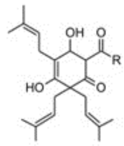
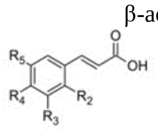
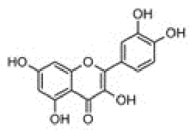
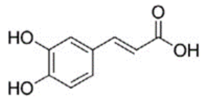
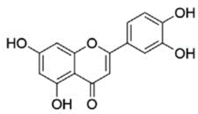
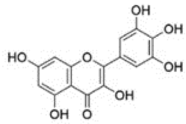
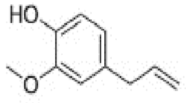
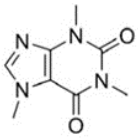
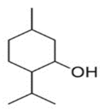
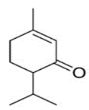
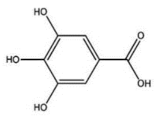
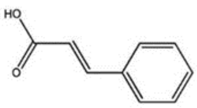
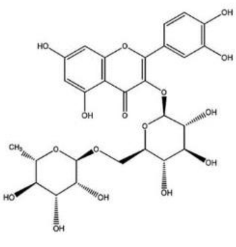
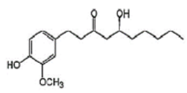
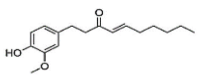
	Components	Chemical structures	References
Hop	**Volatile oils**	 Myrcene  α-Humulene	(Sanz et al., 2019)
	Bitter acids	 α-acids  β-acids	
	Phenolic Acids	 Cinnamic acids	
	Flavonoids	 Quercetin	

Table 3. The main chemical components of herbs used for the extraction of flavors.

Tea	**Phenolic acids**	 Caffeic acid	(Liu et al., 2023)
	Flavonoid	  Luteolin Myricetin	
	Volatile oil	 Eugenol	
	Alkaloids	 Theophylline	
Mint	**Essential Oils**	  Menthol Piperitone	(Bahadori et al., 2018; Salehi et al., 2018)
	Phenolic Acids	  Gallic acid Trans-Cinnamic acid	
	Flavonoids	 Rutin	
Ginger	**Essential oils**	  Gingerol Shogaol	(Hessien et al., 2013; Srinivasan, 2017)

4.4.1. Hop

Hop plants are classified within the Cannabaceae family, and they are scientifically known as *Humulus lupulus* (Arruda et al., 2021), primarily cultivated in colder climates, particularly in northern countries (Almeida et al., 2019). While they originally come from Europe and western Asia, they are actively cultivated in various regions worldwide, including North and South America, Africa, Asia, and Australia (Tardío et al., 2016). Germany is the primary producer in the European Union, accounting for 41.79 tons of the 57.24 tons produced by member states in 2018 (Astray et al., 2020). They are commonly used as basic ingredients in the brewing industry, where they play a key role in creating the bitter flavor and the characteristic hoppy aroma (Di Sotto et al., 2018). The primary significant constituents of hop include the essential oil, the bittering acids (alpha-acids and beta-acids), and the phenolic compounds (flavonoids) (Almeida et al., 2019). The essential oils in hops provide a diverse range of fragrances, including herbal, fruity, spicy, floral, and citrus-like aromas. While α-acids are the main source of bitterness in hop. The total bitterness of the hops is also influenced by a number of additional factors, such as the oxidation by-products of both α- and β-acid. Polyphenols improve the mouthfeel and body (Sanz et al., 2019). In addition to their aromatic qualities, essential oils from hop have been known to have sedative, anxiolytic (anxiety-reducing), and relaxation-inducing effects (Su et al., 2023). Bioactive compounds of hop exhibit various biological activities, including antioxidant, antimicrobial, antifungal, antiviral, anti-inflammatory, and anticancer properties (Di Sotto et al., 2018). Given their health benefits and aromatic qualities, hops could find application in both the food and health industries(Salanță et al., 2023). The non-alcoholic beverage industry is undergoing significant development due to its distinct flavors, ease of consumption, and health-related benefits. This creates the potential for hop-derived products to be among the innovative offerings in this evolving market (Su et al., 2023).

4.4.2. Tea

Tea, scientifically known as *Camellia sinensis*, from the family Theaceae, is an indigenous plant that originated in the regions of East and South Asia. This remarkable plant has received much attention not only for its rich heritage as a traditional beverage consumed worldwide, but also for its numerous medicinal uses (Brimson et al., 2023; Cisneros-Yupanqui & Lante, 2020). Tea is classified into six primary categories, and the distinction among them is determined by the fermentation process applied. These categories encompass green tea (unfermented), white tea (featuring light fermentation), yellow tea (showing mild fermentation), oolong tea (exhibiting semi-fermentation), black tea (displaying full fermentation), and dark tea (undergoing post-fermentation) (Jiang et al., 2020). Due to its appealing color, unique flavor, and energizing impact on the body, tea is the most popular beverage in the world. The chemical constituents of tea have a significant impact on defining the aroma, color, and flavor of the beverage. Additionally, soluble tea powders have the potential to be used as natural enhancements for a wide range of food and beverage products (Baruah et al., 2012). Polyphenols, caffeine, organic acids, and volatile terpenes are the primary chemical constituents that are responsible on the taste and flavor of tea (Senanayake, 2013). The chemical component in tea (catechins, phenolic acids, flavones, flavonols, alkaloids, free amino acids, and pigments) provide a range of biological functions and health advantages, such as antioxidant, anti-inflammatory, anticancer, cardio-vascular protective, hepatoprotective, anti-diabetic, anti-obesity, and neuroprotective properties (Senanayake, 2013; Shang et al., 2021; Yan et al., 2020). However, the composition of tea is influenced by agricultural practices, climate, season, and the specific type and age of the tea plant (Hayat et al., 2015; Liu et al., 2023).

4.4.3. Mint

Mint is a perennial herb widely distributed throughout the world, known for its aromatic and medicinal qualities, and it is classified within the Lamiaceae family as part of the *Mentha* genus (Saqib et al., 2022). The primary regions for the Mentha genus include North Africa, Australia, Europe, and Central Asia (Bouyahya et al., 2020). Due to the significant morphological diversity of mint plants, taxonomists have given them a wide range of various taxonomic names over the last two centuries. The complicated taxonomy of the genus Mentha makes it challenging to identify species within it. Additionally, most mint cultivars can be used to create hybrids (Mamadalieva et al., 2020). There are around 42 species, 15 hybrids, numerous subspecies, and cultivated variations included in the classification of Mentha genus (Tafrihi et al., 2021). The most well-known Mentha species include *Mentha aquatic* L. (watermint), *Mentha spicata* L. (wildmint), *Mentha arvensis* L. (spearmint), *Mentha longifolia* L. (horsemint), *Mentha pulegium* L. (pennyroyal), and *Mentha* x *piperita* L. (peppermint), a naturally sterile hybrid between watermint and spearmint (Silva, 2020). However, the various species of mint differ in several characteristics. These variations include plant height, which ranges from compact to tall. They also extend to the color of the leaves, from light green to dark green with hints of purple. There are also differences in leaf length, ranging from short leaves to elongated leaves. Leaf surfaces also vary, from smooth, glabrous texture to hairy quality. In addition, there are differences in inflorescence shape, which can range from scattered verticillasters to densely packed spike-like inflorescences. Finally, the flower color has a wide spectrum, ranging from almost white to shades of purple (Zeljković et al., 2021). Since ancient times, mint has been valued and widely used for its medicinal and aromatic characteristics (Park et al., 2019). Traditionally, the leaves, flowers, and stems of mint are commonly employed in herbal teas and added to commercial spice blends for various foods to enhance both flavor and taste (Prakash et al., 2016). Currently, mint is widely used for a variety of purposes, and its economic significance is demonstrated by the fact that it can be found in a variety of daily products, such as taste enhancers, cosmetics, medications, and oral hygiene products. In addition, numerous pharmacological and medicinal advantages of the herb have attracted a lot of study interest (Shaikh et al., 2014). Mint has garnered increasing research attention due to its potential therapeutic benefits, which encompass its effects on various bodily systems such as the digestive, circulatory, cardiovascular, urinary, gastrointestinal, and neurological systems. Additionally, mint is recognized for its antibacterial, antioxidant, and anti-inflammatory properties (Silva, 2020). Numerous essential oils were identified in the composition of mint extract, including menthone, menthol, piperitone, and piperitol. Additionally, mint extract includes a number of polyphenols, such as phenolic acids like gallic acid, ferulic acid, cinnamic acid, chlorogenic acid, rosmarinic acid, and caffeic acid, as well as flavonoids like luteolin, diosmin, catechin, quercetin, epicatechin, and rutin (Brahmi et al., 2017; Mimica-Dukic & Bozin, 2008; Park et al., 2019; Salehi et al., 2018). The majority of the active ingredients of mint are concentrated in the leaves, which can be utilized fresh or dried and subjected to solvent extraction or steam distillation to produce mint essential oils (Silva, 2020).

4.4.4. Ginger

Ginger, scientifically known as *Zingiber officinale* Roscoe and part of the Zingiberaceae plant family, has a long history of medicinal and culinary use across the globe. It has been utilized in traditional folk medicine in regions such as Southeast Asia, Greco-Roman traditions, Brazil, Australia, Africa, China, India, Bangladesh, Taiwan, Mexico, Japan, Jamaica, the Middle East, and parts of the United States, where its rhizomes are grown for medicinal purposes (Shahrajabian et al., 2019). Ginger is a popular spice in both food and beverages due to its distinctive spiciness and spicy flavor. It is commonly used in a wide range of meals, carbonated drinks, and as a preservative condiment in sugar syrup (Srinivasan, 2017). Traditional treatments have utilized ginger to treat a variety of ailments and symptoms, including arthritis, rheumatism, headaches, nausea, upset stomach, diarrhea, and colds. It is also used as a carminative, antiflatulent, and digestant. Additionally, ginger is well-known for its pharmacological activity against toxins caused by natural, chemical, and radiation exposure, including its radioprotective, hepatoprotective, nephroprotective, neuroprotective, gastroprotective, and reproductive system-protective properties (Kiyama, 2020). The components of ginger vary depending on its production origin and whether the rhizome is fresh or dried (Ali et al., 2008). More than 400 bioactive chemicals are present in ginger. Gingerols and shogaols are the key phenolic compounds contributors to ginger's bioactivity, however it is also rich in organic acids, terpene, lipids, and fibers. Additionally, the primary volatile oils of ginger extract are mono- and sesquiterpenes, while the non-volatile oils are the phenolic compounds (Dalsasso et al., 2022). Gingerols are responsible for the pungency of fresh ginger, while the pungency of dried ginger primarily results from shogaols, which are dehydrated forms of gingerols. Shogaols are formed from gingerols through thermal processing, and in fresh ginger roots, gingerols are the primary constituents, while dried and heat-processed ginger roots contain shogaols in smaller quantities (Ali et al., 2008; Ghasemzadeh et al., 2018).

5. Thesis objectives

5.1. General aim

The purpose of this thesis is to develop beverages that can be included in the category of functional and sports beverages, offering health advantages, in addition to their principal function of promoting hydration, primarily through the nutritional value, biological properties, and sensory characteristics provided by the selected raw materials (Grape juice, water, lemon juice, herbs, minerals) and to evaluate the physicochemical and sensory properties of the final products.

5.2. Specific objectives

- To create innovative, organic, and functional beverages that fit the preferences of consumers and align with the current trends in the food industry for health-conscious products.

- To develop chemical-free beverages using grape juice as natural sweetener and as a source of red color provided by its anthocyanins, followed by an assessment of their stability.

- To use herbal extracts as natural flavors which enhance both beneficial effects and sensory attributes in grape juice-based drinks.

- To combine natural sources of energy, mineral, and antioxidants in a beverage which can be catalogued by its characteristics as an isotonic drink suitable for athletes.

CHAPTER 2. METHODOLOGY

2.1. Experimental design of the thesis

To extend our knowledge of how grape juice and herbal extracts can contribute to the development of novel beverages, several research projects have been developed, each connected to a specific publication (Table 4).

Table 4. Summary of the experimental design of the thesis

GENERAL AIM	To create natural innovative drinks that offer both health advantages and appealing sensory characteristics by using grape juice and herbal extracts.		
STUDY AND CHAPTER	Study 1 (Chapter 3)	Study 2 (Chapter 4)	Study 3 (Chapter 5)
MANUSCRIPTS	Contribution of Grape Juice to Develop New Isotonic Drinks with Antioxidant Capacity and Interesting Sensory Properties	Elaboration of Organic Beverage Based on Grape Juice with Positive Nutritional Properties	Isotonic Drinks Based on Organic Grape Juice and Naturally Flavored with Herb and Spice Extracts
SPECIFIC AIM	To expand the knowledge about qualities of grape juice and its role in creating sports drinks with functional attributes	To study the potential of developing healthy beverages free of additives using organic grape juice enhanced by herb and spice extracts	To design rich antioxidants isotonic drinks from grape juice enriched with minerals and sensorily improved with herbal extracts
SAMPLES		6 bottles of 500 mL × triplicates	14 bottles of 500 mL × triplicate

2.2. Beverages Preparation

The raw materials employed in the preparation of the beverages, all of which were food grade, are described in Table 4. Each sample for each trial was prepared and analyzed in triplicate, and for each group, a control beverage was made using similar ingredients but without the addition of flavor extracts or salts. The initial experiment of this thesis focused on developing healthy beverages, applying preliminary experiments to identify the suitable formulation. This selection was conducted by sensory assessments with the greatest goal of achieving beverages with the desired sensory characteristics, including acidity, sweetness, color, and flavors. Two groups of beverages were prepared by diluting concentrate red grape juice (47 mL) with two distinct types of mineral water (BA: low-mineral water and BB: high-mineral water). (To evaluate the effect of water mineralization on the physicochemical and sensory characteristics). Lemon juice (7 mL) was used to adjust the pH and provide an acidic taste to the beverages. In order to enhance the flavor of the beverages and provide specific aromas and flavors, we opted for the extraction of flavors from hop, red tea, and dried mint. These extracts were made naturally by crushing the weighted herb (4 g) and blending it with diluted concentrated grape juice (30 mL). To extract the aroma, the mixtures experienced sonification with ultrasound equipment (3300EP Sonica) for 10 minutes, followed by centrifugation (Eppendorf, 5430 R) at 6000 rpm at 20°C for 10 minutes. Subsequently, each extract was filtered using filter papers. The obtained extracts were used through the following combinations: 2 mL of hop extract with 1 mL of tea extract (hop-tea) and 2 mL of hop extract with 0.5 mL of mint extract (hop-mint). Finally, the obtained samples within each of the two groups, A and B, consisted of the following categories: control samples (BAN/BBN) (without any added flavor), samples flavored with hop-tea extracts (BAHT/BBHT), and samples flavored with hop-mint extracts (BAHM/BBHM). In total, there were six distinct sample types.

In the second trial of the thesis, isotonic drinks were designed, maintaining the same beverages composition as those produced in the initial trial. However, in this instance, determined concentrations of salts were introduced into the formulations to make the drinks isotonic and more suited for athletes. Two sets of drinks (A and B) were prepared, and for each of the previous flavored beverage (BAHT, BAHM, BBHT, and BBHM), three different concentrations of salt (sodium chloride/potassium chloride) were added (C1: 0.5 g/L Na; C2: 0.5 g/L Na + 0.2 g/L K; C3: 0.3 g/L Na + 0.2 g/L K. Therefore, for each group, we obtained 3 flavored samples with hop-tea with varying salinities (Group A: BAHTC1, BAHTC2, and BAHTC3; Group B: BBHTC1, BBHTC2, and BBHTC3). Similarly, 3 flavored samples with hop-mint also with different salt concentration were prepared (Group A: BAHMC1, BAHMC2, and BAHMC3; Group B: BBHMC1, BBHTC2, and BBHMC3). The two control samples were without flavors and salts. The prepared samples were thermally treated by autoclave (100°C for 5 min) then they were stored under refrigeration at 4°C until physicochemical and sensory analysis. The experimental design of the two trials is shown in Figure 8.

Table 5. The raw materials used in the preparation of the beverages.

Ingredient	Characteristics	Brand
Grape Juice	Concentrated red grape juice with pH 3.5 and 65 °Brix	Vinos y Bodegas
Water	Low-mineral water with 28 mg/L of dry residues High-mineral water with 278 mg/L of dry residues	Bezoya, Calidad Pascual Solan de Cabras
Lemon Juice	Pasteurized squeezed lemon juice with 40 mg/L of vitamin C and 10 mg/L of sodium.	Hacendado
Hop	Hop cones	Summit
Tea	Red tea	Cafetearte
Mint	Dried mint	Cafetearte
Table Salts	Sodium chloride Potassium chloride	Sal Costa Aranca

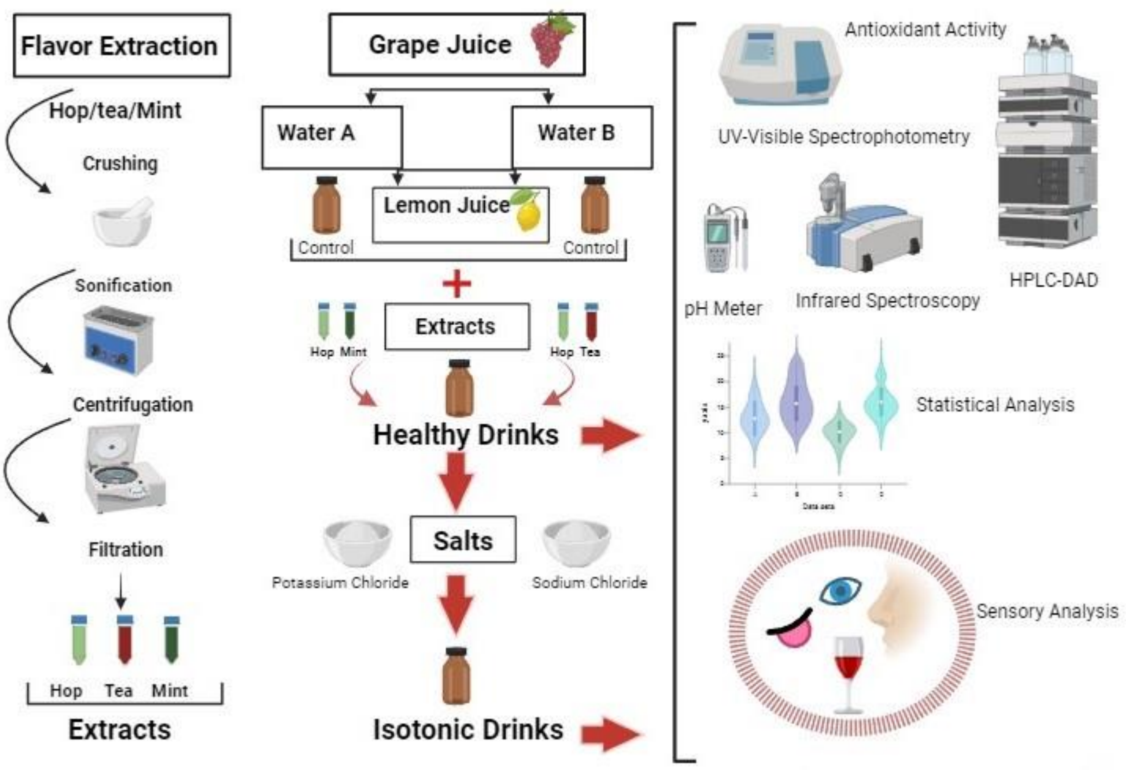


Figure 8. An overview of the experimental design of the thesis.

A third trial was conducted (Appendix I), new isotonic drinks were prepared by using only one type of water, the high mineral water that was used in the previous experiments (Solan de Cabras), combining ginger extract with the other previous extracts and based on preliminary experiments and sensory analysis which were conducted to determine the suitable composition of the isotonic beverages trying different concentrations of grape juice, lemon juice, herb and spice extracts, and salts, the composition in terms of sweetnes, acidity, and salinity was determined being for 500 mL: 40 mL of grape juice, 13 mL of lemon juice, 0.15 g of sodium chloride, and 0.10 g of potassium chloride. In terms of flavor and aroma the extracts used were ginger (3 mL), hop (2 mL), tea (1 mL), and mint (0.5 mL). A total of eight different samples were prepared consisting of four control samples that did not contain salts and four samples containing sodium and potassium (isotonic drinks). The composition of these samples varied based on the types of extracts used, which included samples flavored with only ginger extract and samples flavored with ginger-hop, ginger-tea, and ginger-mint. The control samples were labeled CGLG, CGLGH, CGLGT, and CGLG, while the isotonic drinks were labeled IGLG, IGLGH, IGLGT, and IGLG. These labels corresponded to the following elements: CG: control grape juice; IG: isotonic grape juice; L: lemon juice; GH: ginger-hop; GT: ginger-tea; GM: ginger-mint.

2.3. Color Parameters and Total Phenolic Index

The measurements were performed using an Agilent 8453 spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) with a 1 mm optical path glass cuvette. The absorbance values at 280 nm, 420 nm, 520 nm, and 620 nm were determined. The color intensity (CI) was calculated as the sum of absorbances at 420 nm, 520 nm, and 620 nm. Tonality (T) was determined by calculating the ratio between the absorbance values at 420 nm and 520 nm. The red color (RC) was defined as the absorbance value at 520 nm, while the total phenolic index (TPI) was represented by the absorbance at 280 nm (Burin et al., 2010; Milella et al., 2019). In the fourth article, the CI, RC, T as well as the chromatic parameters, hue (h) and chroma (C), and luminosity (L) were obtained with the DNA Phone Smart Analysis (Parma, Italy).

2.4. Physicochemical Parameters

The Fourier transform infrared spectroscopy, OenoFoss™ (Foss Iberia SA, Barcelona, Spain) was used to identify and quantify some interesting parameters such as sugar concentration, total acid, total soluble solids, malic acid, alpha amino acids, and ammonia. The pH measurements were conducted using a Crison brand pH meter, GLP 21 model (Hach Lange Spain, S.L.U., Madrid, Spain).

2.5. Determination of Anthocyanins

The anthocyanins were identified and quantified using a series 1200 high-performance liquid chromatography (HPLC) set (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array detector. The samples were filtered through a 0.45 µm membrane and injected in a volume of 50 µL. 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) was used 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 (r2 = 0.9999, LOD = 0.1 mg/L) (Escott et al., 2017).

2.6. **Determination of Antioxidant Activity**

The $ABTS^{+}$ method described by Re et al. (Re et al., 1999) was used to evaluate the antioxidant activity (AOX) of the isotonic drinks in the second and third trials. 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) were used. A solution of 7 mM ABTS was mixed with 2.45 mM of potassium persulfate and incubated for 16 hours in the dark to generate the $ABTS^{\cdot+}$ radical solution. The radical solution absorbance was adjusted to 0.70 ± 0.02 at 734 nm. The diluted samples were mixed with the $ABTS^{\cdot+}$ radical solution and incubated for six minutes in the dark. Then, the absorbance was measured at the wavelength of 734 nm. Trolox was used as a standard, and the results were presented as µmols Trolox equivalents/mL. The 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.

2.7. Sensory Evaluation

The sensory evaluations conducted within this doctoral thesis aimed to evaluate the quality of the newly formulated beverages, investigate how the beverages composition can define their sensory attributes, examine the influence of water mineralization on taste and preferences, compare various herbal extracts, and assess the contribution of combining different extracts with grape juice to the flavor and the overall perception of the beverages, and to determine which blended ingredients played a significant role in shaping the preference of the new beverages, particularly in terms of color, taste, and flavor. All sensory assessment of the prepared beverages in the first, second, and third trials involved a panel of eight trained panelists comprising individuals of various genders and ages ranging from 22 to 60. The evaluation took place in the tasting room of the Department of Chemistry and Food Technology of the School of Agricultural, Food and Biosystems Engineering (ETSIAAB) at Universidad Politécnica de Madrid (Spain). Each participant's table was set with glasses of the prepared beverages maintained at a temperature of 12 ± 2°C. An additional glass of water was provided to clean the palate between samples. In the third trial, Aquarius, a popular lemon-flavored isotonic drink in Spain, was used as a reference for comparison with the newly prepared isotonic beverages. An evaluation sheet highlighting the interesting attributes was given to each taster. The panelists used a rating scale ranging from 1 to 5 to assess the intensity of various attributes. They evaluated the appearance of beverages, considering the intensity and hue of the red color which could range from pale to deep red as well as its clarity and turbidity. Aroma attributes were analyzed in terms of both intensity and quality, including herbal, floral, and fruity notes, while also noting any unwanted or less flavors related to reduction and oxidation. In the evaluation of mouthfeel, they took into consideration the strength of sweetness, which could range from subtle to intense, and assessed the presence of bitterness, the level of acidity ranging from gentle acidity to a stronger and pronounced acidity, they also evaluated the saltiness and the beverages body, such as thickness, mouthfeel, and textural properties. Finally, panelists considered the global perception considering both olfactory and gustatory aspects, along with the absence of any defects. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (UPM Ethics Committee) of Universidad Politécnica de Madrid (AIDLAPPMLB-AMB-HUMANOS-20221026 on November 14th, 2022).

2.8. Statistical Analyses

Microsoft Excel 2016 was used to calculate the means and standard deviations (SD) and to generate visualizations and graphical representations of the data. Statgraphics Centurion 18 software V.18.1.06 (Graphics Software Systems) was used for the assessment of analysis of variance (ANOVA) to compare means among groups, the execution of least significant difference (LSD) tests to pinpoint significant group differences, the creation of principal component analysis (PCA), the generation of informative violin charts, and the calculation of Pearson's correlations to estimate relationships between variables. XLSTAT statistical and data analysis solution (New York, USA) was used for the creation PCA in the first experimental work.

CHAPTER 3. ARTICLE 1

Contribution of grape juice to develop new isotonic drinks with antioxidant capacity and interesting sensory properties

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Abstract: Nowadays, the sector of isotonic beverages has developed its market based on fruit juices which provide a sports drink with antioxidant and biological activities in addition to their principal role of rehydration and replacement of minerals and carbohydrates during physical exercise. The consumption of grape juice is increasing worldwide because of its sensory characteristics and nutritional value. It contains mainly water, sugars, organic acids, and phenolic compounds. The phenolic compounds play a major role in the prevention of various diseases through their biological activities linked to antioxidant, anti-inflammation, anticancer, antiaging, antimicrobial, and cardioprotective properties. Several studies have demonstrated that grape juice is able to improve performances of antioxidant activity, protect against oxidative damage and reduce inflammation during sports activities. Polyphenol content also provides a great sensory profile, mainly color which is an important indicator for consumers when choosing beverage products. The contribution of grape juice through its nutritional value and sensory properties makes it an alternative for the development of a new isotonic drink which will be a novel and healthy product in the field of healthy beverages.
Keywords: grape juice; sport; minerals; carbohydrates; polyphenols

1. Introduction

The consumption of fruits and their juices plays an important role in a healthy diet (Tiencheu et al., 2021) because they are a source of free sugars and micronutrient (Ruxton & Myers, 2021). Even more, consumer tendencies to healthy eating habits (Chew et al., 2020) and the request for organic foods (Dyab et al., 2015) led to the production of new drinks from fruit juice as a source of nutrients and bioactive compounds (Gironés-Vilaplana et al., 2016). Nowadays, among challenges facing the food industry is the expansion in the creation of new products and persuading consumers to buy them. In this case, it is essential to recognize the needs and the consumer's demands while developing new and innovative products (Świtalski & Rybowska, 2021).
Several studies focused on the development of functional and healthy beverages based on fruit juice, among them, our previous study, we elaborated natural beverages based on red grape juice as a source of polyphenols, sugars, and with interesting organoleptic properties without chemical additives (Bendaali et al., 2022b). Another study by Najafabadi et al. (Najafabadi et al., 2021) in which a beverage was developed based on jujube fruit which is well known for its content in anthocyanins, organic acids, and phenolic compounds. A mixed fruit beverage was developed through sensory analysis by Bhalerao et al. (Bhalerao et al., 2020) using pomegranate, amla and muskmelon juice (Tiencheu et al., 2021) formulated and studied the nutritional, organoleptic,

physicochemical, phytochemical, microbiological and shelf-life of natural beverage formulated from orange (*citrus sinensis*), lemon (*citrus limon*), honey and ginger (*zingiber officinale*). New Beverages were also created by the team of González-Molina et al. based on lemon juice with elderberry and grape concentrates as a source of bioactive compounds (González-Molina et al., 2012). Accordingly, the sector of isotonic beverage has developed its market based on fruit juices (Galvão et al., 2020; Porfírio et al., 2020). Isotonic drinks are a group of functional beverages defined as products with beneficial effects on human health in addition to their nutritional effect (Świtalski & Rybowska, 2021). Furthermore, from the available recent studies related to isotonic drinks, researchers focused their interest on the development of isotonic beverages using fruit juice with antioxidant and biological activities. An isotonic beverage with functional attributes and as an innovative proposal was developed by Porfírio et al. (Porfírio et al., 2020) based on extracts of peel and pulp of *Myrciaria jaboticaba* which is considered as a richest source of anthocyanins. In other study by the group of Ferreira et al., the phenol extract of jabuticaba peel with whey ultrafiltration permeate were used in the development of an isotonic beverage (Ferreira et al., 2020). Gironés-Vilaplana et al. designed a new isotonic drink with high antioxidant capacity by mixed berries (maqui, açai and blackthorn) and lemon juice (Gironés-Vilaplana et al., 2013).

Given this context, the objective of this review is to provide an overview of the various characteristics of isotonic beverages, the importance of their minerals and carbohydrates content, and the contribution of fruit juice in improving nutritional value and sensory profile, concerning an isotonic beverage based on grape juice with good organoleptic properties.

2. Isotonic beverage

Loss of water and electrolytes and diminution of glycogen in the liver and muscles owing to an increase of the metabolic rate and increased heat, during physical activity, may causes dehydration which influences physical performance (Moreno et al., 2013) and can leads to early tiredness, cognitive changes, sodium deficit, and increases risk of heat-related diseases (Schneider et al., 2011). Hydration improves thermoregulation, assures the preservation of physiological functions, and helps the suitable functioning of the homeostatic mechanisms needed by the exercise (Vanderlei et al., 2015). Generally, water is the first option for hydration before, during, and after sport activities (Schneider et al., 2011). It has been documented that the ingestion of water for rehydration can provoke hyponatremia and its ingredients are insufficient to provide energy (Sadowska et al., 2017). In addition, intake of a high amount of water to replace fluid loss causes a feeling of fullness, urination, a decrease in plasma sodium, and early ending of exercise (Coso et al., 2008). Accordingly, it was mentioned that the best-attained hydration was by a mixture of water, carbohydrates, and electrolytes (Geraldini et al., 2017). In addition, consumers considered sports drinks more beneficial than water in terms of electrolytes, minerals, and physical performance (van Esch & Gadsby, 2019).

Sport drinks are beverages used before, during, and after physical exercises to replace water, electrolytes, and carbohydrates lost during exercise in order to keep the hydration and delay fatigue (Galaz, 2013; Marapana et al., 2017; Presta et al., 2021). They can be hypertonic containing a higher concentration of sugar and salt than those which are found in the human body, isotonic containing similar concentrations of sugar and salt as in the human body, or hypotonic containing lower concentrations of sugar and salt than those in the human body (Adoga et al., 2020; Galaz, 2013). For an adequate hydration, drinks must be isotonic (Urdampilleta & Gómez-Zorita, 2014). It is recommended the intake of isotonic drinks for intensive physical training, while for extremely intensive training, hypertensive drinks are recommended to speed up the restoration of energy reserves by their easily digestible carbohydrate content (Diel & Khanferyan, 2018). Water, carbohydrates, and electrolytes are the main components of isotonic drinks (AbuMoh'd, 2020; Gironés-Vilaplana et al., 2013). They may also contain vitamins, colors, flavors, and natural juices which improve the organoleptic characteristics (Pivnenko et al., 2018), and has a pH value close to 3.0 (Cerezal Mezquita et al., 2020). The taste, acidity, sweetness, and beverage temperature are features that made isotonic beverages more consuming than water, during sport activities (Galvão et al., 2020).

2.1 Mineral content

In current production, isotonic beverages are consist of natural or artificial source of electrolytes salts (Pivnenko et al., 2018), mainly ions of sodium, potassium, magnesium, chloride, and calcium (Stasiuk & Przybyłowski, 2017). Sodium is the main cation in the extracellular space and a large quantity of this cation can be lost through sweating (Evans et al., 2017). Salt is added to sports beverages in order to provide sufficient quantities of sodium and keep up its concentration and the volume of plasma (Zhang et al., 2021). Sodium plays a major role to adjust the balance of water in the body, transport of carbohydrates and proteins to tissues (Styburski et al., 2020), increase the intestinal absorption of carbohydrates (Bonetti & Hopkins, 2010; Coso et al., 2008), aid the active working muscle to contract and relax (Halder & Daw, 2020), and prevent hyponatraemia (AbuMoh'd, 2020), which is a lack of sodium in the body, caused by loss of sodium in sweat, diarrhea, and vomiting (Styburski et al., 2020).

Potassium creates and preserves the constant muscle contraction and nerve impulse, prevents clot of blood and keeps its pH level, helps in the storage of carbohydrates in the muscles (Halder &

Daw, 2020), and sets the intracellular water content by its intervention in the synthetic processes of proteins and carbohydrates (Urdampilleta & Gómez-Zorita, 2014).

Chloride acts an important role in the safety of the osmotic pressure and acid-base balance of the body as well is necessary for the gastric juice (Halder & Daw, 2020; Urdampilleta & Gómez-Zorita, 2014). However, Styburski et al. obtained a level of 342 mg/L sodium in an isotonic drink (Styburski et al., 2020). In other study by Adoga et al., the results of mineral analysis of a commercial isotonic drink showed a sodium concentration of 45.753 mg/100 mL, Potassium, 7.526 mg/100 mL, Calcium, 0.327 mg/100 mL, and Magnesium, 0.326 mg/100 mL (Adoga et al., 2020).

2.2 Carbohydrate content

During intense exercise, muscle glycogen stores and blood glucose decrease which requires a continuous source of carbohydrates to provide energy and avoid fatigue (Schneider et al., 2011). Isotonic drinks are composed of carbohydrates as a source of energy in the form of mono and polysaccharides (glucose, fructose, maltodextrine) (Pivnenko et al., 2018) with a recommended concentration of 6-9 % (AbuMoh'd, 2020). The intake of carbohydrates before exercise slows down and prevents the homeostatic disturbances, giving an adequate plasmatic volume from the start of the exercise, and contribute to offset the loss of carbohydrates stores, enhancing performances during exercises (Bonetti & Hopkins, 2010; Singh et al., 2011).

We have given in table 1 the main composition of some isotonic, energy, and fruit juice available in the market in order to have information about the composition of isotonic drinks and compare their compositions with the other types of beverages. It is clearly observed that there is a difference between the three groups of beverages. The concentration of carbohydrates and salt differs among the six isotonic drinks. carbohydrates concentrations in isotonic drinks (4.4 ± 0.78 g/100mL) are lower than those concentrations present in energy drinks and fruit juices. They range from 2.9 g/ 100 mL to 5 g/100 mL in isotonic drinks, from 9.9 g/100 mL to 17 g/100 mL in fruit juices, and from 11 g/100 mL to 16 g/100 mL in energy drinks. Caloric values are also higher in energy drinks and fruit juices compared to isotonic drinks. Organic grape juice has a high carbohydrate concentration compared to fruit juices (17 g/100 mL). Thus, in our previous work (Bendaali et al., 2022b), to obtain a healthy beverage from grape juice, we diluted sugar content with mineral water because it cannot be considered a healthy beverage if it contains a high concentration of sugars. Therefore, a significant way to improve the impact of grape isotonic juices on health is the dilution of sugars with mineral water which also improves the glycemic index. On the other hand, research shows that simple carbohydrates (sucrose, glucose, and fructose) are the most effective in stimulating fast absorption and promoting carbohydrate oxidation. Thus, the amount and types of carbohydrates used in a sports drink are important to optimize the potential of the drink and improve performance (Singh et al., 2011). In addition, it was mentioned that the consumption of carbohydrates with a low glycemic index before physical exercises has a benefit effect because they perform a slow release of glucose into the blood after digestion providing a sustained source of energy to contracting muscles maintaining muscle glycogen and improving performances. They also promote a low insulin response that is beneficial for substrate metabolism because high level of insulin inhibit fat lipolysis and oxidation (Kaviani et al., 2019). Also, the comparison of sugar, salt, and calories values in the same group (isotonic, energy, or fruit beverages) demonstrates that there is not a large difference between isotonic beverages composition unlike the group of energy drinks and fruit juices. However, salt concentrations are higher in isotonic drinks (0.08 ± 0.02 100 g/mL) and absent in fruit juice. Proteins, fats, and fibers are missing in both isotonic and energy drinks, and they are presented in fruit juices with a mean value of 0.5 ± 0.16 g/100 mL. The stimulants (caffeine and guarana) are found only in energy drinks.

Accordingly, the optimization of carbohydrates and salt concentration for performance is an interest of sport nutritionists and drink manufacturers (Demirhan et al., 2015). The higher concentration provides more carbohydrate and salt but minimize the rate of gastric emptying and can consequently delay the rate of delivery of fluid (Bonetti & Hopkins, 2010; Coso et al., 2008). The needed osmolality value (270-330 mOsm/ kg of water) defined by the European Food Safety Authority (EFSA) for isotonic drinks which is the same osmolality that found in the human body, leads to prevent tiredness, and increase performances after ingestion of the beverage (Pivnenko et al., 2018; Porfírio et al., 2020; Świtalski & Rybowska, 2021) due to the fast absorption of water and ions, which is the main purpose of isotonic drinks consumption, to replenish fluids losing during physical exercises (Stasiuk & Przybyłowski, 2017).

Table 1. Composition of isotonic drinks, energy drinks, and fruit juices collected from the label, and available in 100 mL.

		Carbohydrates (g)	Salts (g)	Calories (KJ)	Proteins (g)	Fat (g)	Fibers (g)	Stimulants
Isotonic Drinks	**Aquarius**	4.4	0.05	80	-	-	-	-
	Powerade	5	0.13	93	-	-	-	-
	Isofresh	4.9	0.08	83	-	-	-	-
	Raw	2.9	0.105	54	-	-	-	-
	Iso On	4.8	0.07	85	-	-	-	-
	Isodrink	4.4	0.06	81	-	-	-	-
Energy Drinks	**Red Bull**	11	0.1	195	-	-	-	Caffeine
	Coca-Cola Energy	10.4	-	177	-	-	-	Caffeine Guarana
	Burn	16	0.05	263	-	-	-	Caffeine Guarana
Fruit juices	**Organic grape juice**	17	-	298	< 0.5	-	-	-
	Peach juice	10.7	-	187	0.3	-	-	-
	Natural orange juice	9.9	-	187	0.7	0.1	0.6	
	Grape and peach juice	12.8	-	234	0.5	0.2	-	-

3. Contribution of grape juice to isotonic drinks

3.1. Composition of grape juice

New dietary guidelines and health professionals are interested in developing foods with lower sugar content or with alternative sweetener sources due to the multiple diseases associated with sugar intake such as obesity, diabetes, cardiovascular disorders, and cholesterol (Moldovan & David, 2020). Zhang et al. mentioned that grape juice sports drink does not need to use sweeteners and acidulant (Zhang et al., 2021). Grape juice is a beverage extracted from different grape varieties, mainly *Vitis vinifera*, *Vitis labrusca*, and *Vitis rotundifolia* species (Granato et al., 2016), by different technological processes (hot press, cold press, and hot break) (Cosme et al., 2018). Its consumption is increasing worldwide because of its sensory characteristics and nutritional value (Toaldo et al., 2013). United States, Spain, China, Italy, France, Turkey, and Chile are the most produced of grape juice (Granato et al., 2016). Grape juice contains water, a high concentration of sugars and organic acids (Chiusano et al., 2015; Cosme et al., 2018; García-Martínez et al., 2021), as well minerals, phenolic compounds, and others nutrients such as vitamins, proteins, fatty acids, and amino acids (Dutra et al., 2021; Gutiérrez-Gamboa et al., 2019). Carbohydrate are found in the forms of fructose and glucose (Naderi et al., 2018). The main organic acids in grape juice are tartaric, malic, and citric acids (Granato et al., 2016), In addition, these acids are used as indicators of microbiological alterations in the beverage because of their impact on its stability (Lima et al., 2014). Phenolic compounds are the most abundant compounds following sugars and acids

(Granato et al., 2016). They play a major role in the prevention of various diseases caused by oxidative stress (Mazrou et al., 2020). The phenolic compounds found in grape juice are those extracted from the grape skins and seeds (Cosme et al., 2018). They classified in flavonoids such as flavanols, flavonols, and anthocyanins and nonflavonoids mainly phenolic acids and stilbene (Lima et al., 2014; Ribeiro et al., 2018). Numerous studies have been identified and quantified the phenolic content from different varieties and cultivars of grape juice (Dutra et al., 2021; Lima et al., 2014; Natividade et al., 2013; Rodrigues et al., 2012; Toscano et al., 2017). The phenolic content differed among various grape juices. Thus, researchers have indicated that the content and the profile of phenolic compounds are dependent on the grape varieties, species, (Toaldo et al., 2013) technology of juice preparation (Lima et al., 2014), geographical origin, ripeness , type of soil, sunlight exposure, the method used for quantification (Granato et al., 2016), farming system of grapes (organic, conventional, and biodynamic) (Butu & Rodino, 2019), culture conditions, and grape tissues as the pulp is rich in phenolic acids and the skin is rich in flavonoids (Moreno-Montoro et al., 2015). However, anthocyanins are the main phenolic compounds in red grape juices, while flavan-3-ols are more abundant in white grape juices (Cosme et al., 2018). It is mentioned that most phenolic compounds in white grapes belong to the non-flavonoid group, including mainly phenolic acids such as gallic, vanillic, syringic, protocatechuic, and ellagic acids, and flavonoids, such as flavanols mainly catechin, epicatechin, procyanidins, and flavonols mainly quercetin and other aglycones (García-Martínez et al., 2021).

3.2. Health benefit of grape juice

The interest of consumers and the food industry in polyphenols have been growing because there is a relationship between their intake and the prevention of various diseases (Mazrou et al., 2020). Accordingly biological activities of polyphenols in grape juice are linked to their antioxidant, anti-inflammation, anticancer, antiaging, antimicrobial, and cardioprotective properties (Xia et al., 2010).They can prevent platelet aggregation, LDL, DNA (Dani et al., 2007), lipid, protein (Rodrigues et al., 2012), and membrane damage oxidation (Moreno-Montoro et al., 2015), reduce adhesion molecule express and limit inflammations (Blumberg et al., 2015) which led to block cellular events predisposing atherosclerosis (Capanoglu et al., 2013), enhance the regulation of blood pressure and vascular reactivity, reduce serum cholesterol and triglycerides (Blumberg et al., 2015), and improve memory function in older adults (Krikorian et al., 2010). They also help to prevent obesity and diabetes by inhibiting specific enzymes (Toscano et al., 2017). Phenolic compounds improve the antioxidant activity by scavenging reactive oxygen and nitrogen molecules, chelating redox-active transition minerals, collaborating with other antioxidants, stimulating antioxidant enzymes and proteins, inhibiting pro-oxidant enzymes, and modulating transcription factors redox-sensitive (Blumberg et al., 2015). Moreno-Montoro et al. have been mentioned that catechin and gallic acid act as free radical scavengers and epicatechin has an antibacterial activity (Moreno-Montoro et al., 2015). In addition to their antioxidant capacity gallic, caffeic and chlorogenic acids act as venous dilators (Lima et al., 2014). Resveratrol has beneficial role to protect against various neoplasias, cardiovascular and neurodegenerative disorders, and viral infections as well help to retard the body aging, reduce the incidence of heart and muscle diseases (Butu & Rodino, 2019). Quercetin and its derivatives have shown anti-inflammation and anticarcinogenic properties when used in the treatment of some types of cancer (García-Martínez et al., 2021).

During extended and intense exercise such as marathon and ultramarathon races, athletes exhibit severe physiological stress that appears in muscle microtrauma, oxidative stress, gastrointestinal dysfunction, and inflammation (Elejalde et al., 2021). According to Martins et al. (Martins et al., 2020), during long and extenuating physical exercises, an oxidative stress is caused owing to a considerable increase of reactive oxygen species (ROS) promoting an imbalance with antioxidant capacity in the body, which led to protein modification, lipid oxidation, DNA damage (Canedo-Reis et al., 2021), inflammation (Toscano et al., 2015) as well chronic diseases including cancer, neurological and cardiovascular diseases (Zeng et al., 2021). Grape and grape derivative products are a source of polyphenols (Elejalde et al., 2021) which are known of high antioxidant activities (Zhang et al., 2017) and can be beneficial against oxidative damage (De Oliveira et al., 2021). In addition, the carbohydrate content is useful for glycogen deposition and improvements of practice during long term exercises (Martins et al., 2020). Therefore, many researchers have studied the beneficial effect of grape juice related to improving performance during physical exercises. Table 2 shows some of the experimental studies carried out and their consequences which have demonstrated that grape juice was able to improve performances and antioxidant activity, protect against oxidative damage and reduce inflammatory. The figure in the graphical abstract shows the contribution of grape juice in the development of isotonic drink effectiveness and its impact on athletes' performances.

Table 2. Different studies demonstrate the impact of grape juice on the performances during physical exercises.

Grape juice	Experiment	Studied properties	Results	Reference
Organic grape juice Bordeaux variety (*Vitis labrusca*)	10 adult male triathletes received organic grape juice (300 ml/day) for 20 days.	Glucose homeostasis Antioxidant status Cutaneous microcirculatory function	The results blood sample that was drawn before (baseline) and after 20 days showed that intake of grape juice improved glucose homeostasis, antioxidant capacity, and microvascular function.	(Gonçalves et al., 2011)
Red grape juice khoshnam variety *Vitis vinifera*	30 male Wistar rats of Parkinson's disease were divided in groups and treated with grape juice, exercise, or grape juice associated with exercise for 30 days	Neurodegenerative effect Parkinson's disease.	Rotations test demonstrated a reduction in the number of rotations in Parkinson's rats treated with grape juice and grape juice associated with exercise.	(Eshraghi-Jazi et al., 2012)
Organic purple grape juice Bordo variety *Vitis labrusca*	12 male Wistar rats were divided into 2 groups (nonexercised and exercised). Rats treated with organic purple grape juice were submitted to an exhaustive exercise bout.	Parameters of oxidative stress Protective impact	Evaluation of oxidative stress parameters showed that organic grape juice had capacity to protect different rat tissues against oxidative damage.	(Corte et al., 2013)
Purple grape juice Isabel, Bordeaux, and Concord varieties *Vitis labrusca*	28 recreational runners of both sexes were divided into 2 groups and received either grape juice or control beverage for 28 days.	Ergogenic impact Oxidative stress Inflammation Immune response Muscle injury	Results of time-to-exhaustion exercise, anaerobic threshold, and aerobic capacity test showed the ergogenic effect of grape juice in recreational runners by increasing performances, time-to-exhaustion, and antioxidant activity, and reducing inflammatory markers.	(Toscano et al., 2015)
Purple grape juice, Isabel, Bordeaux and Concord varieties *Vitis labrusca*	2 groups of 28 healthy adults were assigned and received either grape juice or control beverage during intense and continuous physical exercise for 28 days.	Antioxidant activity, Lipid and glycemic profiles	Evaluation of the nutritional status, blood pressure, and blood collection upon receiving supplementation indicated that grape juice was a source of antioxidants improving antioxidant status and cardiometabolic profile.	(Toscano et al., 2017)
whole red grape juice American burgundy and Isabella grapes	A study was performed with 26 individuals with hypertension distributed into experimental group (supplementation with a daily dose of juice) and control groups (supplementation with a control drink) performed 2 sessions of aerobic exercise on a treadmill, separated by a 28-day period of supplementation with daily dose of grape juice or a control drink.	Impact on blood pressure of individuals with hypertension.	Measurement of blood pressure before, during, and after each exercise session showed that grape juice has a capacity to decrease blood pressure at rest and improve post-exercise hypotension in individuals with hypertension.	(Neto et al., 2017)
Purple grape juice Isabel, Bordeaux, and Concord varieties (*Vitis labrusca*)	14 recreational male runners performed two running tests to exhaustion after ingesting grape juice or a placebo drink (10 ml/kg/day).	Physical performance Oxidative stress Inflammation, and muscle damage	Ergogenic effect of grape juice by increasing time of run and improving of antioxidant activity in recreational runners.	(Toscano et al., 2020)
Purple grape juice Bordeaux variety (*Vitis labrusca*)	12 male volleyball players participated in three different moments with match simulation: without beverage, grape juice, and placebo 44everage for 14 days in a cross-over model.	Oxidative stress Inflammation Muscle damage	Grape juice promoted a reduction in protein oxidation, lipid peroxidation, and DNA damage.	**(**Martins et al., 2020)
Grape juice was Bordeaux variety (*Vitis labrusca*)	20 judo athletes were randomized into 2 groups, and they consumed grape juice or placebo for 14 days in a crossover model	Oxidative stress Muscle fatigue parameters	Grape juice improved parameters of oxidative stress by reducing lipid and DNA damage.	(Goulart et al., 2020)

3.3. Sensory characteristics of grape juice

Another interesting characteristic of grape juice is its great sensory characteristics. Phenolic compounds in grape juice are responsible for its sensory properties (color, flavor, and taste) (Cosme et al., 2018). Phenolic acids affect the organoleptic properties of grape juice (Lima et al., 2014) and assure a low pH value which provides equilibrium between sweet and sour tastes (Cosme et al., 2018). Accordingly, the organoleptic properties are an important factor when choosing food products. Color is the most important characteristic used when choosing beverages (Gérard et al., 2019). In addition, color is used by consumers as an indicator of juice quality (Burin et al., 2010), because there is a strong relationship between color and flavor as consumers can be able to expect the flavor through the color of food products (Bordim et al., 2021; Chandra et al., 2021; Pinto & Vilela, 2021). Nowadays, the replacement of synthetic dyes with natural colorant is a challenge for the sector of food industry (Gérard et al., 2019). the attractive orange, red, and purple colors and water solubility of anthocyanins allow their integration into aqueous food systems and use as natural colorants (Morata et al., 2019; Vidana Gamage et al., 2021). They also participate in multiple chemical reactions such as copigmentation and formation of polymeric pigments which contribute to color changes (Burin et al., 2010). For grape juice, cyanidin, peonidin, delphinidin, petunidin and malvidin are the main anthocyanins responsible for the red color (Tiwari et al., 2009). Their stability is influenced by pH, temperature, oxygen, light, presence of ascorbic acid, and metal ions**,** and high concentration of sugar (Cosme et al., 2018; Ren & Giusti, 2021; Vidana Gamage et al., 2021) . On the other hand, consumption of anthocyanins promotes health benefit by reducing risk of cancer, inflammation, neuronal and cardiovascular diseases , diabetes, obesity, and cognitive function disorders (Tan et al., 2021), owing to their antioxidant anti-cardiovascular, anticancer, anti-inflammatory, anti-thrombotic, anti-ulcer, anti-allergenic, and anti- coagulant activities as well their immunomodulatory, vasodilatory, and analgesic activities (Serea et al., 2022).

4. Conclusion

Since the creation of new, natural, functional, and healthy products based on fruit juices is a challenge for the food industry, grape juice represents a suitable alternative for the development of a new isotonic drink. An isotonic beverage that is designed to rehydrate, replenish electrolytes, and promote energy, could be more effective when enriched with grape juice by diluting the sugar content of grape juice to obtain a beverage with beneficial health properties. Besides rehydration, isotonic drinks acquire more benefits in terms of antioxidant activity, due to the phenolic content, which acts against oxidative stress related to intense sports activity. In addition, grape juice is a natural source of sugars which leads to avoiding adding sweeteners and have an important role in glycogen compensation. Moreover, attractive sensory characteristics, mainly color, which is provided by the anthocyanins content, have a great contribution to make the drink more natural and help to dispense the use of synthetic dyes. Finally, developing new and natural isotonic beverages based on grape juice with antioxidant capacity and interesting sensory properties will be a novel product in the field of healthy beverages.

Author contributions: YB: investigation. YB, CV, CG, and AM: writing—original draft and review and editing. AM and CV: visualization. YB, CV, and AM: validation. AM and CG: conceptualization. All authors have read and agreed to the published version of the manuscript.

CHAPTER 4. ARTICLE 2.

Elaboration of an organic beverage based on grape juice with positive nutritional properties

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Abstract: The present study aimed to develop a natural beverage with interesting phytochemical composition and biological activity, based on grape juice without added sugars or artificial additives. Two groups of blends were made by diluting concentrate grape juice with a sugar content of 65 ºBrix with two different mineral waters (BA: Bezoya with low mineralization; BB: Solan de Cabras with high mineralization). Lemon juice was used for pH correction and mixtures of extractions of hop with tea and hop with mint were used to increase aroma. Samples were stored under refrigeration (4 ºC) then subjected to physicochemical and sensory analysis. The results demonstrated that malvidin-3-O-glucoside pigment was the predominant pigment with a concentration ranging from 75.71 ± 12.49 mg/L to 84.87 ± 1.70 mg/L. The levels of sugars ranged from 79.90 ± 1.37 g/L to 82.37 ± 0.55 g/L and total soluble solids were between 5.47 ± 0.12 ºBrix and 5.77 ± 0.06 ºBrix. Total acids presented a significant difference, ranging from 1.40 ± 0.00 g/L to 1.43 ± 0.06 g/L in BA samples and from 1.10 ± 0.10 g/L to 1.20 ± 0.00 g/L in BB samples. For 20 days, the color increased in all beverages. However, BA drinks presented higher acidity and higher red color intensity than BB drinks, so the type of water and pH influenced the color of beverages. The sensory evaluation showed that the beverage made with low mineral water and flavored with a mixture of hop with tea was preferred.

Keywords: natural beverage; grape juice; organic; anthocyanins; color

Introduction

Currently, the food market is experiencing a great development in the production of natural and functional foods due to the increasing health awareness of consumers and the adoption of healthy eating habits (Chew et al., 2020). The request for organic foods has increased owing to the belief that they might have more benefits than conventional products (Dyab et al., 2015). Accordingly, it has become important to produce new drinks from fruits juices as a source of nutrients and bioactive compounds (Gironés-Vilaplana et al., 2016). Consumption of fruits and vegetables is recommended to prevent disease, especially red fruits such as strawberries, cherries, grapes, and pomegranates, which are characterised by their bioactive component (Gardeli et al., 2019). Fruit and vegetable-based beverages include a variety of polyphenols, oligosaccharides, fibers, and nitrates, which may produce antioxidant, antimicrobial, and antiviral effects (Butu & Rodino, 2019). Nowadays, researchers are interested in the production of foods with lower sugar or alternative sources of sweeteners (Moldovan & David, 2020). Fruit juices could replace sugar sweeteners and contribute to preventing pathologies associated with their consumption (Agulló et al., 2021), such as obesity, diabetes and cardiovascular disease (Styburski et al., 2020). In addition, among challenges facing the food industry is the expansion in the creation of new products and persuasive consumers to buy them. In this case, it is essential to recognize the needs and the consumer's demands while developing new and innovative products (Świtalski & Rybowska, 2021). To respond to consumer demand related to the consumption of natural and healthy products, several studies focused on the development of new and healthy beverages based on fruit juice (Bhalerao et al., 2020; Gironés-Vilaplana et al., 2012, 2013, 2016; González-Molina et al., 2012; Najafabadi et al., 2021; Tiencheu et al., 2021). During physical exercise, oxidative stress is induced because of an imbalance between the production of reactive oxygen species (ROS) and antioxidant capacity in the body, leading to an increase in inflammatory markers, muscle damage, and gastrointestinal dysfunction (AbuMoh'd, 2020; Elejalde et al., 2021; Martins et al., 2020)**.** Grape and its derivatives could be beneficial facing oxidative damage because of the presence of phenolic compounds (De Oliveira et al., 2021). Its carbohydrate content is necessary for glycogen deposition and improvement of practice during long-term exercise (Martins et al., 2020). Concentrated grape juice is a product obtained by physical methods for removing water and increasing the content of soluble solids present in the respective total juice by at least 50 %. By diluting the concentration or dried juice to the initial concentration based on °Brix as reconstitution parameter, reconstituted grape juice is obtained (Dutra et al., 2021). Grape juices consist of water (81 to 86 %) and a high concentration of sugars (glucose and fructose), with high acidity owing to the existence of organic acids that balance the sweet and sour tastes. They present small amounts of minerals, vitamins, and other phenolic aromatic compounds that provide sensory characteristics of grape juices (color, taste, and flavor) (Cosme et al., 2018; Dutra et al., 2021; García-Martínez et al., 2021). Among the phenolic compounds found in grape juice are flavonols (kaempferol, quercetin, and myricetin**)**, flavanols (catechin, epicatechin, and procyanidins), anthocyanins (malvidin, cyanidin, delphinidin, petunidin, peonidin, and pelargonidin), phenolic acids, and the stilbene resveratrol (Burin et al., 2010; Lima et al., 2014; Nadeem et al., 2018; Xia et al., 2010). Consumption of grape juice has positive health advantages due to effective antioxidant, anticarcinogenic, antibacterial, antidiabetic, anti-ageing, and anti-inflammatory activities as well as cardioprotective, hepatoprotective, and neuroprotective effects (Nadeem et al., 2018; B. Wu et al., 2021), which develop endothelial function, increasing the antioxidant capacity of serum and low-density lipoproteins (LDLs), minimizing native plasma protein oxidation, and reducing platelet aggregation (Burin et al., 2010; Dávalos et al., 2005). Moreover, studies have shown the beneficial effects of resveratrol and quercetin in the treatment of cancer and cardiovascular diseases (García-Martínez et al., 2021).

The new drink prepared from grape juice is a natural product in which the use of artificial colorants and flavorings was avoided. No sugars are added, only those of the fruit juice and with great nutritional value and biological activity. Lemon juice is added to the beverage to reduce the pH and provide a pleasant flavor. Lemon is one of the citrus fruits that are characterized by their content of flavanones, vitamin C, minerals, and citric acid, which provide nutritional value in a beverage (Agulló et al., 2021; Gironés-Vilaplana et al., 2013; González-Molina et al., 2012). Anthocyanins have a high potential for utilization as natural colorants to replace synthetic dyes in food systems owing to their attractive colors, water solubility, and health benefits (Brenes et al., 2005; Gérard et al., 2019; Tan et al., 2021). Their stability depends on the pH, lack of vitamin C, high concentration of sugar (Cosme et al., 2018), oxygen, light, temperature presence of ascorbic acid, and metal ions (Moldovan & David, 2020; Vidana Gamage et al., 2021).

Among challenges for academic and industrial investigation is the production of natural flavors, which determine the sensory characteristics of beverages and other food products, because of the growing preference of consumers for sustainable and natural products (Vilela et al., 2019). Since the prepared drink is an organic product without artificial ingredients, natural flavors extracted from spices and herbs (hop, tea, mint) are used to enhance the sensory properties and increase the aroma of the beverage.

2. Materials and method

2.1. Raw materials

Concentrated red grape juice was used with a sugar content of 65 °Brix, pH 3.5 and SO2 < 40 ppm (Vinos y Bodegas, Spain). Dilutions were performed with mineral waters with different mineralization: Bezoya (Calidad Pascual, Ortigosa del Monte, Segovia, Spain) and Solan de Cabras (Beteta, Cuenca, Spain).

Table 1 describes the composition of both waters (taken from the label of water bottles). pH correction and flavoring to correct acidity was done with pasteurised squeezed lemon juice (Mercadona, Spain), with 40 mg/L of C vitamin and 10 mg/L of sodium. The aroma was improved by infusion extractions from organic red tea (Cafetearte, China), organic dried mint (Soria natural, Garray Spain), and hop (Summit, USA).

Table 1 Composition of waters used in the preparation of drinks.

Composition	Bezoya (mg/L)	Solan de Cabras (mg/L)
Dry residue (180 °C)	28	278
Bicarbonate	21	284
Chlorides	0.60	8.3
Calcium	5.26	60
Magnesium	0.91	26.7
Sodium	1.36	4.8
Potassium	-	1
Silica	9.15	7.5
Nitrates	2.8	-
Sulphates	-	21.8

2.2. Extraction

To obtain the extracts, 4 grams of each herb or spice (hop, tea, and mint) were weighed, crushed in the mortar, and mixed with 30 ml of diluted grape juice (Figure 1). The extraction of the aromas and flavors from the three mixtures was performed using ultrasounds (3300EP SONICA, Italy) for ten minutes followed by centrifugation (Eppendorf, 5430 R) at 6000 rpm at 20 ºC for ten minutes. Finally, the extracts were filtered using filter papers and kept refrigerated at 4 ºC until being added to the beverages.

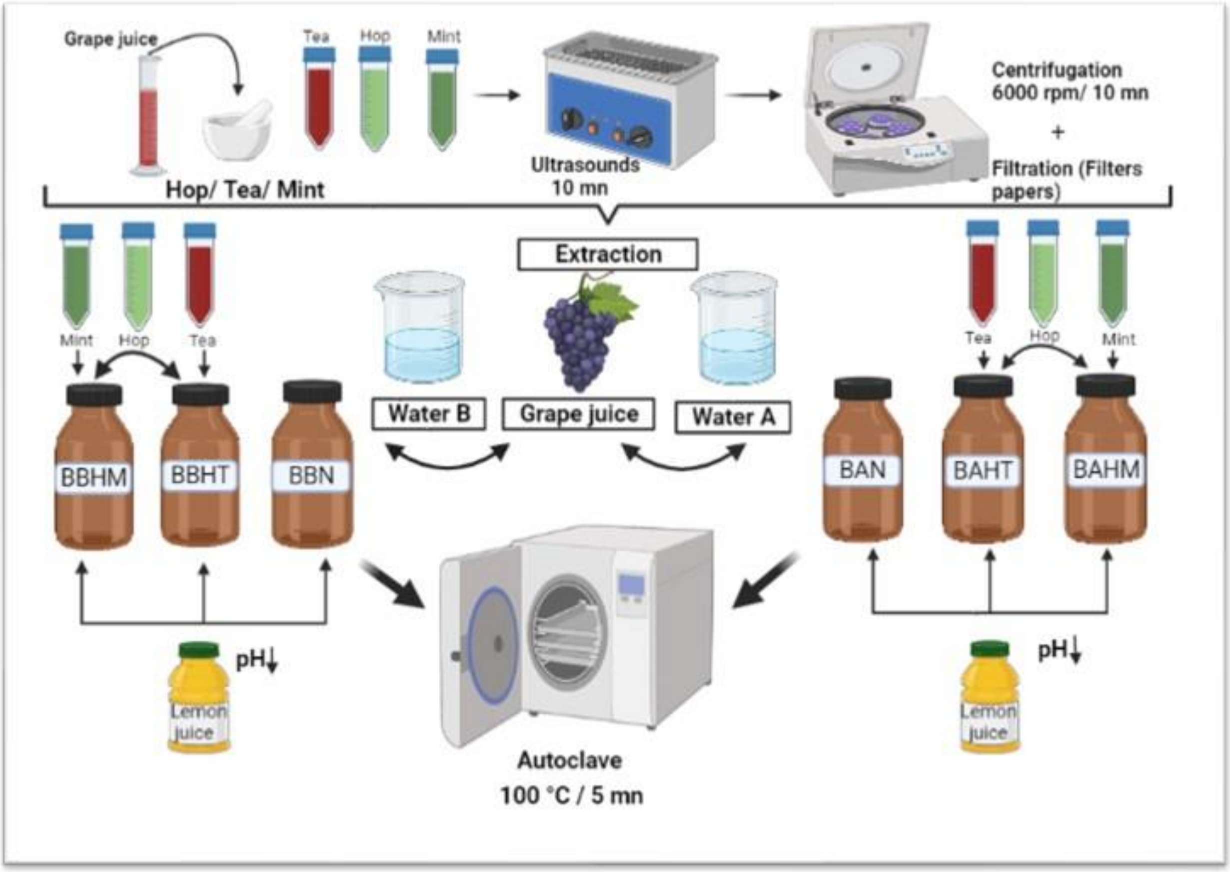

Figure 1 Schematic of flavor extraction and beverage preparation

2.3. Beverage preparation

For the preparation of the beverages, preliminary experiments were carried out, aimed to obtain a product with a suitable sensory profile, in terms of acidity, color, and flavors. Several drinks were prepared with the two types of water previously described (different mineralization) and flavored with different herbs and spices (cardamom, hop, fresh mint, dried mint, black tea, red tea, and green tea). Based on sensory analysis, the appropriate ingredients identified for the final formulation of a natural drink flavored with a mixture of hop-tea and hop-mint.

The natural drink was formulated by diluting grape juice (47 mL) with water (453 mL). Using a bottle of 500 mL for each sample (Table 2). Two groups of beverages were prepared (Figure 1), the first group "A" using natural mineral water with low mineralization (Bezoya) and the second group "B" using natural mineral water with high mineralization (Solan de Cabras). Six types of drink were prepared: four flavored beverages and two beverages as controls without added flavors. To adjust the acidity, 7 mL of lemon juice was added to each drink. Then beverages were flavored with a mixture of hop with red tea and hop with mint. Triplicate solutions were prepared for each experiment and all analytical measurements were performed in triplicate.

Samples were labelled as follows: BAN (control, beverage with water A without flavoring), BAHT (beverage with water A flavored with hop and red tea), BAHM (beverage with water A flavored with hop and mint), BBN (control, beverage with water B without flavoring), BBHT (beverage with water B flavored with hop and red tea) and BBHM (beverage with water B flavored with hop and mint). The 18 bottles of drinks were thermally treated by autoclave at 100 ºC for five minutes. Later, they were kept under refrigeration at 4 ºC until chemical and sensory analysis.

Table 2 Drinks formulation: composition and nomenclature

Group	Samples	Water (mL)	Grape juice (mL)	Lemon juice (mL)	Hops (mL)	Tea (mL)	Mint (mL)
A	**BAN**	453	47	7	-	-	-
	BAHT	453	47	7	2	1	-
	BAHM	453	47	7	2	-	0.5
B	**BBN**	453	47	7	-	-	-
	BBHT	453	47	7	2	1	-
	BBHM	453	47	7	2	-	0.5

2.4. Physicochemical analysis

The Crison brand pH meter GLP 21 model was used for the pH measurements of each sample for 20 days. Sugar concentration (glucose and fructose), TSS (total soluble solids), total acidity, and other parameters were identified with OenoFoss™ equipment (FOSS Iberia, Barcelona, Spain) using Fourier-transform infrared spectroscopy (FTIR).

2.5. Color parameters analysed by UV-visible spectrophotometry

Since the drink was prepared with grape juice, the same wavelengths (280, 420, 520, and 620 nm) were selected for the measurement. For 20 days, the absorbance was determined using an Agilent 8453 spectrophotometer (Agilent Technologies S.L., Madrid, Spain) and a 1 mm optical path glass cuvette. The color intensity, the amount of color present in the juices (CI), was obtained by the sum of absorbances at 420 nm, 520 nm, and 620 nm. The tonality (T) was calculated by the quotient between the absorbance values at 420 nm and 520 nm (Burin et al., 2010). Total phenolic content was the absorbance at 280 nm (Milella et al., 2019).

2.6. Determination of anthocyanins

Anthocyanin determination was according to (Escott et al., 2017). The anthocyanins were identified and quantified with a series 1200 high-performance liquid chromatograph (HPLC)

(Santa Clara, CA, USA), equipped with a diode array detector (DAD). Twenty-microliter samples of previously filtered (0.45 µm membrane) were injected into the HPLC apparatus. Gradients of solvents A (water/formic acid, 95:5 v/v) and B (methanol/formic acid, 95:5 v/v) were used in a reverse-phase Poroshell 120 C18 column (Phenomenex, Torrance, CA, USA) (50 × 4.6 mm; particle size 2.7 µm) as follows: 0–2 min, 15 % B (working flow 0.8 mL/min); 2–10 min, 15–50 % B linear; 10–12 min, 50 % B; 12–13 min, 50–15 % B linear; and 13–15 min, re-equilibration. Detection was performed by scanning in the 400–600 nm range. Quantification was performed by comparison against an external standard at 525 nm and expressed as milligram per liter of malvidin-3-O-glucoside (Extrasynthese, Genay Cedex, France) (r 2 = 0.9999). Identified of anthocyanins were by their retention time and by comparing their UV-visible maximum absorbance. The detection limit was 0.1 mg/L.

2.7. Sensory analysis

The sensory evaluation test was carried out in the tasting room of the Department of Chemistry and Food Technology of the Universidad Politécnica de Madrid. The test was performed with eight participants from both genders who were students and teachers aged between 22 and 60 years. Six glasses of beverages prepared were placed on each participant's table at 12 ± 2 ºC with another glass of water. All the sensory analysis parameters were rated on a scale of 1 (low perception) to 5 (high perception). The attributes evaluated were color intensity, tonality, turbidity, aromatic intensity, aromatic quality, herbaceous, floral, fruity, reduced, rusty, body, bitterness, sweetness, and acidity.

2.8. Statistical analysis

Statgraphics Centurion 18 software V.18.1.06 (Graphics Software Systems, Rockville, MD, USA) was used to calculate means, standard deviation, and analysis of variance (ANOVA). One-way ANOVA between groups was performed with the least significant differences (LSD). Significance was set at $p < 0.05$ for the ANOVA matrix. A principal component analysis (PCA) was carried out on the color and anthocyanins parameters using Addinsoft (2021), XLSTAT statistical and data analysis solution, New York, USA.

3. Results

3.1. Evolution in pH and color

Analysis of pH values of beverages (Figure 2) stored under refrigeration at 4 ºC for 20 days shows a significant difference between the samples ($p < 0.05$) at the beginning, during, and at the end of the storage period. In BA samples, pH values slightly decreased from 3.27 ± 0.01, 3.28 ± 0.01, 3.28 ± 0.01 to 3.22 ± 0.02, 3.24 ± 0.03, 3.26 ± 0.01 in BAN, BAHT, and BAHM, respectively.

The BB samples showed a slight increase in pH value, changing from 3.40 ± 0.00, 3.35 ± 0.01, 3.37 ± 0.01 to 3.41 ± 0.02, 3.45 ± 0.01, 3.45 ± 0.01. in BBN, BBHT, and BBHM, respectively. All the values were within an acceptable range. They are in line with those found by Galvão et al. (Galvão et al., 2020) in an isotonic drink enriched with Cajuína, which ranged from 3.58 ± 0.03 to 2.9 ± 0.02 during the storage period and with those pH values found by Gironés-Vilaplana et al. (Gironés-Vilaplana et al., 2013) of six commercial isotonic beverages, which ranged from 2.64 ± 0.00 to 3.83 ± 0.01.

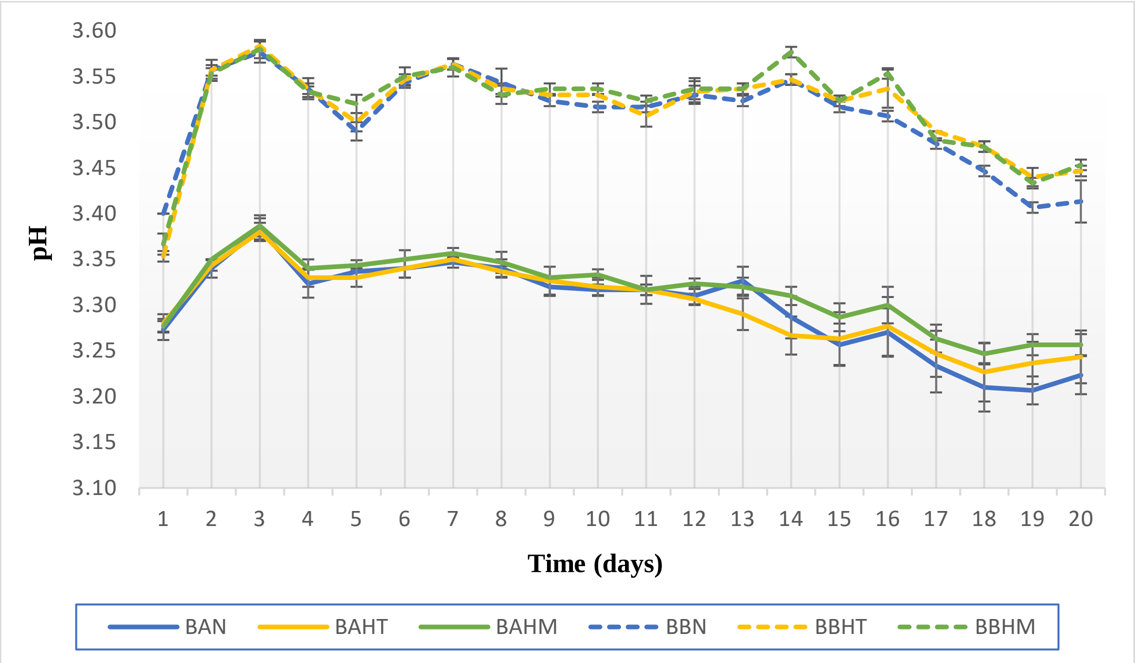

Figure 2 Change in pH values in control beverages and flavored samples for 20 days

Results in Table 3 demonstrate color intensity (CI), total phenolic content, and tonality of the two groups of beverages on the first and last day of period storage. The CI values increased in all beverages during the storage period and were higher in BA samples ranging from 0.43 ± 0.00 to 0.51 ± 0.01 on the first day, and from 0.75 ± 0.11 to 1.11 ± 0.36 on the last day. Total phenolic content was between 2.33 ± 0.04 and 2.45 ± 0.02 on the first day, and between 2.43 ± 0.02 and 2.66 ± 0.12 on the last day with significant difference between samples ($p < 0.05$). For the tonality, values obtained ranged from 0.66 ± 0.01 to 0.77 ± 0.04 on the first day, and from 0.83 ± 0.11 to 0.90 ± 0.07 with no significant difference on the last day.

Table 3. Color characterisation of beverages on the first and last day of the storage period. Values are means ± SD (n = 3). A different letter for the same parameter means significant differences ($p < 0.05$).

Parameters		BAN	BAHT	BAHM	BBN	BBHT	BBHM
Total phenolic content (A280 nm)	**Day 1**	2.36 ± 0.05^{ab}	2.36 ± 0.05^{ab}	2.40 ± 0.00^{bc}	2.33 ± 0.04^{a}	2.36 ± 0.04^{ab}	2.45 ± 0.02^{c}
	Day 20	2.60 ± 0.10^{ab}	2.66 ± 0.12^{b}	2.48 ± 0.10^{a}	2.43 ± 0.02^{a}	2.47 ± 0.06^{a}	2.56 ± 0.12^{ab}
Color intensity (A420 + A520 + A620)	**Day 1**	0.49 ± 0.01^{c}	0.51 ± 0.01^{d}	0.50 ± 0.01^{cd}	0.43 ± 0.02^{a}	0.45 ± 0.01^{b}	0.43 ± 0.00^{a}
	Day 20	1.11 ± 0.36^{a}	0.99 ± 0.16^{a}	0.87 ± 0.24^{a}	0.85 ± 0.11^{a}	0.75 ± 0.11^{a}	0.86 ± 0.17^{a}
Tonality (A420/A520)	**Day 1**	0.66 ± 0.01^{a}	0.68 ± 0.01^{ab}	0.68 ± 0.01^{ab}	0.70 ± 0.00^{bc}	0.72 ± 0.01^{c}	0.77 ± 0.04^{d}
	Day 20	0.90 ± 0.07^{a}	0.90 ± 0.07^{a}	0.83 ± 0.11^{a}	0.84 ± 0.05^{a}	0.86 ± 0.06^{a}	0.90 ± 0.05^{a}

Regarding the absorbance at 520 nm, the length at which anthocyanins absorb, Figure 3 shows an increase of absorbance at 520 nm in all beverages during the storage period. However, the influence of the type of water and acidity on the absorbance of anthocyanins was observed. Drinks with lower pH values and a lower degree of mineralization (BA) presented higher absorbance values than drinks with higher pH values and a higher degree of mineralization (BB).

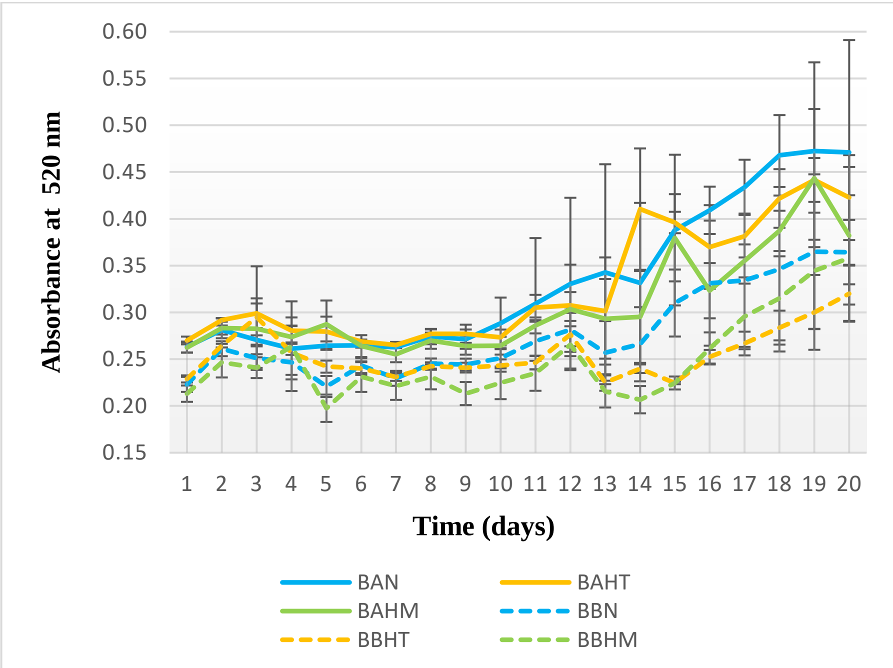

Figure 3 Changes in the absorbance of controls and flavored samples for 20 days at 520 nm

3.2. Physicochemical analysis

The results of the physicochemical analysis using the FOSS analyzer are presented in Table 4. Samples present a significant difference between the two groups except for malic acid, ammonia, and density. It observed that the samples made with weak mineral water and presented a lower pH, were the ones that contained the highest level of sugar content (glucose/fructose) and total acids. The levels of sugars in the beverages ranged from 79.90 ± 1.37 g/L to 82.37 ± 0.55 g/L. TSS were between 5.47 ± 0.12 ºBrix and 5.77 ± 0.06 ºBrix. These results of TSS are close to those of commercial isotonic drinks evaluated by Gironés-Vilaplana et al. (Gironés-Vilaplana et al., 2013). Total acids present a significant difference between BA samples and BB samples, ranging from 1.40 ± 0.00 g/L to 1.43 ± 0.06 g/L in BA samples and from 1.10 ± 0.10 g/L to 1.20 ± 0.00 g/L in BB samples. Alpha amino- acids ranged from 91.53 ± 2.75 mg/L to 98.53 ± 1.80 mg/L and were higher in BB samples.

Table 4 General parameters in beverages measured by FTIR. Values are means with standard deviations, n = 3. Values with the same letter in the same parameter are not significantly different ($p < 0.05$).

Parameters	BAN	BAHT	BAHM	BBN	BBHT	BBHM
Glucose/Fructose (g/L)	82.07 ± 1.27^{bc}	82.37 ± 0.55^{c}	81.03 ± 0.78^{abc}	79.90 ±1.37^{a}	80.57 ± 0.81^{ab}	80.67 ± 0.70^{abc}
Total Soluble Solids (ºBrix)	5.63 ± 0.12^{bc}	5.77 ± 0.06^{c}	5.53 ± 0.12^{ab}	5.47 ± 0.12^{a}	5.63 ± 0.06^{bc}	5.63 ± 0.06^{bc}
Total acids (g/L)	1.40 ± 0.00^{c}	1.43 ± 0.06^{c}	1.43 ± 0.06^{c}	1.10 ± 0.10^{a}	1.20 ± 0.00^{b}	1.20 ± 0.00^{b}
Alpha amino-acids (mg/L)	91.53 ± 2.75^{a}	95.80 ± 1.82^{ab}	91.63 ± 1.19^{a}	91.77 ± 4.48^{a}	98.53 ± 1.80^{b}	97.63 ± 4.05^{b}
Ammonia (mg/L)	28.73 ± 9.00^{a}	39.33 ± 4.31^{a}	38.30 ± 3.82^{a}	34.17 ± 30.73^{a}	42.40 ± 8.23^{a}	45.33 ± 2.71^{a}
Density (g/mL)	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}	1.03 ± 0.00^{a}

3.3. Anthocyanins

The anthocyanin profile of the beverages elaborated was studied by HPLC. The results in Table 5 demonstrated the predominance of malvidin-3-O-glucoside with a concentration between 79.46 ± 8.06 mg/L and 84.87 ± 1.70 mg/L in BA beverages and from 75.71 ± 12.49 mg/L to 84.24 ± 1.40 mg/L in BB beverages, followed by peonidin-3-glucoside with a concentration ranging from 41.39 ± 3.27 mg/L to 43.70 ± 1.02 mg/L and from 38.47 ± 5.80 mg/L to 43.01 ± 0.76 mg/L in BA and BB, respectively. Our results accord with those obtained in the grape juice by Tiwari et al. (Tiwari et al., 2009), which identified the same three major anthocyanins: cyanidin-3-O-glucoside, delphinidin-3-O-glucoside, and malvidin-3-O-glucoside. Another study by Dutra et al. (Dutra et al., 2021) reported the presence of malvidin 3-glucoside, delphinidin 3-glucoside, peonidin 3-

glucoside, and cyanidin 3-glucoside in whole grape juice. (Gironés-vilaplana et al., 2012) also obtained the similar five anthocyanins (delphinidin, cyanidin, petunidin, peonidin, and malvidin) in grape concentrate.

Table 5 Total anthocyanins divided into six beverages. Values are means ± SD (n = 3). Different letters for the same category of anthocyanins mean significant differences ($p < 0.05$).

Anthocyanins (mg/L)	**BAN**	**BAHT**	**BAHM**	**BBN**	**BBHT**	**BBHM**
Delphinidin-3-O-glucoside	24.42 ± 0.50^{a}	21.53 ± 4.16^{a}	22.72 ± 1.30^{a}	24.20 ± 0.26^{a}	18.80 ± 7.70^{a}	20.82 ± 4.33^{a}
Cyanidin-3-O-glucoside	10.55 ± 0.20^{a}	10.03 ± 0.72^{a}	9.96 ± 0.37^{a}	10.34 ± 0.14^{a}	9.42 ± 1.22^{a}	9.38 ± 1.21^{a}
Petunidin-3-O-glucoside	20.33 ± 0.41^{a}	18.52 ± 2.52^{a}	19.25 ± 1.04^{a}	20.24 ± 0.26^{a}	16.89 ± 4.83^{a}	17.74 ± 3.14^{a}
Peonidin-3-O-gucoside	43.70 ± 1.02^{a}	41.39 ± 3.27^{a}	41.67 ± 1.50^{a}	43.01 ± 0.76^{a}	39.04 ± 5.59^{a}	38.47 ± 5.80^{a}
Malvidin-3-O-glucoside	84.87 ± 1,70^{a}	79.46 ± 8.06^{a}	81.09 ± 3.03^{a}	84.24 ± 1.40^{a}	75.71 ± 12.49^{a}	75.77 ± 12.04^{a}
Cyanidin-3-(6” -acetylglucoside)	3.58 ± 0.04^{b}	3.53 ± 0.04^{ab}	3.48 ± 0.05^{ab}	3.50 ± 0.02^{ab}	3.44 ± 0.12^{ab}	3.42 ± 0.14^{a}
Petunidin-3-(6”-acetylglucoside)	4.03 ± 0.01^{a}	3.96 ± 0.10^{a}	3.97 ± 0.07^{a}	3.99 ± 0.02^{a}	3.84 ± 0.26^{a}	3.89 ± 0.14^{a}
Malvidin-3-(6”-acetylglucoside)	12.05 ± 0.21^{a}	11.13 ± 1.15^{a}	11.56 ± 0.45^{a}	11.73 ± 0.24^{a}	10.32 ± 2.24^{a}	10.69 ± 1.40^{a}
Cyanidin-3-(6”-*p*-coumaroylglucoside)	3.49 ± 0.02^{a}	3.42 ± 0.06^{a}	3.45 ± 0.04^{a}	3.46 ± 0.02^{a}	3.38 ± 0.13^{a}	3.36 ± 0.13^{a}
Petunidin-3-(6”-*p*-coumaroylglucoside)	4.21 ± 0.05^{a}	4.01 ± 0.19^{a}	4.12 ± 0.12^{a}	4.15 ± 0.05^{a}	3.91 ± 0.42^{a}	3.93 ± 0.31^{a}
Malvidin-3-(6”-*p*-coumaroylglucoside)	14.85 ± 0.56^{a}	13.55 ± 1.33^{a}	14.36 ± 0.99^{a}	14.32 ± 0.58^{a}	13.18 ± 2.87^{a}	12.78 ± 2.59^{a}

3.4. Sensory analysis

The results of the sensory analysis on sample preference are presented in Figure 4. Flavored drinks were compared sensorially with the control drink in both groups. In general, in cases of an appreciable difference between flavored drinks and control drinks, tasters preferred flavored drinks. As shown in Figure 4, BAHT, BAHM, BBHT, and BBHM presented higher average scores than the control drinks (BAN and BBN). On the other hand, tasters identified a difference between drinks with water A or B. Concerning color intensity, BA drinks were rated slightly higher, with a maximum value of 3.75 ± 0.89 in BAHT. These results agree with spectrophotometric colors measurements (Figure 3). In terms of aromatic intensity and quality, results showed significant differences between samples. BBHM (4.00 ± 0.76) was the most preferred, followed by BAHT (3.63 ± 1.19).

However, samples were slightly herbaceous without significant differences. Fruity and floral parameters showed significant differences; tasters described BA beverages as more floral (2.75 ± 1.04) and fruitier (2.88 ± 0.83) than BB beverages. Samples were seen as a little bitter and with a medium level of sweetness. Tasters identified the lowest acidity in BA beverages with an increase in both flavoring beverages (BAHT and BAHM) compared to the control. Turbidity was lowest in beverages with no significant differences in this parameter due to the filtering process through flavors extraction; the turbidity of grape juice used was <10. Finally, in global perception, tasters demonstrate a great preference for BAHT (3.63 ± 0.92) followed by BAHM (3.38 ± 0.92).

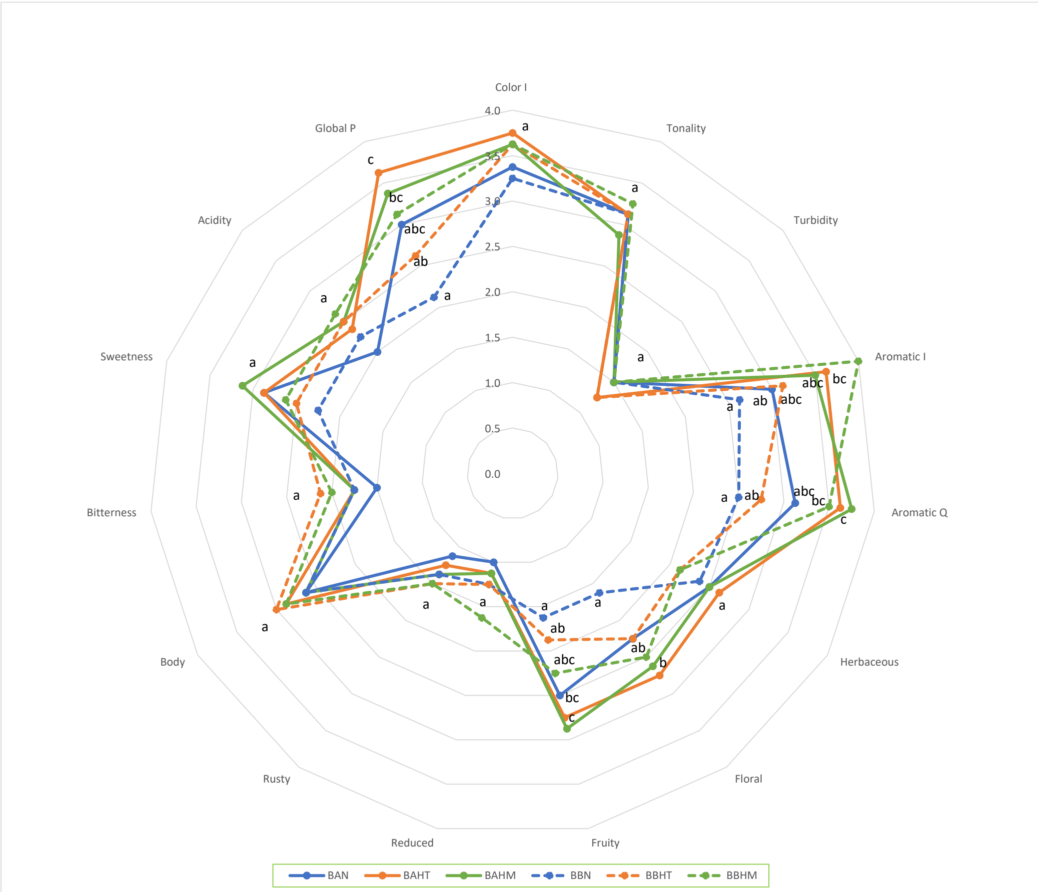

Figure 4. Sensory analysis of flavored beverages compared with the control beverages. The values are the averages from eight tasters. The same attributes with the same letter are not significantly different ($p < 0.05$).

4. Discussion

The samples prepared were subjected to physicochemical and sensory analysis to determine their different properties. Results demonstrated that samples had low pH values (Figure 2). The pH values differed slightly among the samples although the same quantity of lemon juice was added during beverages preparation. The pH was lower for beverages prepared with low-mineralized water (BA) compared to beverages prepared with high-mineralized water (BB). The trend with total acids (Table 4) was the same: BA samples had higher levels of total acids (1.43 ± 0.06 g/L) compared with BB samples. This high acidity in sample drinks could be due to the low pH of the concentrate grape juice (pH = 3.5) used for the preparation of beverages, the presence of tartaric, malic, and citric acids in the grape juice composition (Cosme et al., 2018), and the use of lemon juice, which is characterised by its content of ascorbic acid (vitamin C) and citric acid (Gironés-vilaplana et al., 2012). It should be noted that these values ensure the safety of the beverage by promoting resistance to microbial deterioration including *Clostridium botulinium* (Porfírio et al., 2020). Furthermore, an acidic pH lower than 3.5 is important to obtain the required red color and the stability of anthocyanins (Hani et al., 2019). According to our results (Figure 3), the absorbance at 520 nm (an increase for 20 days) was higher in the BA sample where the acidity was higher (Figure 2). The same trend with CI and total phenolic content with higher values were marked in BA samples (Table 3). These results clearly show that the degree of mineralization of waters influenced the acidity of grape juice beverages and made a significant difference in the absorbance at 520 nm and color between the two groups of beverages. The color characteristic depends on the anthocyanins content, which are responsible for the red color of grape juice and present the most important indicator of grape juice quality (Burin et al., 2010; Dıblan & Özkan, 2021). The stability of anthocyanins is affected by different factors such as the chemical structure, pH, temperature, oxygen, concentration, light, enzymes, presence of co-pigments, and food matrix composition (proteins, carbohydrates, ascorbic acids, minerals, salts, and sugars) (Ren & Giusti, 2021). It was reported that the stability, color intensity, and absorption wavelength of anthocyanins depend on pH of the medium. In lower pH solutions, the predominant form of anthocyanins is the flavylium cation which shows an intense red color. In strong acidic media (pH 1–2), color intensity increases strongly. When pH increases, this form turns to uncolored carbinol pseudo base. The color becomes blue-violet in a basic pH because of the transformation to a quinoidal base form. Consequently, anthocyanins are stable in low pH values (Vidana Gamage et al., 2021)(Morata et al., 2019). Additionally, the color change based on pH conditions is also influenced by the type of anthocyanins, cyanidin shows red color at pH < 3, violet color at pH 7 and 8, and blue color at pH > 11. However, peonidin has higher stability at high pH than other anthocyanidins, at acidic conditions, it shows red color to cherry, and at pH 8 shows a deep blue color (Chandra et al., 2021). Moreover, color stability also depends on the water concentration as the decrease in the concentration of water improves the deprotonation rate of the flavylium, lowering the stability of color (Chandra et al., 2021). Hydration reactions break the pyrylium ring aromaticity producing the loss of absorption properties and the transformation of the structure to an uncolored carbinol-pseudobase which turn to an open chalcone shows a light-yellow color (Morata et al., 2019). Nevertheless, each red fruit has its own anthocyanin content, which makes its properties different from other fruits. Grapes and blueberry show the most diverse profile of anthocyanin pigments provided in the juices (malvidin, delphinidin, peonidin, cyanidin, and petunidin), while only two anthocyanidins are found in strawberry juice (cyanidin and pelargonidin) (Chandra et al., 2021).

According to Hooshyar et al. the glucoside forms of malvidin, delphinidin, cyanidin, pelargonidin, peonidin, and petunidin are the main abundant anthocyanins in red grapes (Hooshyar et al., 2020). Our results (Table 5) present the same types of anthocyanins with different concentrations among samples and with a predominance of malvidin pigment in all samples which is the main anthocyanin found in red grape juice among the six monomeric anthocyanins (Cosme et al., 2018). The concentration of anthocyanins in grape juices depends on cultivars, raw material, processing technology, and heat treatment (Cosme et al., 2018). It was observed that different concentration of monomeric anthocyanins was obtained from different cultivars, and these findings indicate that anthocyanins content of red grape juice depends on cultivar. An investigation of the effects of various clarification treatments on anthocyanins, color, phenolics and antioxidant activity of red grape juice demonstrated that the clarification and the type of clarifying agents affect the anthocyanin content causing a reduction of monomeric anthocyanin content of red grape juice (Dıblan & Özkan, 2021). Also, polyphenols and anthocyanins are sensitive to heat and their degradation is dependent on temperature and can be easily broken during the heat treatment of fruit juices. Thus, a loss of food color during the processing can indicate anthocyanin degradation (Mirzaee et al., 2016). Ayoub et al. studied the influence of ohmic heating at different voltages on the different physicochemical properties of grape juice, results reveal a decrease in the anthocyanins content. Authors attributed the loss of anthocyanins to the instability of these pigments which led to their degradation through the heating process (Ayoub et al., 2020).

After performing PCA analysis on the color absorbance at 520 nm for 20 days, two principal components obtained explain 93.82 % of the total variance: 83.43 % of the variance was explained by the first component (PC1) and 10.39 % of the variance by the second component (PC2). The samples are grouped into two distinct groups (Figure 5). BAN, BAHT, and BAHM samples belong to one group; BBN, BBHT, and BBHM samples are the second group. The first group represent beverages prepared with low mineral water and the second group represent those prepared with high mineral water.

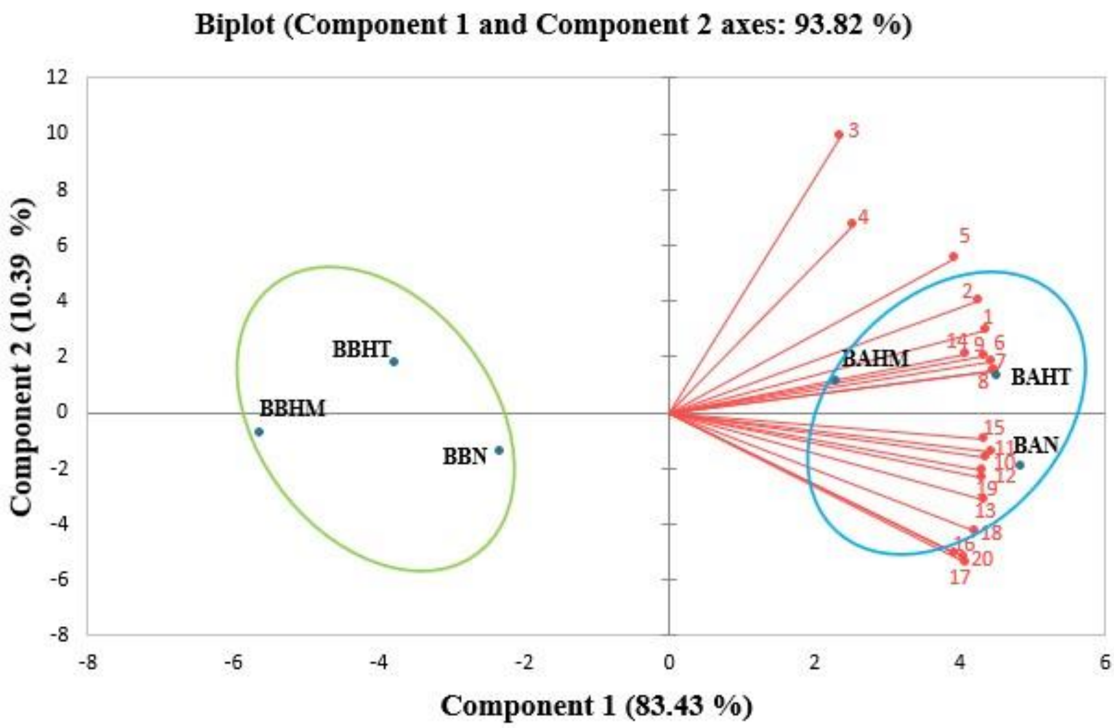

Figure 5. Principal component analysis (PCA) of color absorbance at 520 nm for 20 days.

Concerning PCA analysis on the anthocyanin's parameter (Figure 6), two principal components obtained explain 96.85 % of the total variance. The first component (PC1) explained 91.72 % of the variance and the second component (PC2) explained 15.13 % of the variance. Two groups were differentiated. One of them represents samples prepared with the low mineral water (BAN, BAHT, and BAHM) and the other includes samples prepared with the high mineral water (BBN, BBHT, and BBHM), with different distribution of BBN samples that did not follow the expected trend. Results of PCA analysis showed that already samples can be classified according to their mineral composition and their acidity, which influenced the anthocyanins and the color of the prepared beverages.

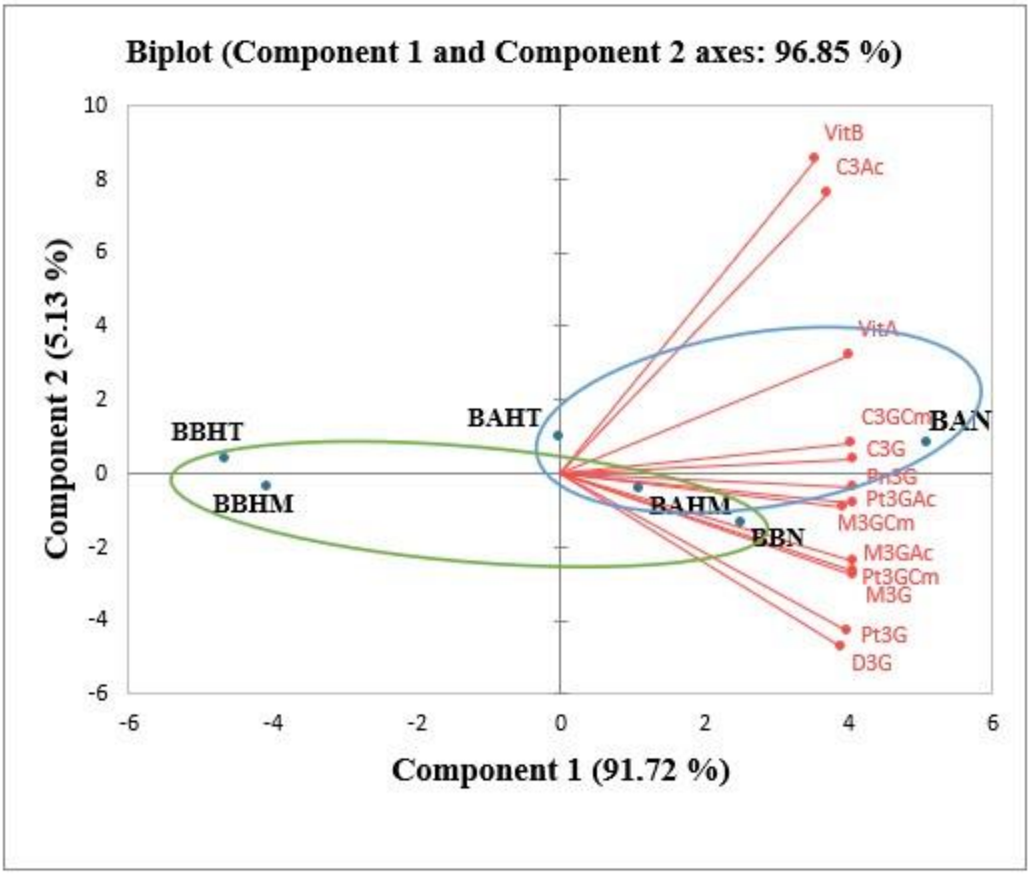

Figure 6. Principal component analysis (PCA) of anthocyanins determined in beverages by LC-DAD**.**

Finally, results of the sensory analysis show that flavored beverages were preferred over control beverages (Figure 4). Panellists gave BAHT the highest mean score, including overall acceptance. This could be due to the preferred sour taste of the formulation, which was positively correlated to the highest level of total acid (1.43 ± 0.06 g/L) (Table 4) among beverages. Tasters greatly accepted the good taste and aroma of the mixture hop-tea (BAHT) added to grape juice, which was characterised by a strong aroma, flavor, and a pleasant taste due to the abundant content of vitamin C in grapes (Ayoub et al., 2020). In addition, color is an important sensory property when choosing a food product, especially in beverage products. In this case, grape juice was preferred as a source of natural colorant due to the high concentration of phenolic compounds that provide sensory characteristics (color, taste, and flavor), namely anthocyanins, which are responsible for the color of grape juice (Cosme et al., 2018). Attractive colors (red, orange, and purple) and water solubility of anthocyanins allow their incorporation into aqueous food systems as natural colorants (Morata et al., 2019). In addition, color is used by consumers to determine the quality of agricultural and food products because of the strong correlation between color and flavor. It has been noticed that the identification of flavor decreases when the colors of food products are different from the expectations of consumers (Chandra et al., 2021). On the other hand, besides their pleasant flavors and aroma, spices have helpful effects on human health and act as natural preservatives (de Souza et al., 2020; Ivanišová et al., 2015; Potortì et al., 2020). According to (Moghaddam et al., 2018), fruits, vegetables, and herbs beverages are considered as health-promoting agents because of their content on bioactive compounds including phenolic compounds, antioxidant agents, and organic acids. This encourages the combination of good sensory properties (color and pleasant aroma) from natural sources and health benefits because of contents rich in phenolic compounds of grape juices and herbs in one product, which could be acceptable to consumers increasingly searching for products free of artificial additives.

5. Conclusion

In this study, new natural beverages were formulated from a combination of concentrated grape juice with two different mineral waters (Bezoya and Solan de Cabras), using lemon juice for pH correction, naturally flavored with herbs and spices extracts. The results of color measurement and pH indicated that beverages prepared with low mineral water (BA) present high acidity and high color intensity. On the other hand, the most interesting in terms of sensory analysis is the beverage prepared with low mineral water and flavored with a mixture of hop and tea (BAHT). The most abundant anthocyanin in prepared drinks was malvidin-3-O-glucoside, followed by peonidin-3-glucoside. Moreover, grape juice, lemon juice, and plant extracts have great potential in the development of a healthy fruit drink due to the antioxidant activity provided by their polyphenolic compounds. This is presented as an alternative for consumers looking for drinks that are less artificial and more beneficial to their health. Additionally, the beverages prepared contribute to sustainable development as they are produced under organic production conditions.

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Conflict of interest: the authors declare that they have no conflict of interest.
Compliance with ethics requirements: this study does not contain any studies with human or animal subjects.
Data availability statement: supplementary data are available upon reasonable request.

CHAPTER 5. ARTICLE 3

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 ± 0.23 to 78.43 ± 0.06 g/L, total soluble solids between 4.23 ± 0.06 and 4.83 ± 0.29 ◦Brix, and total acids from 1.75 ± 0.02 to 2.39 ± 0.08 g/L. Generally, antioxidant activity was higher in the beverages flavored with herb and spice extracts, ranging from 3.28 ± 0.01 to 4.27 ± 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

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, combining water, carbohydrates, and electrolytes in specific amounts and frequency intake is suggested, considering local temperature and substrate type (Geraldini et al., 2017). Isotonic beverages are associated with physical activity and are intended to replace electrolytes, carbohydrates, and other nutrients lost through sweating during physical exercise (Bovi et al., 2017). 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 (Świtalski & Rybowska, 2021). In sports drinks, sodium and potassium are important in replacing the electrolytes of athletes and absorbing them more quickly during training without causing gastrointestinal problems (Ferreira et al., 2021). In addition, the intake of carbohydrates is necessary as a source of energy in isotonic drinks (Pivnenko et al., 2018) to offset the loss of carbohydrate stores and improve performance during exercises (Bonetti & Hopkins, 2010). 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 (D'Angelo, 2019). 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 (Cerezal Mezquita et al., 2020; Ferreira et al., 2021; Gironés-Vilaplana et al., 2013; Porfírio et al., 2020). associated with many human health benefits, mainly antioxidant activity (Lima et al., 2015), which helps to prevent different diseases associated with oxidative stress, including cancers and cardiovascular and neurodegenerative diseases (Cosme et al., 2018). The identification of the phenolic composition of grape juices from different varieties and cultivars revealed that phenolic content in grape juices includes flavonoids (flavanols, flavonols, flavanones, anthocyanins) and non-flavonoids (phenolic acids, stilbenes) (Dutra et al., 2021; Lima et al., 2014; Rodrigues et al., 2012; Toscano et al., 2017). The phenolic content in grape juices depends on the grape variety, grape maturity, geographical origin, soil type, and sunlight exposure (Cosme et al., 2018). A review by Granato et al. analyzed the information reported in the literature concerning the differences between organic, biodynamic, and conventional grape and mentioned that biodynamic and organic grape juices have very similar quality traits and there is a trend that organic juices present higher contents of bioactive compounds as compared to the 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 (Granato et al., 2016).

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 (Skąpska et al., 2020). 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 (Pinto et al., 2022). In this trend, the search for organic products has increased (Granato et al., 2016). Accordingly, our previous work (Bendaali et al., 2022b) concerned the elaboration of healthy organic beverages by diluting the concentrated red grape juice in mineral water to obtain products with low sugar content and avoiding chemical additives using herbs and spice extracts to improve the sensory profile of the beverages.

Color and flavor are essential sensory characteristics that affect the product appearance and acceptance of foods products (Sowbhagya & Chitra, 2010). In addition, color is an indicator consumers choose in anticipating the flavor and taste of food or beverages (Pinto & Vilela, 2021).

The most-used natural food pigments are anthocyanins, carotenoids, betalains, and chlorophylls (Cortez et al., 2017). However, the classification of natural colorants is based on their source, water or oil solubility, and chemical structure, whereas the latter is the most commonly used to classify natural colorants (Nedamani, 2021). 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) (Chung et al., 2016). 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) (Morata et al., 2021). 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 (Enaru et al., 2021; Morata et al., 2019; Tan et al., 2021). 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 (El-Sayed & Youssef, 2019).

The aim of this study was to develop sports beverages based on formulations of the healthy beverages prepared previously (Bendaali et al., 2022b) by adding determined concentrations of salts to make them as 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 (hop, 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 analyzes 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 Vinos y Bodegas company (Ciudad Real, Spain). This juice is an organic product with a sugar content of 65 °Brix, pH 3.5, and SO2 < 40 ppm. Lemon juice was used in the formulation with the purpose of correcting the acidity of the isotonic drinks. It is 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 hop (Summit) were used for the extraction of natural flavors to improve the sensory profile of the isotonic drinks. Table salts: sodium chloride (Sal Costa) and potassium chloride (Aranca), without any additives, from the same Spanish supermarket (Mercadona). The ingredients were diluted using two types of mineral water with different mineralization: low mineral water Bezoya (Calidad Pascual) with 28 mg/L of dry residues, and high mineral water (Solan de Cabras) with 278 mg/L of dry residues. All ingredients used for the elaboration of the isotonic drinks were food-grade.

2.2. Isotonic Drinks 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 (Bendaali et al., 2022b) 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 hop-tea (hop = 2 mL; tea = 1 mL) or hop-mint (hop = 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 by autoclave at 100°C for five minutes. Later, they were kept under refrigeration at 4°C until subjected to physicochemical and sensory analysis. All samples were prepared and analyzed in triplicate.

Table 1. Drinks formulation: composition and nomenclature.

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

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 (Bendaali et al., 2022b).

2.3. Color Parameters and Total Phenolic Index

During the storage period, red color (RC), color intensity (CI), and tonality (T) were evaluated spectrophotometrically according to Burin et al. (Burin et al., 2010) using an Agilent 8453 spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) and a 1-mm optical path glass cuvette according to the following formula:

$$\mathbf{CI} = \mathbf{A}_{420} + \mathbf{A}_{520} + \mathbf{A}_{620};\ \mathbf{T} = \mathbf{A}_{420}/\mathbf{A}_{520};\ \mathbf{RC} = \mathbf{A}_{52}$$

The total phenolic index (TPI) of the prepared isotonic drinks was analyzed with the same instruments at 280 nm (Milella et al., 2019).

2.4. pH Measurement

The pH evolution was determined and studied in both groups of beverages during the storage period with the 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 a refractometer HI 96812 model (Hanna Instruments, Romania)

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) (Agilent Technologies, Palo Alto, CA), 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. (Re et al., 1999), using diammonium salt (ABTS) [2.2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] (Thermo Fisher, Kandel, Germany), potassium persulfate (Sigma Aldrich, St. Louis, MO, U.S.A.), and Trolox (Fisher Scientific, Massachusetts, USA). First, the $ABTS^{\cdot+}$ radical was produced by mixing ABTS stock solution (7 mM) with 2.45 mM of potassium persulfate in darkness at room temperature for 16 hours 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 $ABTS^{\cdot+}$ 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. $ABTS^{\cdot+}$ inhibition percentage was calculated by the formula $I = [(AB - AA)/AB] \times 100$, where I = $ABTS^{\cdot+}$ 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 Universidad Politécnica de Madrid (Spain) following the reference procedure of (Escott et al., 2017).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 tasting glasses of 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 Universidad Politécnica de Madrid (AIDLAPPMLB-AMB-HUMANOS-20221026 on November 14th 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. Chemical composition of beverages measured by FTIR Parameters.

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

Table 2 shows the chemical composition of prepared beverages measured by 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 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 observed values (7.83 °Brix) by Porfírio et al. (Porfírio et al., 2020) 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. (Gironés-Vilaplana et al., 2013). 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, and 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, malic acids in these beverages were from the grape juice because analysis 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 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 acids 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. 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 3. Color characterization, TPI, and pH of the prepared beverages at the beginning and end of the storage period.

SAMPLES		R C	C I	T	pH	TPI
BAN	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	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	**Biggening**	0.25 ± 0.01^{b}	0.47 ± 0.01^{cd}	0.70 ± 0.01^{bc}	3.45 ± 0.01f	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	**Biggening**	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 ± 0.01e	2.53 ± 0.04^{de}
BBHMC3	**Biggening**	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).

Regarding color parameters, all samples showed an increase in values during storage. At the beginning, samples showed CI values ranging from 0.44 ± 0.02 to 0.52 ± 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 samples of Group B. The comparison with our previous work (Bendaali et al., 2022b) demonstrated that 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 be 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 contained the highest salt concentration (C2= 0.7 g/L), presented CI higher values at the end of storage. However, in Group A, CI values were higher for the control (without salts) and samples that contained low salt concentration (C1/C2= 0.5 g/L). In the case of the total polyphenol index, values were between 2.44 and 2.66 at the beginning and from 2.44 to 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 and from 0.77 to 0.90 at the end of the storage period. Regarding herb extracts used for flavoring, the comparison of flavored isotonic drinks with the controls and the comparison of hop-tea beverage values with hop-mint beverage values demonstrated that the extracts did not have a strong effect as there is not a large difference among 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 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 2.53 to 3.75 (Leśniewicz et al., 2016). Therefore, Ferreira et al. (Ferreira et al., 2021) 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. (Gironés-Vilaplana et al., 2014) 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.

3.3 Anthocyanins Content

Anthocyanins were identified in the grape-juice-based isotonic drinks by HPLC at the beginning and at the end of the storage period to evaluate the change of the anthocyanin profile. The results revealed the presence of a wide range of anthocyanins with a significant difference between samples ($p < 0.05$). Figure 1 and Figure 2 show the anthocyanin content classified in the beverages 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 ± 0.99 mg/L and 115.16 ± 4.45 mg/L of non-acylated anthocyanins and 17.73 ± 0.17 mg/L and 16.79 ± 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 ± 5.45 to 119.46 ± 0.99 mg/L and decreased from 2.86 ± 0.22 to 5.37 ± 0.03 mg/L at the end. The same pattern was observed for the acylated anthocyanins: samples had a content between 9.92 ± 0.78 and 17.73 ± 0.17 mg/L at the beginning and a content of 0.33 ± 0.04 to 0.58 ± 0.01 mg/L at the end of the storage period.

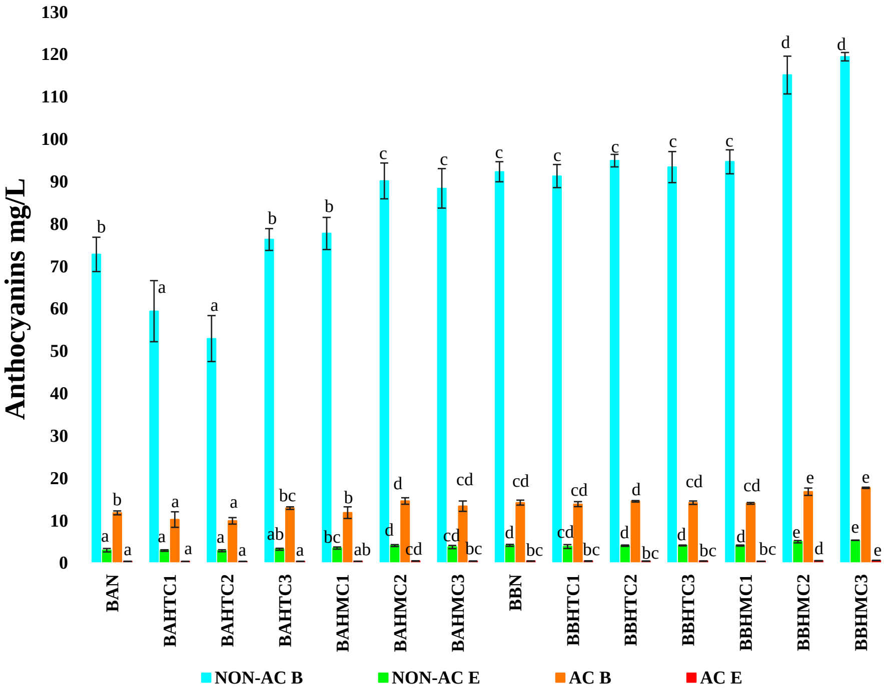

Figure 1. Changes in the anthocyanin content of samples determined with HPLC–DAD and grouped by anthocyanin families at the beginning and the end of the storage period. Different letters indicate a significant difference between means ($p < 0.05$). Non-AC = Non-acylated anthocyanins; AC = Acylated; B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

A PCA was carried out for the anthocyanin content at the beginning and the end (Figure 3 A and B). The distribution is explained by the first two principal components. PC1 is positively contributed 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 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 anthocyanins content.

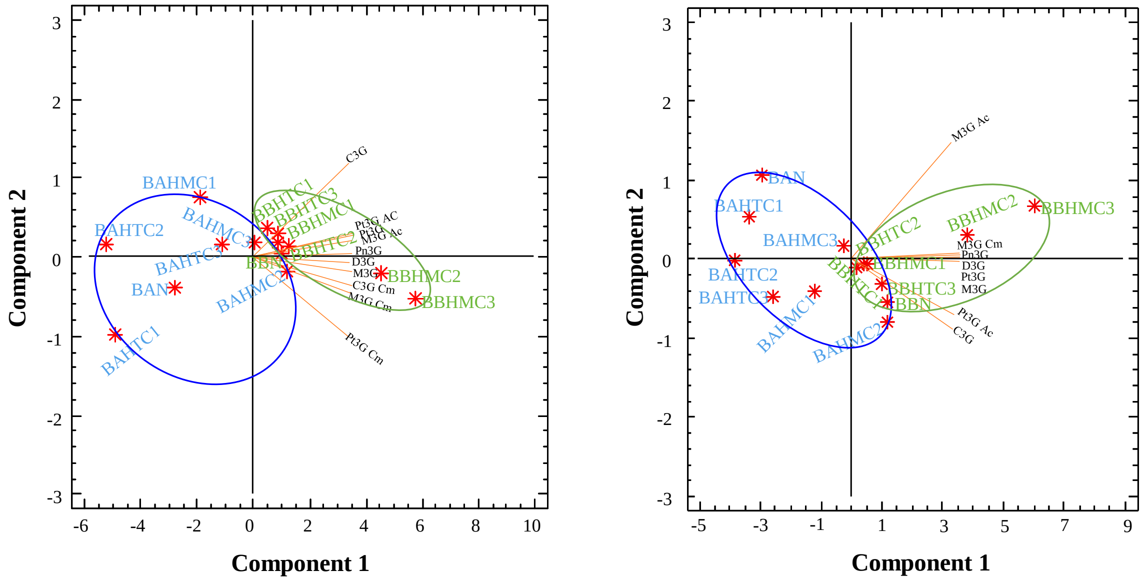

Figure 2. PCA of anthocyanin pigments present in the beverages at the beginning (A) and at the end (B). **Abbreviations:** D3G: Delphinidin-3-O-glucoside; C3G: Cyanidin-3-O-glucoside; Pt3G: Petunidin-3-O-glucoside; Pn3G: Peonidin-3-O-glucoside; M3G: Malvidin-3-O-glucoside; Pt3G Ac: Petunidin-3-(6″- acetylglucoside);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

3.4. Antioxidant Activity

The results of antioxidant activity measured by 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 marked in the beverage containing the mixture of hop and tea (BAHTC3 followed by BBHTC1 and BBHTC3). However, the lowest value was observed in control Group A. In general, samples that contained spice extracts showed good antioxidant capacity. Numerous studies based on natural sources of the bioactive compound in the preparation of isotonic drinks measured their antioxidant capacities, using different analytical methods. Gironés-Vilaplana et al. (Gironés-Vilaplana et al., 2013) 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 Andes berries prepared by Estupiñan et al. (Estupiñan et al., 2011) was from 0.58 ± 0.01 to 0.99 ± 0.05 µmols TE/mL. Ferreira et al. (Ferreira et al., 2021) prepared an isotonic beverage based on whey permeated with carotenoid extract powder from pequi and evaluated its antioxidant capacity by ABTS and DPPH, which was 10.79 µmols TE/100 mL and 73.38 µmols TE/100 mL, respectively.

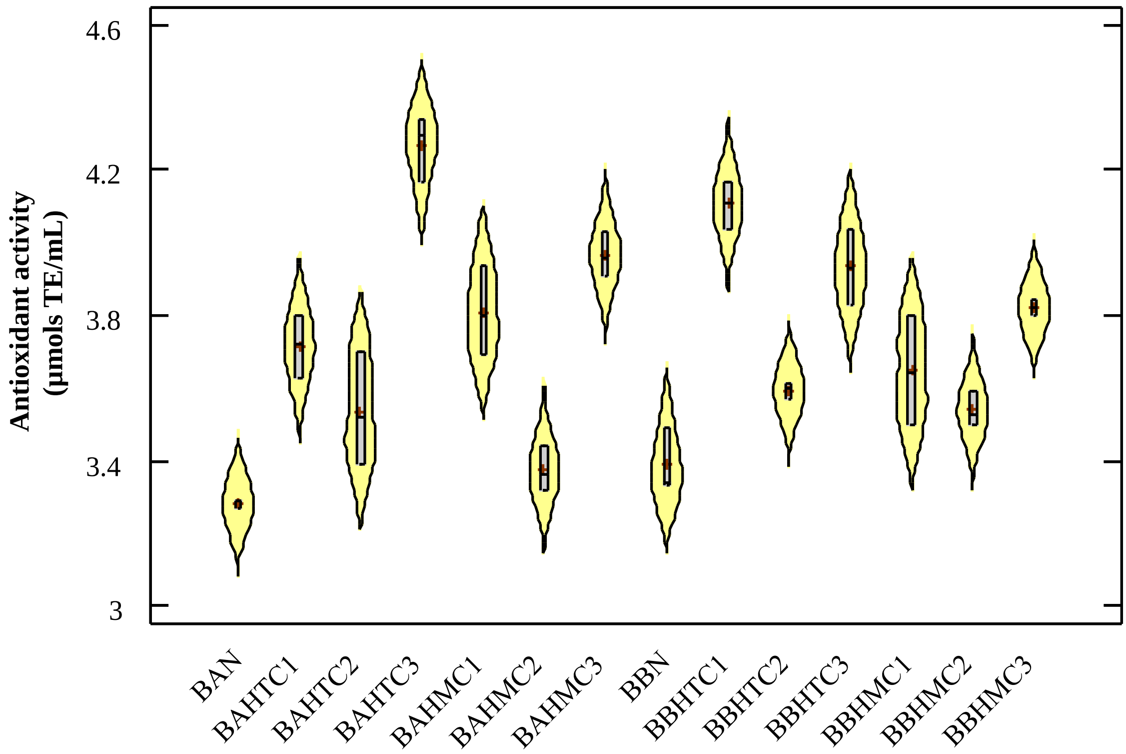

Figure 3. Violin chart for the antioxidant activity measured in the prepared beverages.

3.5. Sensory Evaluation

The results of the sensory analysis are presented in Table 4. In general, panelists preferred flavored isotonic drinks compared to the control drinks. 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 done 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, 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, 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 was detected. Regarding body, BBHMC1 had the highest score (3), and the controls had the lowest scores. On the other hand, 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, where 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	BAHTC 1	BAHTC 2	BAHTC 3	BAHMC 1	BAHMC 2	BAHMC 3	BBN	BBHTC 1	BBHTC 2	BBHTC 3	BBHMC 1	BBHMC 2	BBHMC 3
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 (AbuMoh'd, 2020; Ferreira et al., 2021). For sports nutritionists, the optimization of carbohydrates and salt concentration is important to improve athlete performance (Demirhan et al., 2015). Studies 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 reducing the rate of gastric emptying and thereby delay the rate of delivery of fluid (Coso et al., 2008; Singh et al., 2011). Isotonic drinks are characterized by an acidic taste and a pH of around 3 (Cerezal Mezquita et al., 2020). During the storage period, all samples showed low pH values (3.25-3.50) (Table 3). These pH values were correlated with total acids values which were higher in the beverages of group A that presented the lowest pH values comparing 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 others acids such as tartaric and citric acids (Granato et al., 2016). Acidic conditions ($pH < 4$) are also used as indicators of beverage quality, which enhances microbiological stability and consumer safety (Galvão et al., 2020). The color of grape juice is an important characteristic observed by consumers as an indicator of grape juice quality (Chen et al., 2020; Cosme et al., 2018). Results of the color analysis (Table 3) demonstrated that the newly elaborated isotonic beverages with natural anthocyanins of grape juices, although with slight differences between the two groups, can present an attractive color for consumer acceptance. 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 (Pinto et al., 2022). 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 (Bendaali et al., 2022b). On the other hand, results of 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. (González-Molina et al., 2012) interpreted the decrease in anthocyanin concentration in blends of lemon juice with elderberry concentrate and lemon juice with grape concentrate by the high vitamin C content in lemon juice, which affects the stability of the anthocyanins, either by their degradation through condensation at the fourth position or a free radical mechanism, or by the degradation of ascorbic acid to dehydroascorbic acid, furfurals, and hydrogen peroxide, which attack anthocyanins. Bingöl et al. (Bingöl et al., 2022) 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 copigmentation of anthocyanins with flavanols that could lead to a change in the color expression (Gironés-Vilaplana et al., 2014; Gironés-vilaplana et al., 2012). 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 (Gironés-Vilaplana et al., 2016). Generally, anthocyanins are more stable under acidic conditions (Bendokas et al., 2020) and unstable in neutral and alkaline pH (Vidana Gamage et al., 2021). Therefore, copigmentation is considered a mechanism of anthocyanin stabilization by the interaction with colorless or yellowish copigments through intramolecular copigmentation, intermolecular copigmentation, metal complexation, or self-association (Morata et al., 2019). This results in a stable structure that preserves the flavylium chromophore from nucleophilic attack by water, leading to a reduction of colorless hemiketal and chalcone formation (Liu et al., 2019; Tan et al., 2021). Thus, researchers have used copigmentation as a natural way to improve the stability of anthocyanins based mainly on phenolic acids (hydroxycinnamic and hydroxybenzoic acids) (Bendokas et al., 2020). Moreover, Vidana Gamage et al. (Vidana Gamage et al., 2021) 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 (hop, tea, and mint) is to obtain a natural isotonic drink characterized by the 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. 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 (Bendaali et al., 2022a) 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 (Corte et al., 2013; Gonçalves et al., 2011; Goulart et al., 2020; Martins et al., 2020; Neto et al., 2017; Toscano et al., 2020; Toscano et al., 2015, 2017). 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 (Embuscado, 2015). Herbal supplements have long been used to treat problems caused by stress, inflammation, and sleep, but their effects on post-exercise recovery have not been measured directly. However, results of studies and investigations at the cellular level suggested that some of these compounds have the potential to improve post-exercise recovery (O'Connor et al., 2022). 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 (Cosme et al., 2018). 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 (Galvão et al., 2020). This palatability depends on the balance of sugars, acids, and salt (Coso et al., 2008). Likewise, hop is characterized by its bitterness, flavor, and aroma due to its essential oil fraction, which contains a complex group of volatile compounds (Martins et al., 2020). As well, it has interesting antioxidant, antimicrobial, and antifungal activities (Arruda et al., 2021) and can be used as a natural preservative to extend the shelf life of food products (Baji et al., 2019). 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) (Swarnalakshmi et al., 2019). 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 (Baruah et al., 2012). 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 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 loss 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 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 the 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. The new isotonic drinks flavored naturally with herb and spice extracts were sensorily preferred. 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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CAHPTER 6. GENERAL DISCUSSION

6.1 Main finding

The objective of this Doctoral Thesis was to design beverages that incorporate key elements in line with current trends in the food industry such as innovation, natural ingredients, organic components, functionality, health, and sensory appeal. Therefore, the first study of this thesis has looked at various aspects of isotonic beverages, including their purpose, composition, and the importance of their ingredients and has investigated how grape juice can improve the quality of these beverages by showing the chemical composition of grape juices and the health benefits of their consumption, especially in conjunction with physical activity. Furthermore, the interesting sensory properties that can be imparted to the beverages were presented. In the second paper, we used organic grape juice and mineral water to prepare natural and healthy beverages that were acidified with lemon juice and naturally flavored with herb and spice extracts avoiding the use of chemical additives. In the third article, we wanted to develop isotonic drinks based on the formulation of the beverages in the previous paper by adding the necessary concentration of salts. Finally, the fourth article (Annex I) was also about the formulation of isotonic drinks but by designing new formulations introducing changes in the previous ones (articles two and three) and using new ingredients. However, in each of the prepared beverages, our focus was directed toward examining their chemical and nutritional composition, observing the changes in color, and assessing how the ingredients interacted with one another, including the effects of water mineralization, pH, and the influence of salts on anthocyanins. Furthermore, sensory evaluation played a crucial role in the development of these beverages, especially since they were novel products. It was essential to assess how the combination of grape juice with herbal and spice extracts could improve the sensory characteristics and overall acceptance of these beverages.

6.1.1. Nutritional aspect

As previously mentioned, our primary goal is to develop additive-free beverages. Our first research has shown that grape juice has significant potential as a base for isotonic drinks designed to enhance the performance of athletes by providing a source of energy and replenishing minerals lost during physical exercise. Grape juice is particularly suitable for this purpose because it is rich in sugars, organic acids, minerals, vitamins, and amino acids (Gutiérrez-Gamboa et al., 2019). Glucose and fructose are the main sugars found in grape juices. They give grape juice a sweet taste and serve as a source of energy. Grape juice is commonly used as a sweetener in various industries due to its sweetness (Kersh et al., 2023). Furthermore, various organic acids are found in grape juice, which contribute to its acidity and overall taste. The two most important organic acids in grape juice are tartaric acid and malic acid (90% of total organic acids). Grape juice may also contain small amounts of other organic acids, such as succinic acid, citric acid, acetic acid, and lactic acid. The balance of these organic acids, along with the sugars, and other aromatics components, is responsible for giving grape juice its unique taste (Cosme et al., 2018; Kersh et al., 2023; Lima et al., 2014). Furthermore, Grape juice has attained considerable attention among grape products, because it does not contain ethyl alcohol and is rich in phenolic compounds (Granato et al., 2016). However, the composition of grape juice may vary depending on different factors including grape species, varieties, region, climate, soil, degree of maturation, technology method of juice elaboration, thermal treatments, enzymes, culture conditions, agronomical procedures, and the type of farming system (conventional, organic, and biodynamic) (Butu & Rodino, 2019; Coelho et al., 2018; Granato et al., 2016; Moreno-Montoro et al., 2015).

Furthermore, in the second study grape juice was used to create healthy beverages with natural sweetness and low sugar content by diluting the concentrated grape juice (65 °Brix). Sugar consumption has been associated with negative health effects, including the risk of type 2 diabetes, cardiovascular disease, and obesity. Consequently, there has been a growing focus on reformulating products and developing low-sugar alternatives. Consumers have responded positively to this trend, increasing their interest in healthier products that have low-sugar content (Martins et al., 2021). On the other hand, during the third study, the previous healthy beverages were developed into isotonic drinks by adding salts (sodium and potassium) to their formulations. The isotonic drinks were within the range of the recommended concentration of carbohydrates (6-8%) from grape juice that help to increase performance by balancing the depletion of the body's carbohydrate stores (Bonetti et al., 2010; Ferreira et al., 2021). In addition, grape juice sports drink does not require sweeteners or additives, except for sodium chloride. The added sodium chloride increases the sodium content of the drinks without affecting their flavor (Zhang et al., 2021). Therefore, salt was included in the drinks (0.5-0.7g/L) to supply the required electrolytes (sodium and potassium) essential in sports beverages, mainly sodium which aids to compensate sodium loss through sweating during exercise and to improve the transport of glucose in the intestine, which ultimately facilitates water absorption (Coso et al., 2008).

In our first study, we conducted a comparison between certain commercial isotonic beverages, energy drinks, and fruit juices. The findings revealed that the carbohydrate concentration of isotonic drinks was significantly lower (up to 186.36%) compared to energy drinks and fruit juices. Conversely, isotonic drinks tend to have slightly higher salt levels than energy drinks and completely lack salt, as seen in fruit juices. Furthermore, proteins, fats, and dietary fibers are notably absent in both isotonic and energy drinks, while they are present in fruit juices. Finally, stimulants like caffeine and guarana are only found in energy drinks. However, increased carbohydrate and salt concentration in beverages can slow down fluid intake by affecting the rate of gastric emptying. Therefore, optimizing the precise concentration of carbohydrates and salts is crucial for sports nutritionists and beverage manufacturers. Due to their osmolarity which is similar to the body fluids and depend on the concentration of carbohydrates (6-8%) and sodium (450-1100 mg/L) (ANVISA, 2010), isotonic drinks are particularly suitable for maintaining adequate hydration during physical activity as they are rapidly absorbed (Demirhan et al., 2015; Sugajski et al., 2023; Urdampilleta & Gómez-Zorita, 2014). Additionally, lemon juice, which is high in citric acid and ascorbic acid (Gironés-vilaplana et al., 2012) which was used as an acidulant, as well as the organic acids found in grape juice all contributed to the low pH values of the prepared drinks (pH < 3.5). The acidity in beverages is an important factor that ensures the microbial safety by preventing the proliferation of pathogenic bacteria such as *Clostridium botulinium* (Galvão et al., 2020; Porfírio et al., 2020).

6.1.2. Contribution of Polyphenols

In the first study, we discussed the benefits effect that grape juice can bring to the beverages mainly those that are related to its antioxidant, anti-inflammatory, anticancer, anti-aging, antimicrobial, and cardioprotective properties (Xia et al., 2010). These benefits are linked to its secondary metabolites, representing by the abundant polyphenol content which are classified to flavonoids such as flavanols, flavonols, flavanones, proanthocyanin, and anthocyanins, and non-flavonoid compounds such as phenolic acids and stilbenes (da Silva et al., 2016; Dutra et al., 2021; Kersh et al., 2023; Lima et al., 2014; Natividade et al., 2013; Rodrigues et al., 2012; Toscano et al., 2017). However, anthocyanins are the main phenolic components in red grape juice, and each grape species and variety has its own particular combination of anthocyanins, whereas flavan-3-ols are the most common antioxidant in white grape juice (Cosme et al., 2018). The skin of grapes is the primary source of polyphenols, and the concentration of these compounds varies depending on the variety, maturity, soil, climate, geographic origin, environmental conditions, cultivation, and exposure to disease. In addition, other elements such as processing conditions, techniques, thermal treatments, and enzyme use can affect the concentration and composition of these compounds (Guler, 2023).

Regarding physical exercise, we referenced several experimental studies and their outcomes. These studies have shown that grape juice can enhance performance and antioxidant activity, protect against oxidative damage, improve glucose homeostasis, decrease blood pressure, and reduce inflammation (Corte et al., 2013; Eshraghi-Jazi et al., 2012; Gonçalves et al., 2011; Goulart et al., 2020; Martins et al., 2020; Neto et al., 2017; Toscano et al., 2020; Toscano et al., 2015, 2017).

The measurement of total phenolic index and antioxidant activity of the elaborated beverages during the second and the third study revealed that they are a good source of phenolic compounds and antioxidants which can be beneficial for athletes in combating oxidative stress mainly when they were enriched with herb and spice extracts which also known as natural sources of polyphenols. The antioxidant activity values measured in the prepared isotonic drinks ranged from 3.28 ± 0.01 to 4.27 ± 0.09 µmols Trolox equivalent/mL being higher in the beverages flavored with herb and spice extracts compared to control samples. However, the combination between hop and tea extracts resulted in high antioxidant activities in the third article, while the samples which contained the mixture ginger-hop showed the highest antioxidant activity value (4.92 ± 0.06 µmols TE/mL) in the fourth article. Likewise, the formulation of functional beverages using natural sources of bioactive compounds and the evaluation of their antioxidant activity have been highlighted significant objectives in numerous research studies (de Castro et al., 2021; Estupiñan et al., 2011; Ferreira et al., 2021; Gironés-Vilaplana et al., 2013; Sahraee et al., 2022; Todaro et al., 2023).

6.1.3. Stability of anthocyanins and color evolution

Given that the anthocyanin content in grape juice makes a major contribution to the attractive red color of the prepared beverages, we sought to identify these compounds and evaluate their stability and color changes in the beverages. The HPLC analysis revealed the presence of two group of anthocyanins: non-acylated anthocyanins encompassing delphinidin-3-O-glucoside (D3G), cyanidin-3-O-glucoside (C3G), petunidin-3-O-glucoside (Pt3G), peonidin-3-O-glucoside (Pn3G), and malvidin-3-O-glucoside (M3G), and acylated anthocyanins, which included petunidin-3-(6″-acetylglucoside) (Pt3G Ac), cyanidin- 3- (6″- acetylglucoside (C3G Ac), malvidin-3-(6″-acetylglucoside) (M3G Ac), Cyanidin-3- (6″-*p* coumaroylglucoside (C3G Cum), Petunidin-3-(6″-*p* coumaroylglucoside) (Pt3G Cum), and Malvidin- (6″-*p* coumaroylglucoside) (M3G Cum).

In general, our findings from the second and third articles indicated that non-acylated anthocyanins were more abundant than acylated ones. Non-acylated anthocyanins made up 77.77%, 86.37%, and 72.44% of the total content of anthocyanins in the second, third, and fourth articles, respectively. Malvidin pigments were also the predominant type, accounting for 47.54% of the total anthocyanin content in the second article, 52.46% in the third article, and 46.74% in the fourth article. The glucoside forms of cyanidin, malvidin, delphinidin, peonidin, and petunidin are the most abundant anthocyanins in grapes (Tiwari et al., 2009). Nevertheless, the stability of anthocyanins can be influenced by various factors, including their chemical structure, pH levels, temperature, enzymatic activity, type of anthocyanin pigment, exposure to oxygen and light, the presence of co-pigments, and components of the food matrix such as ascorbic acid, salts, sugars, minerals, sulfites, antioxidants and proteins (Enaru et al., 2021; Khoo et al., 2017; Ren & Giusti, 2021; Vidana Gamage et al., 2021). Since color characteristic depends on the anthocyanins content, our findings in the second article showed that the level of minerals and pH of water had an impact on the acidity of the beverages and therefore on the color. This was evident when we used two distinct types of water (high and low mineral water), and both groups contain an equal amount of lemon juice. A noticeable increase in color intensity was observed during the 20 days of storage (up to 126.56%) with observed differences in both pH values and color intensity between the two groups of beverages. Higher color intensity values were marked in the beverages with low mineralization and high acidity. The same observation was for the anthocyanins content which were higher in the samples prepared with low mineral water compared to those in the other group. Generally, anthocyanins are more stable under acidic conditions and unstable in neutral and alkaline pH (Bendokas et al., 2020; Vidana Gamage et al., 2021). Depending on the pH of the solution, anthocyanins are found in four different chemical forms (Enaru et al., 2021; Tang et al., 2023), including neutral quinoidal base (purple or blue), flavylium cation (red), carbinol or pseudobase (colorless), and chalcone (colorless) (Cai et al., 2022). The flavylium cation is red colored when the pH is from 1 to 3, the colorless carbinol pseudobase appears at pH 5, and in the basic pH (7-8) the quinoidal base is formed of with blue purple color (Bueno et al., 2012).

When the results from the second and third articles were compared in terms of color intensity, it became clear that the addition of salt to the beverages had an impact on the color characteristics. The water mineralization impacted the pH of samples, as observed previously, but the beverages with low mineralization did not exhibit the highest values of color intensity. It was observed that most samples made with high-mineral water had higher color intensity values compared to the samples made with low-mineral water mainly at the end of the storage period contrary to what had been seen in the previous study except the control sample which presented the highest value in the group (A) followed the sample with the highest value in group (B). Additionally, the comparison between the three different added salt concentrations showed that the effect of salt on the color of beverages varied among samples depending on the salt concentration and the composition of each sample. At the beginning of storage, the highest value of color intensity in group (A) was in the sample containing hop-mint and a high concentration of salt (C2), however at the end of storage, the highest value was in the control sample followed by the sample containing hop-mint and low salt concentration (C3). In group (B), the color intensity was observed in the two samples containing hop-mint extracts with high and low concentrations of salts (C2 and C3) at the beginning of storage, while at the end of storage, the highest value was found in the sample which contained hop-tea extract and with high concentration of salt (C2). In beverages made with high-mineral water, a high salt concentration (C2) led to greater color intensity, which also increased significantly during storage (up to 129.39%). Conversely, in beverages prepared with low-mineral water, control sample without salt exhibited high color intensity, with color intensity increasing up to 123.58% during storage. In addition, it was also observed that the samples contained hop-mint extracts showed high color intensity values in both groups (following the highest values). The anthocyanins content was shown to be higher in the beverages of group (B) with high concentrations in the samples containing hop-mint extracts in both groups. Furthermore, in the fourth article (Appendix I) whereas the difference between each sample and its control was the addition of salts, the difference in anthocyanin content and color (high or low value) among samples could be due to the positive or negative effect of the salts. The effect of salt on phenolic composition and anthocyanins is controversial in the literature with different assessments of the positive and negative effects (Trivellini et al., 2014). However, the samples that contained the combination of ginger and tea extracts resulted in high anthocyanin content and color intensity values.

According to the analysis of the anthocyanins content over the storage period in the second artice, there was a decrease which showed a negative correlation with the increase in color intensity. Other studies was explained the reduction in the anthocyanins content by the high ascorbic acid content present in lemon juice, which affects the stability of the anthocyanins, either by causing their degradation through condensation at the fourth position or a free radical mechanism, or by leading to the degradation of ascorbic acid to dehydroascorbic acid, furfurals, and hydrogen peroxide, which attack anthocyanins (Bingöl et al., 2022; González-Molina et al., 2012). Furthermore, the increase in color intensity, even in the presence of anthocyanin degradation, could be due to the formation of alternative-colored polymers or co-pigmentation of anthocyanins with other phenolic compound which could lead to a change in the color expression (Gironés-Vilaplana et al., 2014; Gironés-vilaplana et al., 2012). Therefore, the presence of other phenolic compounds from herbs and spices as well as the minerals in the beverages could interact with anthocyanin pigments and influence the color expression. However, copigmentation is a recognized method for enhancing the stability of anthocyanins by involving interactions with colorless or yellowish copigments. These interactions can take various forms, including intramolecular and intermolecular copigmentation, metal complexation, or self-association. Researchers have utilized copigmentation as a natural way to improve the stability of anthocyanins (Bendokas et al., 2020; Liu et al., 2019; Morata et al., 2021). Moreover, the copigmentation which based on intermolecular, electrostatic, hydrogen-bonding, and hydrophobic interactions, has been a successful technology used in the food industry to change food color to preserve or recreate natural color intensity or to produce new hues. In this respect, it has been discovered that the addition of organic acids, flavonoids, alkaloids, polysaccharides, proteins, and amino acids increases the stability and may even change the bioactivity of anthocyanins (Gençdağ et al., 2022). In addition, acylation contributes to the stability of anthocyanins helping in the intramolecular copigmentation, protecting the flavylium form, and making an acidic medium that preserves color stability. Consequently, acylated anthocyanins are notably more stable than non-acylated ones (Vidana Gamage et al., 2021).

6.1.4. Sensory quality

The visual aspect of food products is a crucial factor in consumer decision-making. For example, in the case of beverages, color is a fundamental attribute that influences purchasing choices and perception of flavor (Gérard et al., 2019). Consumers, as they adopt more sustainable lifestyles, are favoring natural, healthy, innovative, and delicious options that are both nutritious and environmentally friendly. Additionally, they look for products that minimize the use of chemical preservatives and processing technology (Pinto et al., 2022). In the case of isotonic drinks, they are often consumed more frequently than water during sports exercises because of their appealing taste attributes such as sweetness, acidity, mouthfeel, and temperature (Galvão et al., 2020). To achieve these desired sensory qualities, we blended grape juice and herb-spice extracts. These components significantly contributed to creating more natural drinks and help to dispense the need for synthetic additives. Consequently, when we conducted sensory evaluations of the prepared healthy beverages and natural isotonic drinks (as detailed in the second and third articles, and Appendix I), it became evident that the herb-spice extraction improved the overall sensory characteristics of grape juice. Hence, the panelists exhibited a more favorable overall perception of the naturally flavored beverages compared to their respective control groups (Article 2). In terms of water used for preparing these beverages, drinks prepared with low mineral water had higher global perception scores compared to those made with high mineral water. However, the combination of hop-tea in group (A) was the preferred mixture, whereas in group (B) the preferred mixture was hop-mint. In terms of color intensity, the beverage prepared with low mineral water and flavored with hop-tea mixture received the highest score. Regarding aroma, the intensity was higher in the beverage flavored with hop-mint in group (B), while the highest score for aroma quality was marked in the drink flavored with hop-mint in group (A). Furthermore, in the context of isotonic drinks (Article 3), the flavored beverages received the highest overall perception scores when compared to the control samples. The hop-mint mixture had the highest score of global perception in both groups, however, in group (A) the combination hop-mint was preferred in the beverages with low concentration of sodium and potassium (C3), while in group (B), both beverages with low concentrations of salts (C1, containing only sodium, and C3, containing both sodium and potassium) had the highest score. The sample containing a hop-mint mixture with a high concentration of salt (C2) and low mineral water was shown to have a higher color intensity. The two beverages with salt concentration C1, one flavored with hop-tea in group (A) and the other with hop-mint in group (B), were found to have a higher aroma intensity, however, the latter also had the highest aroma quality score. On the other hand, the fourth article revealed that the beverages containing the combination of ginger extract with hop, tea, and mint in grape juice-based isotonic drinks showed higher global perception scores compared to those flavored with ginger alone, with a preference of the mixture ginger-mint among the others. The highest color intensity was observed in the isotonic drinks which contained ginger-mint and ginger-tea and the highest aromatic quality was found in the isotonic drink flavored with ginger-mint. The comparison with the commercial isotonic drinks showed that it had the lowest values in terms of aroma intensity, herbaceous, floral, body, salinity, and acidity, while it was identified as the most sweetener and fruiter. Hence, the incorporation of a variety of herbs and spices as natural flavorings, along with their combination with grape juice and other ingredients in beverage formulations, has been demonstrated to have a significant impact on taste, aroma, color, and overall sensory perception. This enhancement of the sensory profile would allow these beverages to be accepted by consumers as innovative and natural beverages. Moreover, several studies have shown that incorporating herbal extracts into fruit juices can have a positive impact on their sensory characteristics. Yellow, orange, and red fruit juices are often supplemented with herbal extracts to improve their natural qualities without causing any changes in flavor or color (Maleš et al., 2023). A tasty and nourishing pineapple and milk whey-based beverage was formulated, incorporating different amounts of mint extract which resulted in an improvement in the beverage's overall acceptability and organoleptic scores mainly with an increase in mint extract from 0 to 1% (Kumar et al., 2017). Cinnamon, mint, ginger, and clove extracts demonstrated the potential to be used in developing a palatable and functional cucumber juice, enhancing its sensory characteristics, mainly taste and flavor, which were enhanced mainly by clove, and the color characteristics were enhanced by mint and cinnamon (Saad et al., 2021). In another study, mixed beverages containing organic apple juice with cardamom tea were prepared and the formulations showed good overall impressions and acceptability (de Souza et al., 2020).

6.2. Conclusion

The mains conclusions derived from this thesis can be summarized as follows:

- The combination of grape juice and natural flavors from herbs and spices seeks to provide consumers with unique and differentiated beverages, with new flavors, aromas, and colors which represent an innovation in the food industry and meet the consumer demand for more natural, healthier, and less artificial products.

- Grape juice plays a significant role in the creation of low-sugar beverages by reducing its sugar content (around 80 g/L), thereby eliminating the need for added sugar. This sugar content serves as a source of energy. As well as it contributes to the palatability of beverages primarily through the balance between acidity and sweetness.

- Phenolic compounds found in grape juice contribute to the sensory attributes, including color, taste, and flavor of the beverage. Specifically, anthocyanins play an important role in creating a natural, attractive red color for drinks, replacing synthetic colorings.

- The red color of grape juice is attributed to its anthocyanin content, and the stability of these anthocyanins can be affected by various factors, resulting in color changes with an increase during storage period up to 129%.

- The results showed that the composition of beverages, especially the mineralization of water, pH, and the presence of salts, can affect the color of anthocyanins, resulting in color variations between beverages based on their composition.

- Isotonic drinks made from grape juice offer functional products with antioxidants and biological activities in addition to their principal role of rehydration and replacement of minerals and carbohydrates during physical exercise.

- Herbs and spices have the potential to serve as natural flavor enhancers, improving the sensory characteristics of beverages when they are blended with grape juice. This combination contributes to an improved overall evaluation of the beverages, enhancing their overall taste and aroma.

6.3. Future research lines

This thesis had the goal of developing functional beverages using natural ingredients. These beverages were designed by combining grape juice as the primary component with herb and spice extracts to improve their overall quality. The research delved into examining the physicochemical characteristics, their biological effects, and their sensory attributes. Likewise, potential future research could explore alternative approaches in both the production methods and the selection of ingredients for these beverages. Given that these products combine health advantages with an appealing sensory profile, it would be interesting to incorporate additional fruit juices and plant extract ingredients as natural flavor enhancers in the beverage formulations. In addition, fortifying these drinks with protein sources would enhance their functionality and nutritional content. This enhancement is essential for aiding muscle recovery and overall performance, as both athletes and physically active individuals need to ensure they receive sufficient protein to maintain a balance between muscle protein synthesis and breakdown (Orrù et al., 2018). On the other hand, better results can be obtained if the safety of newly developed beverages is further investigated by analyzing their microbial properties. This can ensure that the beverages are free of microorganisms and determine how long they can be kept and how they need to be stored to avoid spoilage. In addition, it is possible to conduct comparisons between different preservation methods and under different storage conditions to determine the most effective technique. Another option is to use white grape juice instead of red grape juice and add anthocyanin extract. Emerging non-thermal technologies such as high hydrostatic pressure, pulsed electric fields, ultra-high pressure homogenization ultrasound, irradiation, and pulsed light can also be used in anthocyanin extraction to increase yields, speed up the process and improve anthocyanin extraction efficiency and maintain antioxidant capacity (Morata et al., 2021). Finally, to assess the potential positive impact of these beverages, it would be advisable to conduct in vivo and clinical studies. These investigations would serve to confirm their biological activity and assess their bioavailability.

CHAPTER 7. REFERENCES

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CHAPTER 8. APPENDEX I

ARTICLE 4

Physicochemical, Antioxidant activity, and Sensory Properties of Grape Juice-Herbs Extract Based Isotonic Beverages.

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Abstract

The aim of this work was to develop isotonic drinks free of chemical additives and based on grape juice as a source of carbohydrates, pigments and rich in antioxidants, mixed with herb and spice extracts to improve the taste of the drinks. The color evolution, pH and acidity, sugar content, anthocyanin content, antioxidant activity and sensory properties were analyzed. The results of color evolution demonstrated an increase in color intensity in all samples over four weeks. The determination of anthocyanins revealed the presence of non-acylated and acylated anthocyanins, with malvidin pigments predominating (71.75-73.05%). Antioxidant activity values ranged from 3.97 to 4.92 µmols TE /mL and were positively correlated with anthocyanin content, howevers anthocyanins were negatively correlated with pH. Sensory evaluation showed that the combination of grape juice with herb and spice extracts promoted the use of natural sources to enrich sports drinks with antioxidants and improve their sensory profile.

Keywords: Grape juice; ginger; hop; mint; tea; antioxidants.

1. Introduction

Functional foods comprise substances that possess biological and physiological activities, resulting in health advantages that go beyond the fundamental nutritional properties (Banwo et al., 2021). Growing consumers interest in healthy nutrition is one of the key reasons supporting the development of the functional food market (Mudgil & Barak, 2019). Fruits have a functional role in health and exercise because they are rich in bioactive phytochemical components (phenolic compounds) in addition to their macro and micronutrients such as carbohydrates, minerals, fiber, and vitamins (Naderi et al., 2018).

Isotonic drinks are functional beverages that contain on their composition minerals and carbohydrates, with an osmolarity value similar to the osmolarity of the human body (270 to 330 mOsm kg–1 of water) which helps to replenish quickly minerals lost during physical activities and contributes to avoiding dehydration (Stasiuk & Przybyłowski, 2017). Moreover, due to its typical palatability (sweetness, acidity, taste intensity in the mouth and temperature of the drink), the isotonic drink is consumed more frequently than water during sports exercises (Galvão et al., 2020). Thus, flavorings and synthetic dyes with sensory characteristics similar to fruit are used during the development of isotonic drinks. However, studies have demonstrated the toxicity of synthetic dyes and their harmful effects on health as well as the consumers concerns about the chemical additives in these beverages (Porfírio et al., 2020). Accordingly, researchers are interested in the development of new isotonic drinks characterized by organoleptic properties from natural sources and replacing synthetic colorants, especially anthocyanins and carotenoids (Bendaali et al., 2023; Bovi et al., 2017; Cerezal Mezquita et al., 2020; Porfírio et al., 2020). In addition, the purpose of using these natural sources is also related to their known biological activities, especially antioxidant activity, since physical exercise can cause oxidative stress and muscle damage (D'Angelo, 2019). Regarding flavors, herbs and spices have been used since the old years as flavoring agents. Recently, the interest in the use of these substances as sensory and functional additives has been renewed due to their recognized antioxidant and preservative benefit effect (Mitchell et al., 2013). Many studies have looked at the use of plant extracts to improve the health and sensory properties of fruit juice-based beverages (Skąpska et al., 2020)

The composition of grape juice consists of water and a combination of various metabolites, including a high proportion of sugars, organic acids, minerals, and phenolic and aromatic compounds (Dutra et al., 2021). The combination of sweet and acidic taste in grape juice enhances consumer appeal (Kersh et al., 2023). Moreover, the color of the grape juice, which is related to the anthocyanin content, is the most important quality indicator for consumers (Cosme et al., 2018). Grape juices are rich in phenolic compounds such as flavanols, flavonols, anthocyanins, phenolic acids, and stilbenes. In a previous section of our review, we discussed how grape juice's antioxidant and anti-inflammatory qualities can improve physical performance during physical activity, as demonstrated by a number of experimental investigations (Bendaali et al., 2022a).

The aim of this work was to develop isotonic drinks which based on natural and organic products, avoiding chemical additives, using red grape juice as a source of sugars and natural colorings, and enhancing the sensory profile with mixtures of herb and spice extracts (ginger, hop, tea, mint). The beverages were studied and evaluated for their physicochemical characteristics and sensory acceptance.

2. Materials and Methods

2.1. Raw Materials

Organic concentrated red grape juice provided by the Spanish company Mostosa (Ciudad Real, Spain), pasteurized squeezed lemon juice (Hacendado, Mercadona, Spain), ground ginger (Ducros, Mercadona, Spain), red tea, dried mint (Cafetearte, Madrid, Spain), hop (Summit, Spain), and table salt: sodium chloride (Sal Costa) and potassium chloride (Aranca) were diluted in mineral water (Solan de Cabras) with 278 mg/L of dry residues (Mercadona, Spain) to prepare the new isotonic drinks. All ingredients used for the elaboration of the isotonic drinks were food grade.

2.2. Preparation of Isotonic Drinks

Preliminary experiments were conducted to determine the composition of isotonic beverages with different concentrations of grape juice, lemon juice, herb and spice extracts, and salts to obtain isotonic beverages characterized by suitable sensory properties in terms of color, acidity, taste, and aroma.

The composition of prepared isotonic drinks for 500 mL was: 80 mL/L of grape juice, 26 mL/L of lemon juice, 0.30 g/L of sodium chloride, and 0.20 g/L of potassium chloride. Two groups of beverages were prepared, the first group contained isotonic beverages and the second group contained control beverages (without the addition of salts). The beverages were flavored with herb and spice extracts: ginger (6 mL/L) + hop (4 mL/L)/ tea (2 mL/L)/ mint (1 mL/L). The samples were labeled as follows: CGLG, CGLGH, CGLGT, CGLGM, IGLG, IGLGH, IGLGT, IGLGM, the label of each sample is composed of three sections, the first section refers to the type of sample: control grape juice (CG) and isotonic grape juice (IG); the second refer to lemon juice (L), and the third section of the label refer to the type of extracts: ginger (G), ginger-hop (GH), ginger-tea (GT), and ginger-mint (GM). The extraction of flavors and aromas from herbs and spices were performed by crushing the weighted amount of the herb/spice (5 grams of ginger, 4 grams of hop/tea/mint) and mixing each crushed spice or herb with 30 mL of diluted grape juice. Ultrasounds (3300EP SONICA) for 10 min followed by centrifugation of the mixtures (Eppendorf, 5430 R) at 6000 rpm at 20°C for 10 min were carried out to obtain the extracts. The extracts were filtered using filter paper and kept refrigerated at 4°C until added to the beverages.

All samples were prepared and analyzed in triplicate thermally treated by autoclave at 100°C for five minutes then they were kept under refrigeration at 4°C until subjected to physicochemical and sensory analysis.

2.3. Physicochemical Characteristics

The equipment OenoFoss™ (Foss Iberia SA, Barcelona, Spain) using Fourier transform infrared spectroscopy (FTIR) was used to quantify and identify sugar concentration and total acidity in the beverages.

2.4. pH Measurement

The Crison brand pH meter GLP 21 model (Hach Lange Spain, S.L.U., Madrid, Spain) was used for the pH measurement of each sample.

2.5. Anthocyanins Determination

The anthocyanins in the prepared beverages were determined according to Escott. et al (Escott et al., 2017) using a series 1200 high-performance liquid chromatography (HPLC) (Agilent Technologies, Palo Alto, CA), equipped with a diode array detector. A volume of 50 µL of filtered samples was injected and the concentrations were calculated with a calibration curve of malvidin-3-O-glucoside (r2 = 0.9999, LOD = 0.1 mg/L).

2.6. Color Assessment

The color parameters: color intensity ($\mathbf{A}_{420}$ + $\mathbf{A}_{520}$ + $\mathbf{A}_{620}$), tonality ($\mathbf{A}_{420}$/$\mathbf{A}_{520}$), red color (A_{520}) luminosity (L), hue (h), and chroma (C) were obtained with the DNA Phone Smart Analysis (Parma, Italy).

2.7. Antioxidant Activity

The $ABTS^{+}$ method described by Re et al. (Re et al., 1999) was used to evaluate the antioxidant activity (AOX) of the prepared isotonic drinks. For this assay, a solution of 7 mM ABTS was mixed with 2.45 mM of potassium persulfate to create the $ABTS^{\cdot+}$ radical solution. After incubation for 16 hours in the dark, the radical solution absorbance was adjusted to 0.70 ± 0.02 at 734 nm using ethanol. The diluted samples (30 µL) were mixed with the $ABTS^{\cdot+}$ radical solution (3 mL) and incubated for six minutes in the dark. Then, the absorbance was measured at the wavelength of 734 nm. Trolox was used as a standard, and the results were presented as µmols Trolox equivalents/mL.

2.8. Sensory Analysis

The sensory evaluation of the prepared drinks was carried out in a room provided with white light and constant 21 °C at the Chemistry and Food Technology Department of the School of Agricultural, Food and Biosystems Engineering (ETSIAAB) at Universidad Politécnica de Madrid (Spain). The trained panel consisted of eight tasters representing both sexes and ages between 22 and 60 years. Each taster received four glasses of isotonic beverages, four glasses of control samples, and a glass of a popular isotonic drink in Spain (Aquarius, lemon-flavored isotonic drink) which was chosen for comparison with the new natural isotonic beverages. The tasters used a scale from 1 (low perception) to 5 (high perception) to rate the intensity of the attributes (Color intensity, tonality, turbidity, aromatic intensity, aromatic quality, herbaceous, floral, fruity, reduction, oxidation, body, bitterness, sweetness, acidity). Finally, they evaluated the overall impression, considering olfactory and gustatory aspects, as well as the lack of defects. The significant differences between samples were determined by ANOVA analysis ($p < 0.05$) and are shown in the radar chart obtained. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (UPM Ethics Committee) of Universidad Politécnica de Madrid (AIDLAPPMLB-AMB-HUMANOS-20221026 on November 14th, 2022).

2.9. Statistical Analysis

Statistical analyses were performed with Microsoft Excel 2016 and Statgraphics Centurion 18 software V.18.1.06 (Graphics Software Systems). The samples were compared by their means (± standard deviations) and through one-way ANOVA with the least significant difference (LSD) test. Significance was set at $p < 0.05$.

3. Results

3.1. Physicochemical Characteristics

The analysis of the physicochemical characteristics of the isotonic drinks prepared with grape juice and herb and spice extracts using the FOSS analyzer (Table 1) shows a significant difference ($p < 0.05$) between the samples. The total acidity was from 1.38 to 1.48 g of tartaric acid/ L, this acidity led to the lower pH values in the beverages which were between 3.70 and 3.74, being slightly higher in the controls. Leśniewicz et al. (Leśniewicz et al., 2016) evaluated the pH of six commercial isotonic drinks and the values ranged from 2.58 to 3.75. The pH values of commercial isotonic drinks and berries and lemon juice-based isotonic drinks measured by Gironés-Vilaplana et al. were between 2.35 and 3.83 (Gironés-Vilaplana et al., 2013). Lemon juice was added to grape juice with the aim of lowering the pH because the acidity is recommended for isotonic drinks (pH < 4) to ensure their microbiological stability and safety by limiting the growth of microorganisms (Ferreira et al., 2021). Grape juice and lemon juice are rich sources of organic acids. The main organic acids in grape juices are tartaric, malic, citric, lactic, acetic, and succinic acids whereas tartaric and malic acids present 90% of the total acidity of grape juices (Granato et al., 2016). However, lemon juices are known for their rich content of ascorbic acid and citric acids (Gironés-vilaplana et al., 2012). Sugars are also an important component in isotonic drinks, the concentration ranged from 66.97 to 69.13 g/ L in the prepared drinks. This sugar content is from grape juice which is used as a natural source of sugars because it is known for its high content of sugars mainly glucose and fructose (Kersh et al., 2023).

Table 1. Physicochemical characteristics of prepared beverages.

	CGLG	CGLGH	CGLGT	CGLGM	IGLG	IGLGH	IGLGT	IGLGM
Sugar (g/L)	69.13±0.06^{c}	67.27±0.31^{ab}	68.17±0.91^{abc}	67.97±0.97^{abc}	68.90±0.20^{c}	68.40±0.5^{bc}	67.10±1.04^{a}	66.97± 1.01^{a}
pH	3.74±0.01^{cd}	3.75±0.01^{d}	3.75±0.01^{d}	3.75±0.00^{d}	3.70±0.01^{a}	3.72±0.01^{b}	3.74± 0.02^{cd}	3.73± 0.00^{bc}
Total Acidity (g.L)	1.45±0.01^{cd}	1.41±0.03^{ab}	1.42±0.02^{bc}	1.38±0.01^{a}	1.48±0.03^{e}	1.47±0.01^{de}	1.43± 0.01^{bc}	1.45± 0.01^{cd}

Note: Values are means with standard deviations, n = 3. Different letters in the same parameter indicate statistical differences ($p < 0.05$) between samples. Total acidity is expressed as tartaric acid.

3.2. Anthocyanins Content

The typical color of red grape juice is due to the presence of anthocyanins. Figure 1 A shows the anthocyanin pigments which were identified and quantified in the prepared beverages by HPLC. The anthocyanin profile included acylated and non-acylated anthocyanins with the highest amount of non-acylated anthocyanins (71.75-73.05%) comprising delphinidin-3-O-glucoside (D3G), cyanidin-3-O-glucoside (C3G), petunidin-3-O-glucoside (Pt3G), peonidin-3-O-glucoside (Pn3G), and malvidin-3-O-glucoside (M3G), whereas malvidin pigments were predominating followed by delphinidin pigment, with the highest values in CGLGT sample (3.36±0.05 mg/L). However, these pigments were also the predominant acylated anthocyanins malvidin-3-(6″-acetylglucoside) and malvidin-(6″-*p* coumaroylglucoside). Accordingly, the glucoside forms of cyanidin, malvidin, delphinidin, peonidin, and petunidin are the most abundant anthocyanins in grapes (Tiwari et al., 2009), whereas, the most found anthocyanins in grapes juices were cyanidin and malvidin, and the lowest was peonidin (Kersh et al., 2023). The identification of anthocyanins in grape juices from different varieties in several research works (Silva et al., 2019; Lima et al., 2014, 2015; Natividade et al., 2013) demonstrated the presence of the same forms of anthocyanins. However, the concentration of anthocyanins in grape juice depends on the raw materials factors such as the variety of grapes used and the processing technologies such as high temperatures during the extraction and pasteurization, as well as the different forms of storage (Burin et al., 2010; Cosme et al., 2018). On the other hand, when comparing the quantitative profile of the prepared drinks (Figure 1 B), in some case statistically significant differences ($p < 0.05$) were observed between the total content of the identified anthocyanins. The high content was found in the control sample which contained the extract of ginger-tea (CGLGT), which could be due to the anthocyanins from tea extract. It is mentioned that red and purple tea contains two major pigments which are delphinidin and cyanidin (Jiang et al., 2013; Saito et al., 2011; Terahara et al., 2001). However, the isotonic beverage which contained the same extracts (IGLGT) had lower content of anthocyanins than the control. As for the sample which contained ginger-hop extracts, it is observed also that the control sample (CGLGH) had higher total content than the isotonic beverage (IGLGH). Unlike the control samples which contained only ginger extracts (CGLG) and ginger-mint extracts (CGLGM), they had lower content than the isotonic ones.

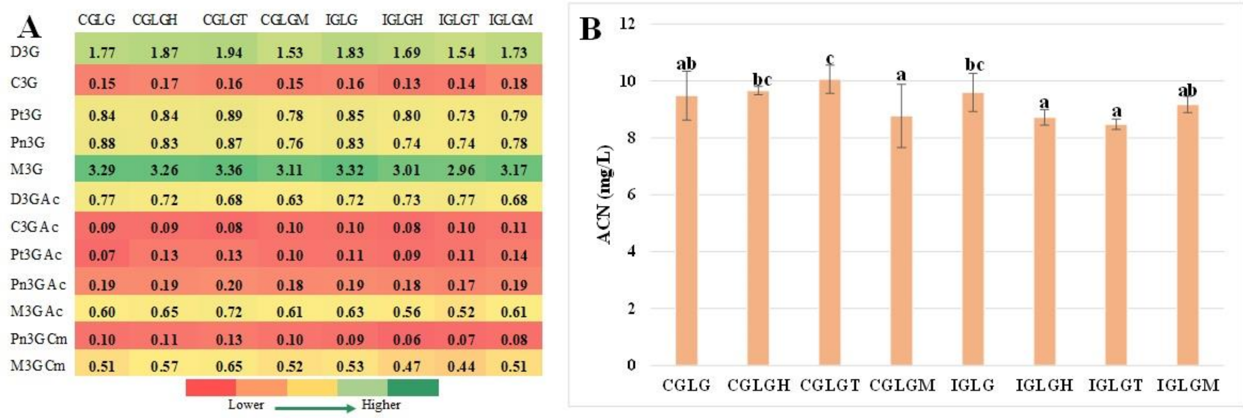

Figure 1. Individual anthocyanins pigments divided in the prepared beverages represented as heatmap (A). Change in total anthocyanins content among samples (B). Different letters indicate a significant difference between samples ($p < 0.05$). **Abbreviations:** D3G:Delphinidin-3-O-glucoside; C3G: Cyanidin-3-O-glucoside; Pt3G:Petunidin-3-O-glucoside; Pn3G:Peonidin-3-O-glucoside; M3G: Malvidin-3-O-glucoside; D3G Ac:Delphinidin-3-(6″-acetylglucoside); C3G Ac: Cyanidin-3-(6″- acetylglucoside); Pt3G Ac: Petunidin-3-(6″-acetylglucoside); Pn3G Ac: Peonidin-3-(6″- acetylglucoside); M3G Ac: Malvidin-3-(6″-acetylglucoside); Pn3G Cm: Peonidin-3-(6″-*p* coumaroylglucoside; M3G Cum: Malvidin-3-(6″-*p* coumaroylglucoside).

3.3. Color Assessment

Color is one of the most important quality factors for consumer acceptance (Chen et al., 2020). The color parameters were determined and evaluated for four weeks (Table 2). Chromatic parameters and luminosity presented in the chromatic circle (Figure 2) showed that all beverages had a red color from grape juice (anthocyanins) with chroma, hue, and luminosity values ranging between 42.68 and 46.46, 38.58 and 40.68, and 22.57 and 28.53 respectively. Besides, all samples showed an increase in color intensity, red color, and hue, being inversely proportional to luminosity which tended to decrease. However, the chroma, luminosity, and tonality values were rather constant. The increase or stability of color intensity has been stated in other studies in anthocyanin-containing beverages that were interpreted by the formation of other colored polymers, or co-pigmentation between anthocyanins and other flavonoids (Gironés-Vilaplana et al., 2014; Gironés-vilaplana et al., 2012).

Table 2. Color parameters evolution for four weeks in the prepared beverages.

Samples	Weeks	Color Intensity[1]	Red Color[2]	Tonality[3]	Chroma[4]	Hue[4] (°)	Luminosity[4] (L)
CGLG	1	4.67±0.06^{ab}	1.86±0.02^{b}	1.10± 0.01^{bc}	45.81±0.92^{d}	39.15± 0.33^{ab}	27.67±0.78^{bc}
	2	4.90±0.04^{a}	1.96±0.02^{a}	1.09± 0.00^{abcd}	45.39±0.35^{bc}	38.96± 0.00^{a}	25.53±0.45^{ab}
	3	4.91±0.04^{b}	1.95±0.02^{b}	1.10± 0.00^{bc}	44.53±0.13^{abc}	39.53±0.00^{ab}	25.37±0.38^{ab}
	4	5.04±0.08^{ab}	1.99±0.03^{abc}	1.09±0.01^{a}	43.23±1.06^{ab}	40.49±0.66^{c}	23.93±0.91^{abc}
CGLGH	1	4.73±0.04^{bc}	1.87±0.02^{b}	1.10±0.01^{bcd}	43.17 ± 0.59^{a}	39.15 ± 0.33^{ab}	26.37 ± 0.55^{ab}
	2	4.80±0.05^{a}	1.91±0.01^{a}	1.09±0.00^{bcd}	44.11 ± 0.56^{a}	38.96 ± 0.00^{a}	26.00 ± 0.53^{ab}
	3	4.87±0.07^{b}	1.93±0.03^{ab}	1.09±0.01^{ab}	43.43 ± 0.67^{a}	39.34 ± 0.33^{a}	25.30 ± 0.70^{ab}
	4	4.97±0.03^{ab}	1.96±0.01^{ab}	1.10±0.01^{ab}	43.04 ± 1.10^{ab}	40.30 ± 0.33^{bc}	24.47 ± 0.51^{abc}
CGLGT	1	4.90±0.06^{c}	1.96±0.03^{c}	1.08±0.01^{a}	43.22±0.60^{ab}	38.58 ± 0.33^{a}	24.87 ± 0.50^{a}
	2	4.74±0.26^{a}	1.90±0.11^{a}	1.08±0.01^{ab}	44.64±0.37^{ab}	38.96 ± 0.00^{a}	26.57 ± 1.94^{ab}
	3	4.93±0.13^{b}	1.96±0.06^{b}	1.09±0.01^{ab}	44.20±0.63^{ab}	39.34 ± 0.66^{a}	25.13 ± 1.15^{a}
	4	5.19±0.20^{b}	2.05±0.07^{c}	1.09±0.00^{a}	42.09±1.16^{a}	39.72 ± 0.33^{ab}	22.57 ± 1.63^{a}
CGLGM	1	4.49 ± 0.22^{a}	1.78 ± 0.08^{a}	1.09 ± 0.01^{b}	43.85 ± 0.56^{ab}	38.77 ± 0.33^{a}	28.53 ± 1.76^{c}
	2	4.71 ± 0.18^{a}	1.88 ± 0.07^{a}	1.10 ± 0.00^{d}	46.29 ± 0.41^{cd}	38.96 ± 0.00^{a}	27.40 ± 1.39^{b}
	3	4.71 ± 0.15^{a}	1.87 ± 0.05^{a}	1.11 ± 0.01^{cd}	46.30 ± 0.96^{d}	39.53 ± 0.58^{ab}	27.37 ± 1.15^{c}
	4	4.97 ± 0.05^{ab}	1.95 ± 0.02^{ab}	1.11 ± 0.00^{b}	43.72 ± 0.35^{ab}	40.49 ± 0.33^{c}	24.73 ± 0.31^{bc}
IGLG	1	4.89 ± 0.10^{c}	1.96 ± 0.04^{c}	1.07 ± 0.01^{a}	44.02 ± 0.60^{ab}	38.77 ± 0.33^{a}	25.23 ± 0.84^{a}
	2	4.80 ± 0.10^{a}	1.94 ± 0.04^{a}	1.07 ± 0.00^{a}	46.46 ± 0.41^{d}	38.77 ± 0.33^{a}	26.60 ± 0.78^{ab}
	3	4.86 ± 0.05^{ab}	1.95 ± 0.02^{b}	1.08 ± 0.00^{a}	45.92 ± 0.67^{cd}	38.96 ± 0.00^{a}	26.00 ± 0.20^{ab}
	4	5.10 ± 0.18^{ab}	2.01 ± 0.07^{bc}	1.09 ± 0.01^{a}	42.68 ± 0.42^{ab}	39.53 ± 0.00^{a}	23.47 ± 1.40^{ab}
IGLGH	1	4.88 ± 0.12^{c}	1.95 ± 0.04^{c}	1.08 ± 0.01^{a}	44.34 ± 0.98^{bc}	39.15 ± 0.33^{ab}	25.47 ± 1.08^{a}
	2	4.84 ± 0.12^{a}	1.94 ± 0.04^{a}	1.09 ± 0.01^{abc}	45.65 ± 0.72^{cd}	38.96 ± 0.57^{a}	26.03 ± 1.01^{ab}
	3	4.88 ± 0.05^{b}	1.95 ± 0.03^{b}	1.09 ± 0.02^{ab}	45.57 ± 0.77^{bcd}	39.34 ± 0.66^{a}	25.73 ± 0.45^{ab}
	4	5.11 ± 0.09^{ab}	2.01 ± 0.04^{bc}	1.10 ± 0.02^{ab}	43.48 ± 0.88^{ab}	40.11 ± 0.58^{abc}	23.67 ± 0.92^{abc}
IGLGT	1	4.65 ± 0.05^{ab}	1.84 ± 0.02^{ab}	1.11 ± 0.01^{cd}	45.27 ± 0.29^{cd}	39.91 ± 0.66^{c}	27.83 ± 0.47^{bc}
	2	4.79 ± 0.09^{a}	1.90 ± 0.05^{a}	1.10 ± 0.01^{d}	45.33 ± 0.77^{bc}	39.53 ± 0.58^{b}	26.57 ± 1.00^{ab}
	3	4.81 ± 0.03^{ab}	1.90 ± 0.02^{ab}	1.12 ± 0.01^{d}	45.40 ± 0.32^{bcd}	40.30 ± 0.88^{b}	26.53 ± 0.32^{bc}
	4	4.89 ± 0.22^{a}	1.92 ± 0.08^{a}	1.11± 0.01^{b}	44.13 ± 1.65^{b}	40.68 ± 0.57^{c}	25.53 ± 2.11^{c}
IGLGM	1	4.72 ± 0.07^{bc}	1.87 ± 0.03^{b}	1.11 ± 0.00^{d}	45.83 ± 0.43^{d}	39.53 ± 0.00^{bc}	27.43 ± 0.64^{bc}
	2	4.94 ± 0.11^{a}	1.96 ± 0.04^{a}	1.10 ± 0.01^{d}	44.33 ± 0.79^{a}	38.96 ± 0.00^{a}	25.10 ± 1.05^{a}
	3	4.88 ± 0.07^{b}	1.93 ± 0.02^{ab}	1.12 ± 0.00^{d}	45.98 ± 1.57^{d}	39.72 ± 0.33^{ab}	26.07 ± 0.86^{ab}
	4	5.01 ± 0.00^{ab}	1.97 ± 0.01^{abc}	1.11 ± 0.01^{b}	43.67 ± 1.26^{ab}	40.49 ± 0.33^{c}	24.40 ± 0.36^{abc}

Note: Values are means ± SD (n = 3). A different letter for the same parameter in the same week means significant differences between samples ($p < 0.05$). [1] Obtained from the sum of the following wavelengths: A 420 nm, A 520 nm, and A 620 nm; [2] was the absorbance at 520 nm; [3] quotients between the absorbance values at 420 and 520 nm; [4] From CIELab and CIECh coordinat.

Concerning color intensity, the results showed that the beverage which contained the extractions of ginger and tea (control) had the higher color intensity values among samples (except week 2). The comparison of the isotonic drinks with their controls demonstrated that in the case of the beverages that contained ginger-hop and ginger-mint extracts the color intensity was higher in the isotonic drinks, while in the case of the mixture ginger-tea, the control was more intense than the isotonic drink. Regarding beverages that contained only the extract of ginger, the color intensity was higher in the isotonic drink in the first and the last week. The results of color intensity were correlated with the results of red color for all samples for four weeks.

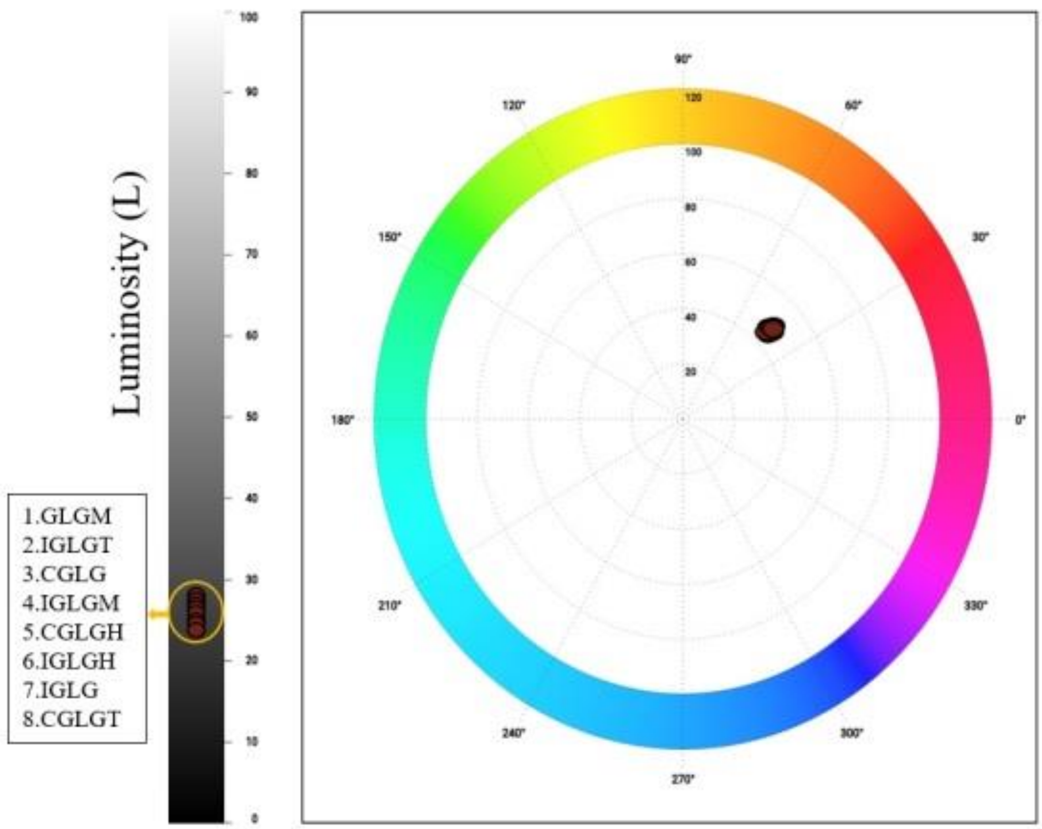

Figure 2. Beverage’s chromatic parameters and luminosity.

3.4 Antioxidant Activity

In grape juice antioxidant activity is due to the presence of phenolic compounds such as anthocyanins, phenolic acids, and resveratrol (Cosme et al., 2018). Figure 3 presents the antioxidant activity of grape juice-based isotonic drinks and their controls measured by the ABTS method. The difference between samples could be due to the presence of other phenolic compounds from herbs and spices, which are known for their content of these compounds responsible for antioxidant activity. Therefore, the antioxidant activity of the prepared beverages was enhanced by the addition of herb and spice extracts. Ginger is known for its rich content of phenolic compounds which act as potent antioxidants and most studies have been focused on their phenolic composition and antioxidant activity (Mustafa & Chin, 2023). All the prepared beverages contained ginger extracts either alone or combined with other extracts. However, the combination between ginger and hop extracts showed the highest antioxidant activity. For controls, the highest value (4.92 ± 0.06 µmols TE/mL) was in the sample that contained ginger-hop extract (CGLGH) followed by the sample that contained only ginger extract (CGLG). In isotonic drinks, the same samples which contained ginger and ginger-hop extracts (IGLGH and IGLG) had higher values (4.12 µmols TE/mL) than those samples that contained ginger-tea and ginger-mint (4.10 and 3.97 µmols TE/mL, respectively). Nevertheless, these newly isotonic beverages with antioxidant activity from grape juice and herb and spice extracts can be useful for athletes to improve their antioxidant defense and prevent oxidative damage provoked during physical exercises.

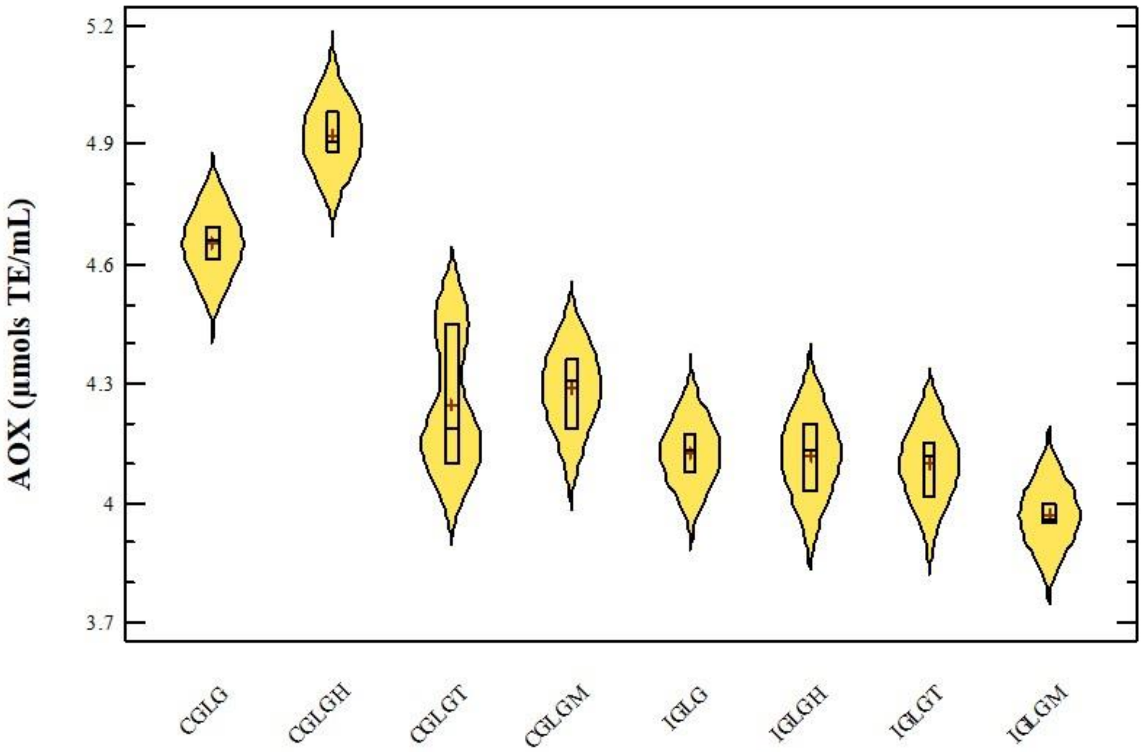

Figure 3. Violin chart for the measured antioxidant activity by ABTS in the prepared beverages.

3.5 Sensory Evaluation

According to the sensory evaluation results (Figure 4), there is no significant difference between samples in values of aromatic intensity, fruity, turbidity, reduction, oxidation, body, bitterness, and acidity attributes. The commercial isotonic drink (AQ) had the lowest values in terms of color intensity, tonality, herbaceous, floral, reduction, oxidation, body, bitterness, and acidity. However, in terms of sweetness, AQ was more sweetened than the prepared drinks as AQ had a value of 3.63 while the prepared drinks showed lower values ranging from 2.13 to 2.75. Tasters also marked the commercial isotonic drink as the fruitier among all samples. In relation to color intensity, values ranged from 3.25 to 3.75 without significant differences in the prepared beverages, and the lowest value (1) was in AQ. In this case, tasters were more attracted to the red color of grape juice. On the other hand, the sample flavored with ginger-mint extracts (IGLGM) showed the highest values of aromatic intensity and aromatic quality followed by the samples flavored with ginger-hop extracts (IGLGH and CGLGH). The samples which contained ginger-hop extracts were higher in terms of herbaceous, acidity, (IGLGH), and floral attributes (CGLGH). Low values were marked for reduction, oxidation, body, and bitterness in all beverages without significant differences. With regard to salinity, tasters classified the prepared isotonic drinks as saltier, as it was expected, followed by the commercial isotonic drink and then the beverages without salt.

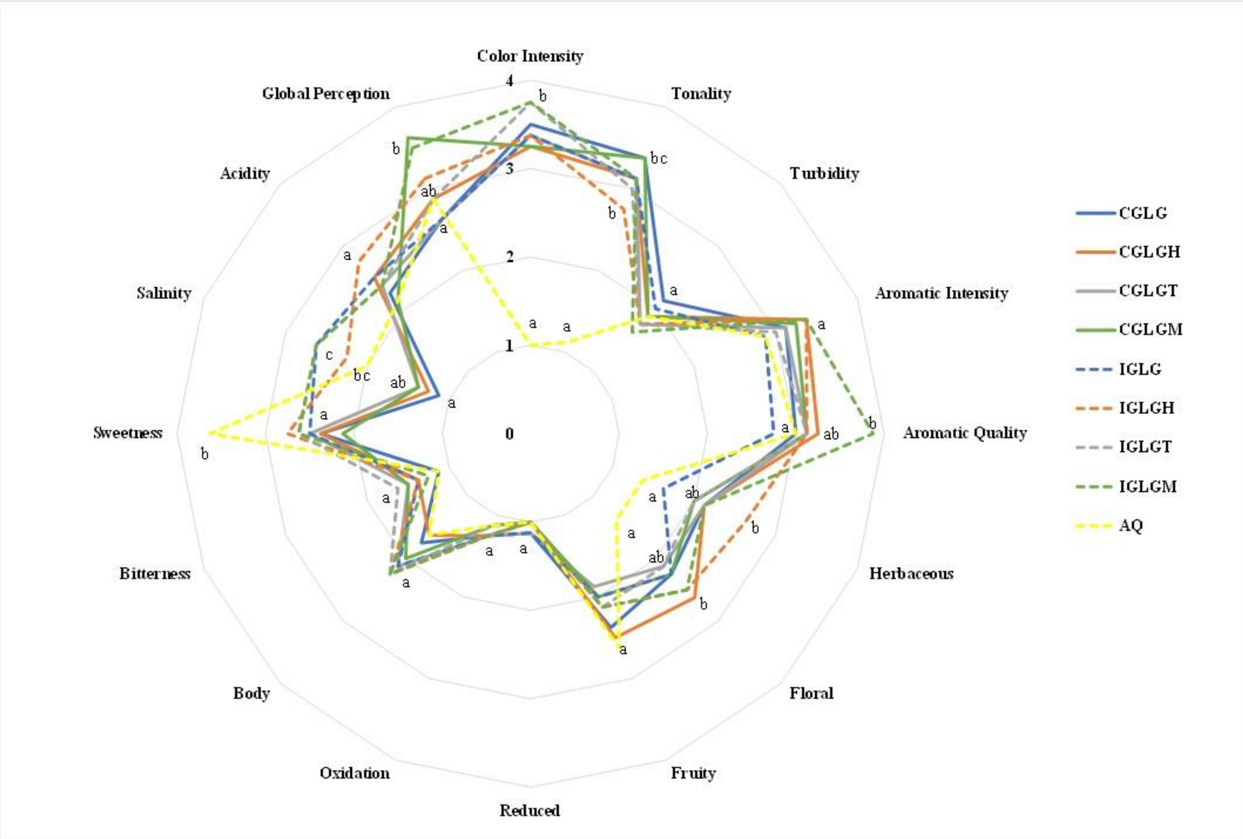

Figure 4. Two-dimensional star plot of beverages-tasting descriptors. Different letters indicate a significant difference between samples for each parameter ($p < 0.05$).

4. Discussion

In the formulation of isotonic beverages, grape juice was used as a base to avoid the addition of sugar and chemical additives. However, the samples had a sugar content from grape juice that is in the range of recommended concentration values for this type of isotonic sports drinks (6 to 8%) (ANVISA, 2010). Carbohydrates play an important role in sports drinks by providing energy to working muscles, maximizing gastric emptying, and improving absorption from the intestines

(Idangodage et al., 2023). However, a carbohydrate concentration greater than 80 g/L results in a slowing of gastric emptying and intestinal absorption during exercise (Pound & Blair, 2017). In addition to carbohydrates, minerals are also an important component of isotonic drinks, especially sodium and potassium, which are lost in large quantities through sweat during physical activity (Styburski et al., 2020). Therefore, sodium and potassium have been included in the formulation of prepared isotonic drinks with certain concentrations, which are within the range recommended by legislation (ANVISA, 2010). In addition, grape juice also contributes to the attractive red color of isotonic beverages due to the non-acylated and acylated anthocyanins present in the beverages. However, to determine the relationship between the color parameters, anthocyanins, antioxidant activity and pH, a Pearson correlation was performed. The coefficients between each pair of variables are shown in the Pearson correlation curve (Figure 5). These correlation coefficients range from −1 to +1 and measure the strength of the linear relationship between the variables. Color intensity was positively correlated with red color and anthocyanins, which was expected since anthocyanins are responsible for the red color in grape juice. This correlation was evident in the CGLGT sample, which had the highest total anthocyanin content (Figure 1 B) and the highest values for color intensity and red color (Table 2). However, color intensity and anthocyanins correlated negatively with chroma, hue, tonality, luminosity, and pH, which correlated positively with each other. These negative correlations indicate that an increase in one variable causes a decrease in the others. The negative correlation of anthocyanins, red color and color intensity with pH can be explained by the flavylium cation, which is the predominant cation under acidic conditions and shows the red color in solutions with low pH. Therefore anthocyanins are considered as colorimetric pH indicators (Morata et al., 2019). Besides the color properties of anthocyanins, they are also known for their biological effects, mainly related to antioxidant activity (Bendokas et al., 2020). Thus, there was a positive correlation between anthocyanin content and antioxidant activity of the prepared beverages. The positive correlation between anthocyanins and antioxidant activity was also observed by Zhang et al. who indicated that anthocyanins contribute significantly to the total antioxidant capacity of grape juice during storage. However, different types of phenolic compounds, as well as other factors, components and the synergistic effect between them, may influence the antioxidant capacity (Zhang et al., 2021). On the other hand, the difference between each sample and its control in this study is the addition of salts (sodium chloride and potassium chloride), so the difference in anthocyanin content, color, and antioxidant activity could be due to the positive or negative effect of the salts. According to Trivellini et al. (Trivellini et al., 2014), the effects of salt on phenolic composition and anthocyanins are controversial in the literature, with different assessments of the positive and negative effects. Zhang et al. (Zhang et al., 2021) mentioned that there is little information on the effects of sodium chloride on fruit juice quality during storage. They studied the effects of sodium chloride at different concentrations (0, 17, 42.5 and 85 mmol/L) on the quality of grape juice during storage. The result showed that the samples containing sodium chloride had higher levels of polyphenols, anthocyanins, and antioxidants than the control samples, and that sodium chloride helped to delay the decline in total anthocyanins and antioxidant activity compared to the control samples. Antioxidant activity was higher in the control beverages than in the isotonic beverages, which may also be related to the presence and effect of salts (sodium and potassium) on the molecules responsible for antioxidant activity in these beverages. Unsimilar results were obtained by Siti Rashima et al. (Rashima et al., 2017), whereas the treatment of the bitter gourd juice by sodium chloride solution showed higher anthocyanins content and antioxidant activity than the control. However, in a study of *Hibiscus* flowers, salt had a negative effect on the anthocyanin content, leading to a noticeable reduction in color intensity (Trivellini et al., 2014). Moreover, the high concentration of sodium chloride led to a degradation of total monomeric anthocyanins and color properties of extracted anthocyanins from red radish compared with control (Chen et al., 2019). Hubbermann et al. tested the effect of different concentrations (1, 3, and 5%) of sodium chloride on the stability of anthocyanins and the color of elderberry and black currant concentrate and demonstrated its negative influence which increased with increasing its concentration (Hubbermann et al., 2006). However, the stability of anthocyanins can be influenced also by other multiples factors such as relative humidity, light, pH, temperature, sugars, vitamin C, oxygen levels, sulfur dioxide or sulfites, enzymes, and co-pigments (Enaru et al., 2021). In terms of color, all the beverages had red color from grape juice presenting slight differences in values of the color parameters which could be differed depending on the type of extracts used in each beverage or could be affected by the added salts which appeared through the comparison between each sample with its control. In addition, the effect of salts was also observed in our previous works, while in the prepared beverages which did not contain salts (Bendaali et al., 2022b), the color intensity was higher in the beverages prepared with low mineral water and had low pH values than the beverages prepared with high mineral water, however, these beverages were developed into isotonic beverages (Bendaali et al., 2023) by adding different concentrations of salts to the previous formulations. It was observed that these added salts led to a change in color parameters, giving the samples with high mineral water high color intensity values, and the color intensity was affected by the presence of salts, high concentration, and low concentration. Thus, salts can affect anthocyanins, color intensity, and antioxidant activity through several mechanisms depending on their concentrations and sample composition. Finally, the sensory analysis showed that the beverages flavored with ginger-mint were preferred among samples because they had high values of global perception (3.63). Furthermore, the beverages which contained ginger extract mixed with mint, hop, or tea extracts were the most preferred than those that contained only ginger. Herbs and spices have been used as natural flavors in several studies and they were well accepted due to their contribution to improving the sensory properties of products (Dyab et al., 2015; El-Sayed & Youssef, 2019; Saad et al., 2021). Hence, the sensory profile of the newly prepared beverages was enhanced by the combination between fruit juices and herbs and spice extracts. This combination would allow these isotonic beverages to be accepted by consumers mainly because consumers are more tending to prefer products without artificial additives (Porfírio et al., 2020).

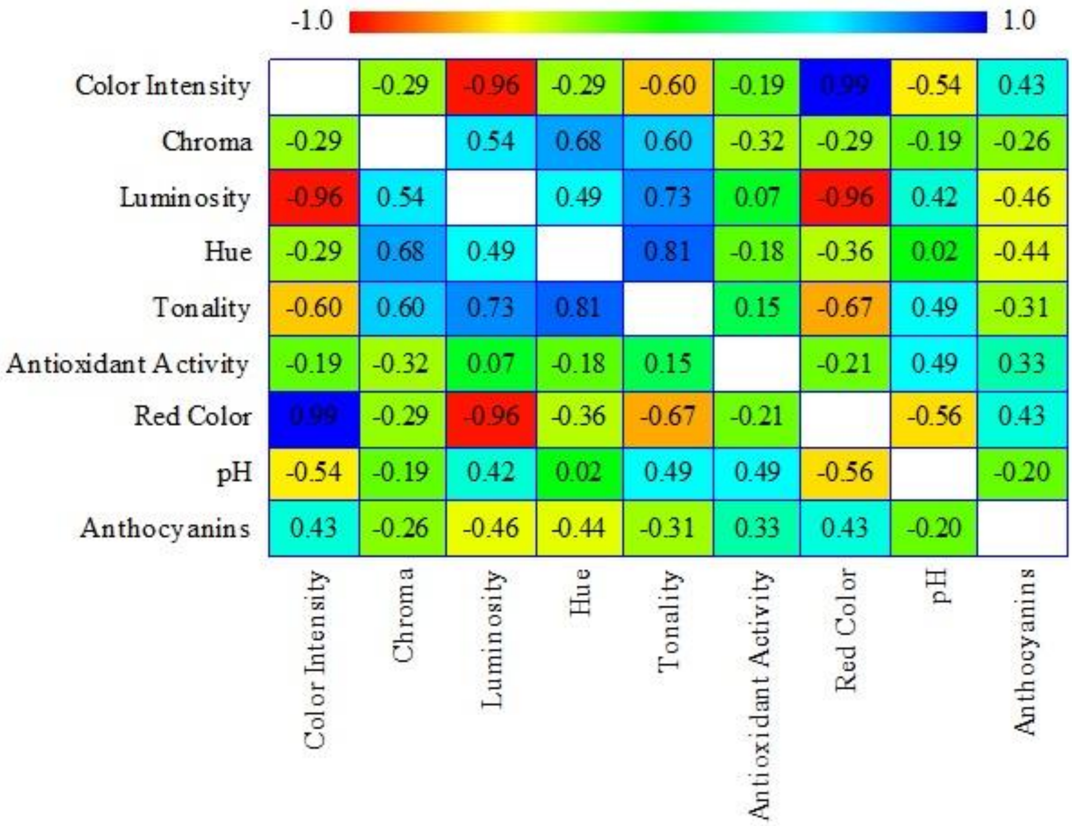

Figure 5. Pearson's correlations considering the key variables for color parameters, anthocyanins, pH, and antioxidant activity.

Conclusion

The functional properties of isotonic drinks can be improved by using grape juice which is rich in antioxidants helping athletes to avoid oxidative damage during intense sports exercises. In addition, the use of grape juice helps to avoid added sugars making the beverages a natural source of carbohydrates and energy. Moreover, ginger, hop, tea, and mint extracts can enhance the sensory properties of the beverages giving them typical flavor and aroma in addition to the sweet and acidic taste, and attractive red color of grape juice. These beverages can be novel, natural, and functional products that reflect the trends and challenges in the food and beverages industry.

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