

Use of red grape juice (concentrated and treated by UHPH) as a base to produce isotonic drinks

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Abstract. The physical-chemical composition, sensory characteristics, and nutritional value of the grape juice can provide sports drink (isotonic beverage) with antioxidant compounds that complement the beneficial effect of these drinks to the rehydration and replacement of minerals and carbohydrates, during physical activity. Grape juice contains mainly water, sugars, organic acids, and phenolic compounds. By diluting the sugar content of the must to 40-50 g/L, it will allow us to obtain a drink with beneficial properties for health, avoiding the addition of sweeteners. Phenolic compounds play an important role in the prevention of various diseases through their biological activities related to antioxidant, anti-inflammatory, anticancer, anti-aging, antimicrobial and cardioprotective properties. Several studies have shown that grape juice allows to improve the performance of the activity, protect against oxidative damage, and reduce inflammation during, sports activities. The polyphenolic substances present in grape musts provide sensory characteristics of interest, mainly color and aroma, important indicators for consumers when choosing this type of beverage. The anthocyanin content of musts from red varieties, gives the drink a more natural and attractive character for the consumer without the use of synthetic dyes. It is important to recognize the demand of consumers for new innovative, and healthy products, so we focus on the development of a natural, functional drink using red grape musts as a base (concentrated or treated with UHPH) as a source of polyphenols and sugars, showing interesting organoleptic properties without chemical additives, and that allows to replenish electrolytes and energy.

1 Introduction

Consumption of fruits and their juices plays an important role in a healthy diet (1) because they are a source of free sugars and micronutrients (2). Even more, consumer tendencies to healthy eating habits (3) and request for organic foods (4) led to the production of new drinks from fruit juices as a source of nutrients and bioactive compounds (5). Nowadays, among the challenges facing the food industry are expansion in the creation of new products and persuading consumers to buy them. In this case, it is essential to recognize the needs and consumers' demands while developing new and innovative products (6).

The physical-chemical and nutritional characteristics of red grape juices have allowed the development of healthy drinks, with a certain functional character (7) and can provide unbeatable characteristics (polyphenols and sugars) to isotonic drinks, free of additives and with optimal sensory characteristics.

Sports drinks are beverages used before, during, and after physical exercises to replace water, electrolytes, and carbohydrates lost during exercise (8,9) in order to keep hydration and delay fatigue (10). 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 (9,11). For adequate hydration, drinks must be isotonic (12). Intake of isotonic drinks for intensive physical training is recommended, while for extremely intensive training, hypertonic drinks are recommended to speed up the restoration of energy reserves by their easily digestible carbohydrate content (13). Water, carbohydrates, and electrolytes are the main components of isotonic drinks (14,15). They may also contain vitamins, colors, flavors, and natural juices that

improve the organoleptic characteristics (16), and have a pH value close to 3 (17). Taste, acidity, sweetness, and beverage temperature are features that made isotonic beverages more consumable than water during sport activities (18).

1.1 Contribution of grape juice to isotonic drinks

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 (19). Zhang et al. (20) mentioned that grape juice sports drinks do not need to use sweeteners and acidulants. Grape juice contains water, high concentration of sugars and organic acids (21,22,23), and minerals, phenolic compounds, and other nutrients such as vitamins, proteins, fatty acids, and amino acids (24,25). Carbohydrates are found in the form of fructose and glucose (26). The main organic acids in grape juice are tartaric, malic, and citric acids (27). In addition, these acids are used as indicators of microbiological alterations in the beverage because of their impact on its stability (28). Phenolic compounds are the most abundant compounds followed by sugars and acids (27). They play a major role in prevention of various diseases caused by oxidative stress (29). The phenolic compounds found in grape juice are those extracted from grape skins and seeds (21). They are classified into flavonoids such as flavanols, flavonols, and anthocyanins, and non-flavonoids mainly phenolic acids and stilbene (28, 30). The phenolic content differed among various grape juices. The content and profile of phenolic compounds are dependent on grape varieties, species, (31) technology of juice preparation (28), geographical origin, ripeness, type of soil, sunlight exposure, method used for quantification (27), farming system of grapes (organic, conventional, and biodynamic) (32), and culture conditions. Grape tissues in the pulp are rich in phenolic acids, and the skin is rich in flavonoids (33). However, anthocyanins are the main phenolic compounds in red grape juices, while flavan-3-ols are more abundant in white grape juices (21). 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, flavonoids such as flavanols mainly catechin, epicatechin, procyanidins, and flavonols mainly quercetin and other aglycones (22).

The interest of consumers and the food industry in polyphenols has been growing, because there is a relationship between their intake and prevention of various diseases (29,34). Accordingly biological activities of polyphenols in grape juice are linked to their antioxidant, anti-inflammation, anticancer, anti-aging, antimicrobial, and cardioprotective properties (35). They can prevent platelet aggregation, LDL, DNA

(36), lipid, protein (37), and membrane damage oxidation (33), reduce adhesion molecule expression and limit inflammations (38), which block cellular events predisposing atherosclerosis (39), enhance the regulation of blood pressure and vascular reactivity, reduce serum cholesterol and triglycerides (38), and improve memory function in older adults (40). They also help to prevent obesity and diabetes by inhibiting specific enzymes (41). Phenolic compounds improve 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 (38).

In Moreno-Montoro et al. (33), it was mentioned that catechin and gallic acid act as free radical scavengers, and that epicatechin has an antibacterial activity. In addition to their antioxidant capacity, gallic, caffeic, and chlorogenic acids act as venous dilators (28). Resveratrol plays a beneficial role in protection against various neoplasias, cardiovascular and neurodegenerative disorders, and viral infections as well as helps to retard body aging and reduces the incidence of heart and muscle diseases (32). Quercetin and its derivatives have shown anti-inflammation and anticarcinogenic properties when used in the treatment of some types of cancer (22).

During long and extenuating physical exercises, oxidative stress is caused owing to a considerable increase of reactive oxygen species (ROS) promoting an imbalance in antioxidant capacity in the body, which leads to protein modification, lipid oxidation (42), DNA damage (43), inflammation (44), and chronic diseases including cancer and neurological and cardiovascular diseases (45). Grape and grape derivative products are a source of polyphenols (48) which are known of high antioxidant activities (46) and can be beneficial against oxidative damage (47). In addition, the carbohydrate content is useful for glycogen deposition and improvements of practice during long term exercises (42). Therefore, many researchers have studied the beneficial effect of grape juice related to improve performances during physical exercises. Figure 1 shows some 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 inflammation.

In a first study, a drink was made from grape juice, a natural product in which the use of artificial colors and flavors was avoided. No sugars were added, only those of the fruit juice and with great nutritional value and biological activity. Lemon juice was added to the drink to lower the pH and provide a pleasant taste. Since the prepared drink was an organic product without artificial ingredients, natural flavors extracted

from spices and herbs (hops, tea and mint) were used to enhance the sensory properties and increase the aroma of the drink. Subsequently, grape juice was chosen again to design an isotonic drink according to its known bioactivity and interesting nutritional composition.

2 Materials and methods

2.1 Raw materials

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 containing 40 mg/L of vitamin C and 10 mg/L of sodium. Organic red tea, dried mint, and hop were used for the extraction of natural flavors to improve the sensory profile of the isotonic drinks. Salts for food use: sodium chloride and potassium chloride, without additives. The ingredients were diluted using two types of mineral water with different mineralization: low mineral water Bezoya 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 (48) except for the addition of a measured amount of salts to the formulations to obtain beverages that are characterized as isotonic drinks for athletes' consumption. Two groups of isotonic beverages were prepared (Fig. 1) 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. 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 physico-chemical and sensory analysis. All samples were prepared and analyzed in triplicate.

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 (48).

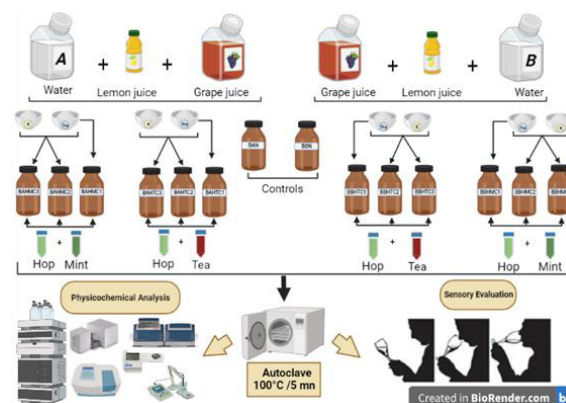


Figure 1. Schematic representation of the experimental set-up for the elaboration of isotonic drinks.

2.3 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. Total soluble solids were measured with a refractometer HI 96812 model (Hanna Instruments, Romania).

2.4 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). The test was conducted by eight panelists from both genders, aged between 22 and 60. 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. IC, 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).

2.5 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 and Discussion

3.1 Nutritional composition

Table 1 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 Porfirio et al. (37) in two formulations of isotonic drinks based on an extract of *Myrciaria jaboticaba* and close to those of some commercial isotonic drinks found by Gironés-Vilaplana et al. (28).

Table 1. Chemical composition of beverages measured by FTIR Parameters.

	Sugar (g/L)	Total Soluble Solids	Total Acid (g/L)	Malic Acid (g/L)	Alpha Amino acids	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
BAH TC1	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
BAH TC2	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
BAH TC3	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
BAH MC1	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
BAH MC2	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
BAH MC3	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
BBH TC1	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
BBH TC2	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
BBH TC3	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
BBH MC1	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
BBH MC2	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
BBH MC3	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

Regarding total acids, higher values were found in Group A, which ranged between 2.15 ± 0.20 and $2.39 \pm$

0.08 g/L, and the content in Group B was between 1.75 ± 0.02 and 1.98 ± 0.07 g/L. The main values of malic acid differed slightly among samples, being between 1.37 ± 0.15 and 1.63 ± 0.12 g/L. 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 Antioxidant activity

The results of antioxidant activity measured by ABTS assay are shown in Fig. 2. 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 the 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. (5) designed new isotonic beverages based on berries (maqui, Açai: 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. (50) was from 0.58 ± 0.01 to 0.99 ± 0.05 μmols TE/mL. Ferreira et al. (49) prepared an iso-tonic beverage based on whey permeated with carotenoid extract powder from pequi and evaluated its antioxidant capacity by ABTS and DPPH, which was 0.79 μmols TE/100 mL and 73.38 μmols TE/100 mL, respectively.

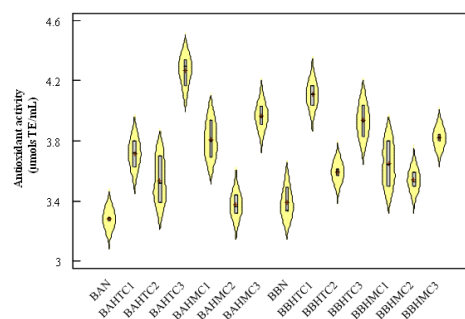


Figure 2. Violin chart for the antioxidant activity measured in the prepared beverages.

3.3 Sensory evaluation

The results of the sensory analysis showed that the panelists in general preferred the formulated isotonic

drinks to the control drinks. Whites (BAN and BNN) had lower average global perception scores. However, the samples made with stronger mineralized waters and medium salt levels (BBHMC3) had the best overall perception. Regarding IC, it was slightly higher than the rest (maximum value of 3.88 ± 0.64 out of 5) in the BAHMC2 sample. In terms of aromatic intensity, the tasters gave the samples prepared with 0.5 g/L Na (BAHTC1 and BBHMC1) the highest values while the control (BBN) obtained the least value, being again the best score for the samples elaborated with waters of greater dry extract for the aromatic quality. The herbaceous, floral, and fruity flavors were identified by the tasters, showing no significant differences between the samples. It is interesting to note that the tasters identified bitterness in all drinks as zero or almost zero, and a medium level of sweetness, with the highest score being 2.5 ± 0.93 . The sample identified with the highest level of salinity was BBHMC2, with a maximum value of 3 ± 1.07 due to the high concentration of salt added to this drink ($C2 = 0.7$ g/L). There were no significant differences in the acidity of the samples.

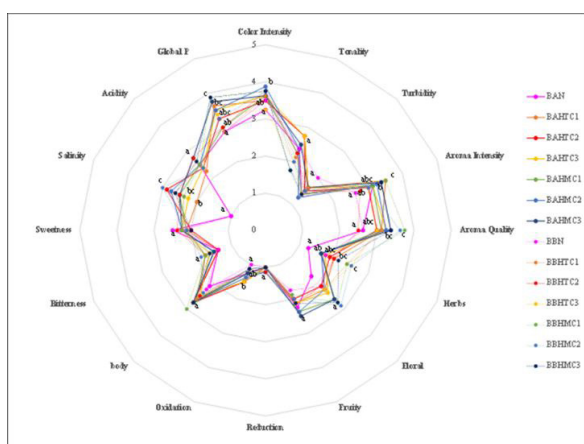


Figure 3. Sensory evaluation represented as a heatmap (A). Colors range from green (lowest score) to red (highest score). Two-dimensional star plot of beverages-tasting descriptors (B). Different letters indicate a significant difference between samples ($p < 0.05$).

4 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 the grape juice, lemon juice, herbs, and spices used in their formulation, which is important owing to their antioxidant effect against oxidative stress. The elaborated isotonic drinks had anthocyanins from grape juice in the formulation that contribute to an attractive RC and avoid the use of synthetic dyes. However, their stability was influenced by pH, water mineralization and salt concentrations, giving more IC 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 are environmentally friendly and have no negative effects on consumers as their formulation is based on organic and natural ingredients that present a novel product in the field of nutrition, sports, and healthy beverages.

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