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ORIGINAL ARTICLE

Assessment of Nutritional and Antioxidant Activity of Sport Drink Enriched with *Spirulina platensis*

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KEYWORDS

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ABSTRACT: In this study, a sports drink was prepared with the addition of different concentrations of *Spirulina platensis* (0.05%, 0.125%, and 0.25% w/w). Spirulina biomass was extracted by ethanol, ethanol/water, and water and added to a sports drink. During storage time (21 days, 4°C), the chemical properties of these drinks such as antioxidant activity, ascorbic acid, and total sugar were evaluated. The lowest and highest amount of total sugar also was observed in the control sample, and sports drink containing 0.25% aqueous extract, respectively. After 21 days of storage, total sugar decreased by 40%, 27.25% and 38% for samples containing 0.25% alcoholic, water and hydroalcoholic extracts. The ascorbic acid content of sports drinks was significantly higher ($p < 0.05$) in the treatment sample in the comparison with control sample. Content of ascorbic acid was highest in sports drinks containing 0.25% aqueous extract and decreased (up to 1.3mg/100cc) with storage. The addition of spirulina increased the antioxidant activity of the sports drink. Among the sports drinks containing alcoholic, water and hydroalcoholic extracts, minimum control of free radicals, respectively pertained to the samples containing 0.05% of Spirulina extract (18.31, 23.33 and 22.11) after 21 days of storage. Also, the antioxidant properties of the sports drink samples decreased during storage time. According to the findings of this research, the addition of spirulina extract to sports drinks improves their nutritional and antioxidant characteristics.

INTRODUCTION

Biochemical changes before, during, and after exercise are important for athletes' physiological control because blood responses in exercise physiology are permanent elements to bring exercise performance and competition to a high level. Athlete nutrition has been shown to benefical impact on athletic performance and is the most important factor in achieving optimum-level response. Furthermore, a balanced diet, nutrition strategies are also important in improving athletic performance [1]

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The goal of post-workout nutrition is to recover lost energy. Carbohydrates help maintain blood glucose levels during exercise as well as muscle glycogen replenishment and rapid recovery after exercise. Moreover, it is recommended to consume simple carbohydrates with high glycemic indexes for glycogen synthesis, which plays an important role in athletic performance [2].

The effects of fatigue appear gradually when an athlete engages in any exercise. Fatigue reduces an athlete's ability and intensity during training and even takes them out of competition. Sports drinks are produced to replace lost water, electrolytes, and nutrients by athletes [3].

Sports and energy drinks have been widely consumed over the past two decades and have been a common drink in various social classes and ages. The term "energy drink" refers to a beverage that, in addition to caffeine, combines with other energy boosters, such as taurine, herbal extracts, and vitamins [4]. Sports drinks are popular beverages with a high content of electrolytes, sugar and other isotonic nutrients (in similar proportions to blood levels). It is produced to replace water, electrolytes and nutrients lost by athletes. However, sports drinks are different from energy drinks.[5] Sports drinks should contain 40-80 g L^{-1} of carbohydrates and more than 400 mg L^{-1} of sodium. In addition, it is vital to consider the osmotic absorption capacity of drinks. Sports drinks are mainly designed to be isotonic. Drinks containing sugar/sweetener, electrolyte, and other permitted compounds have an osmolality of 250-330 mM L^{-1}. Hypertonic drinks containing sugar/sweetener, electrolyte, and other permitted compounds have an osmolality of more than 340 mM L^{-1}. Also, hypotonic sports drinks contain sugar/sweetener, electrolytes, and other permitted compounds. The osmolality is less than 250 mM L^{-1} [6].

Microalgae is a fine resource of digestible proteins, fiber, vitamins, beta-carotene, iron, omega 3, and 6 fatty acids [7]. Therefore, edible microalgae are very important because they improve the immune system and gastrointestinal function, and protect the skin and hair [8].

Spirulina platensis is a commercial photosynthetic cyanobacterium killed in many tropical, semitropical, and temperate climates for human and animal nutrition [9]. This commercial product is used in the pharmaceutical and food/chemical industries due to it's valuable nutrients as a rich source of protein and vitamins [10].

Spirulina platensis is used as an important sustainable food in biological food and pharmaceutical compounds. It has many nutrients, including protein (55-70%), carbohydrates (30%), and fats (8%) [linoleic acid, oleic acid, gamma-linolenic acid, eicosapentaenoic acid, stearidonic acid, arachidonic acid, docosahexaenoic acid], amino acids and (Lysine, methionine and cysteine) vitamins (E, D, B, ascorbic acid, especially B_{12}) minerals (iron, potassium, zinc, calcium, selenium, magnesium, chromium, copper, phosphorus, manganese, and sodium), dietary fibers (3%), phytochemicals, and phycocyanin pigment [11].

Spirulina has three pigments: 1) chlorophyll (1.7% relative to cellular organic compounds), 2) carotenoids and xanthophylls (0.5% relative to organic matter), and 3) two types of phycobiliprotein c (phycocyanin and allophycocyanin), which make about 29% of cellular proteins and major fats, such as galactosyl diglyceride and phosphatidylglycerol [12].

In the past decades, powder *Spirulina platensis* has been used to produce various food products, such as soups, pasta sauces, snacks, drinks, chocolates, candies, biscuits, cakes, and enriched flours [13, 14].

Algae are a well-known resource of phenolic compounds and there is a positive correlation between the concentration of these compounds in extracts and their antioxidant activity. The main active compound of *Spirulina platensis* called phycocyanin pigment is a 70 antioxidant ingredient that creates the characteristic blue-green color [15, 16]. Algae contain high amounts of phenolic compounds which 173 participate in the antioxidant power of their extracts[17].

Takyar et al., (2019) suggested the impacts of a *Spirulina platensis* extract on the characteristics of rainbow trout fillets stored for 16 days. They reported that high antioxidant capacity of extract of *Spirulina platensis* (77.12 and 0.46 based on DPPH and FRAP) and it's extract can control the chemical properties of fillets such as thiobarbituric acid, peroxide value and free fatty acid [18]. Other studies showed that β-carotene, fiber, ash, and protein contents improved in extrudates

containing 8% of spirulina extract by 1260, 140, 36 and 34%, respectively [19].

In previous reports, *Spirulina platensis* extract has been investigated to antioxidant activity based on different method, such as ORAC(12.3µmolTE/gDW) [20], ABTS(6.743µmolTE/gDW [20]; 97.44 200µg mL^{-1}[21]), DPPH(1 µmolTE/Gdw [20]; 68.41 200 µg mL^{-1}[21]), FRAP(13µmolTE/gDW) [20].

Another research evaluated the effect of spirulina biomass on the quality of bread pasta and showed that pasta enriched spirulina (20 g/100 g of flour) was slightly changed in quality properties [22].

Due to its high carbohydrate, mineral, and vitamin content, spirulina can be added to sports drinks. Therefore, this study aimed to add spirulina to sports drinks at different levels and its effects on total sugar, ascorbic acid and antioxidant activity of the product during 20 days of storage at 4°C.

MATERIALS AND METHODS

Materials

Glucose, fructose, maltodextrin, sodium chloride, potassium chloride, citric acid, monosodium phosphate and sodium citrate were purchased from Sigma Chemical Co (St. Louis, MO, USA). 2,6-dichlorophenolindophenol, Fehling-A and Fehling-B solution were purchased from Merck Co (Darmstadt, Germany). Spirulina powder was obtained from Shahid Beheshti University.

Spirulina extraction

Water, ethanol, and water/ethanol (50/50 v/v) were used as a solvent to extract dried spirulina powder. For this purpose, the spirulina powder was mixed with solvent in a ratio of 4 to 1 (solvent to powder) and immersed at 60°C for 2h. The solution was sonicated in an ultrasound equipment (KQ3200DE, Kunshan Ultrasonic Instrument Jiangsu, China) providing a maximum power of 100 W at 25°C for 30 min and immersed in solvent for 24 h. After that, the liquid phase was filtered through a Büchner funnel and tissue filter (Whatman no.1). The isolated liquid phase was concentrated by a rotary dryer at 40°C and then dried under vacuum in a freeze dryer (Labconco, USA) at -60°C. The powder obtained from the drying step was used for other experiments [23].

Preparation of sports drink

Different samples of sports beverages were produced by incorporating spirulina extract, fruit flavor, maltodextrin (20g), sodium citrate (0.4g) citric acid (0.6g), salt and sucrose/glucose mixtures as the sweetener. Treatment samples were formulated with 0.05, 0.125 and 0.25 (%v/v) spirulina extract. Beverage samples were filled into bottles and heated at 80°C for 10min in a water bath (Shimaz co, SHWBT 5) then cooled to 25°C. Control and treatment samples were stored in a refrigerator until evaluation. The control sample was produced similarly but without incorporation of extract.

Ascorbic acid

Ascorbic acid was evaluated by ISIRI Method No. 5609. Ascorbic acid was determined based on the extraction of ascorbic acid sample, using oxalic acid solution along with acetic acid and calibration with 2,6-dichlorophenolindophenol until a light pink color appears.

Antioxidant activity

DPPH radical scavenging activity of samples was measured using 1,1- diphenyl-2-picrylhydrazyl by the method of -Brand Williams(1995). A 0.1 mM DPPH solution in ethanol was prepared and 100 µL of this solution was added to 100 µL of the samples. At last, after incubation in darkness for 30 min, the absorbance was measured at 517 nm. Lower absorbances of the reaction mixture indicated higher free radical scavenging activities. The ability to scavenge the DPPH radical was calculated using the following equation

$$\text{DPPH radical scavenging effect (\%)} = ((A_1-A_2)/A_1)\times 100$$

where A_1 is the absorbance of the control reaction and A_2 is the absorbance of sample reaction.

Total sugar

Total, nonreducing and reducing sugars were determined according to Fehling's method. The method uses two

different solutions called Fehling A and B, respectively. The Fehling-A solution contains 34.64 g L^{-1} of copper sulfate (CuSO4.5H2O) in distilled water. The Fehling-B solution contains 173 g L^{-1} of potassium sodium tartrate ($KNaC_4H_4O_6$. $4H_2O$) and 125 g L^{-1} of NaOH in distilled water [24].

Statistical analysis

ANOVA and Duncan's Post Hoc tests were used to compare means of chemical properties of samples at the 5% significance level. Statistical analysis was conducted using GraphPad Prism 6 (GraphPad Software Inc., 2236 Avenida de la Playa, La Jolla, CA 92037, USA).

RESULTS AND DISCUSSION

Ascorbic acid

Vitamin C (ascorbic acid and (L)-Dehydroascorbic acid) is an essential nutrient with high antioxidant properties that protects humans against free radicals and different diseases. Ascorbic acid is the nutritional quality index in food processes. Citrus fruits, kiwifruit, guava, broccoli, Brussels sprouts, bell peppers and strawberries are rich sources of ascorbic acid [25].

Figure 1 shows the effect of different concentrations of spirulina extract on ascorbic acid of sports drinks during 21- days of storage. Adding algae to the drink resulted in a significant increase in ascorbic acid levels ($p<0.05$). The highest amount of ascorbic acid was 0.25% in all three extracts. On the other hand, the ascorbic acid content of sports drink decreased in different extracts during storage. In the hydroalcoholic extract, there was no significant difference between %0.125 and 0.05 samples on day 21 ($p>0.05$). The results showed that the ascorbic acid of control sample had a declining trend during storage time; there was no significant difference until the fourteenth day.

Studies recently stated that enzymes such as ascorbic acid oxidase, peroxidase, and polyphenol oxidase help reduce the ascorbic acid of vegetables and fruits in during storage[26-28].

Ascorbic acid levels are reduced due to its sensitivity to light, low water activity and presence of oxygen. Therefore, the decrease in ascorbic acid during storage time is justifiable [29].

Spirulina is a rich source of amino acids, unsaturated fatty acids, carbohydrates, pigments, minerals such as iron and calcium, and vitamins including E, C, and B_{12} [30]. Hence, studies applying micro-algal biomass for food products also have reported improvements in nutritional factors [22, 31]. The Spirulina incorporation resulted in an increment of ascorbic acid and antioxidant activity in pasta [22]. Another research reported that nutritional value of baby food increased in different specimens containing spirulina biomass[32].

Another study was performed on the physicochemical properties of sports drinks containing 3% lemon juice and 1.8% isomaltulose during six months of storage at 20°C. The results showed that ascorbic acid levels decreased from 158 to 108 mg L^{-1} during storage time[33].

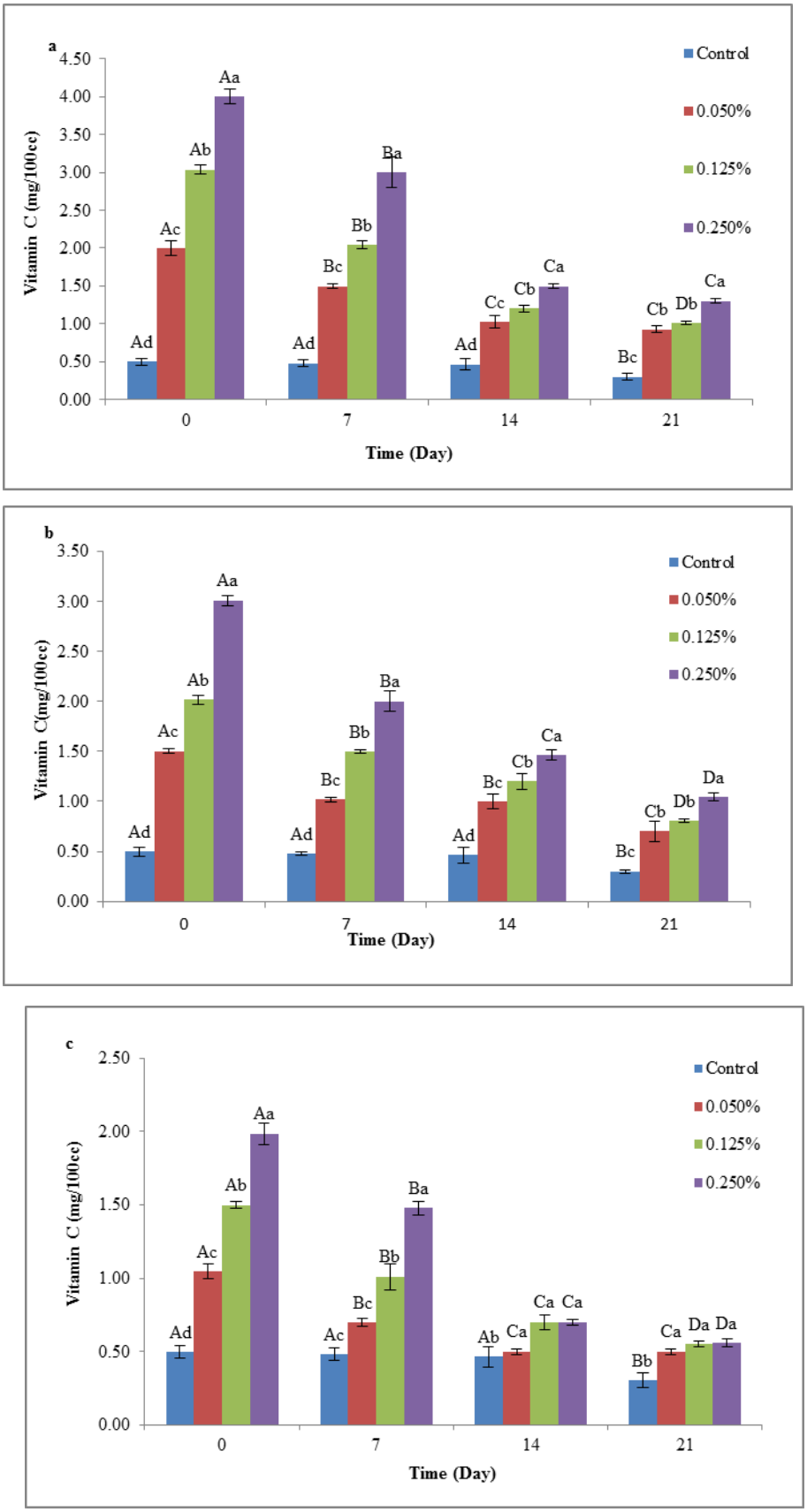

Figure 1. Ascorbic acid content of sports drinks a) water b) hydroalcoholic and c) alcoholic extracts. Different small letters and capital letter show significant difference between means of different concentrations and one sample during storage respectively $P < 0.05$.

Antioxidant activity

Antioxidants are compounds that prevent the reaction of free radicals, leading to reduced damage or cell death, cancer and cardiovascular disease [34].

Figure 2 shows the effect of algae extracts on the antioxidant activity of the sports drink. The use of spirulina extracts increased the antioxidant activity of sports drinks. Also, antioxidant properties are declining during storage time. In the aqueous extract, there was no significant difference between 0.125 and 0.25% extract on days 7 and 21 ($p>0.05$). The control sample had the lowest antioxidant activity. However, antioxidant activity of sports drink containing 0.05%, 0.125%, and 0.25% aqueous extract were less than 45% on day 7($p<0.05$).

Spirulina is a rich resource of several natural compounds, including carotene, xanthophyll, phytopigments, and phycocyanin, associated with its antioxidant properties [35]. Hence, studies applying spirulina biomass for different beverages also have reported improvements in antioxidant properties [36, 37].

The effects of spirulina biomass on antioxidant capacity for the sports drink in this research are consistent with another study, as the addition of 2-6% spirulina into bread increased the antiradical properties of the cookies from 11.8 to 13.37, 15.1 and 16.51 for the control sample and cookies containing 2% ,4% and 6% spirulina powder, respectively [38].

The antioxidant activity of algae was attributed to the interaction between free radicals and phenolic compounds in the extract. On the other hand, phenolic compounds in algae are variable, and their amounts in the extract depend on the type and condition of extraction. Moreover, other research has demonstrated that phenolic compounds can bind to cellular sugars and increasing the sugar content of product resulted in antioxidant properties improve. Also, sodium nitrate or phenylalanine in *Spirulina maxima* causes the levels of phenolic compounds to reach 4.51-16.96 mg g^{-1} cell dry matter[39].

Hexane extracts carotenoids and chlorophyll II. Also, methanol extracts sugars, organic acids, and phenols with lower molecular weight, and ethyl acetate extracts phenol compounds with low molecular weight. Therefore, it has been shown that the amounts of phenolic compounds extracted from algae when using hexane and ethyl acetate were lower compared to methanol. This can be attributed to the presence of lighter molecules in hexane and ethyl acetate. Nevertheless, phenolic compounds with higher antioxidant activity are present in methanol, such as caffeic acid, chlorogenic acid, salicylic acid, synaptic acid, and trans-cinnamic acid [40].

The antioxidant activity of orange juice increased with incorporated aqueous extracts of spirulina and stevioside, as reported by Abu-Taweel et al., 2019. They stated that the antioxidant activity of different samples is 71.09%, 94.54% and 92.95% for spirulina biomass, treatment and control samples, respectively[41].

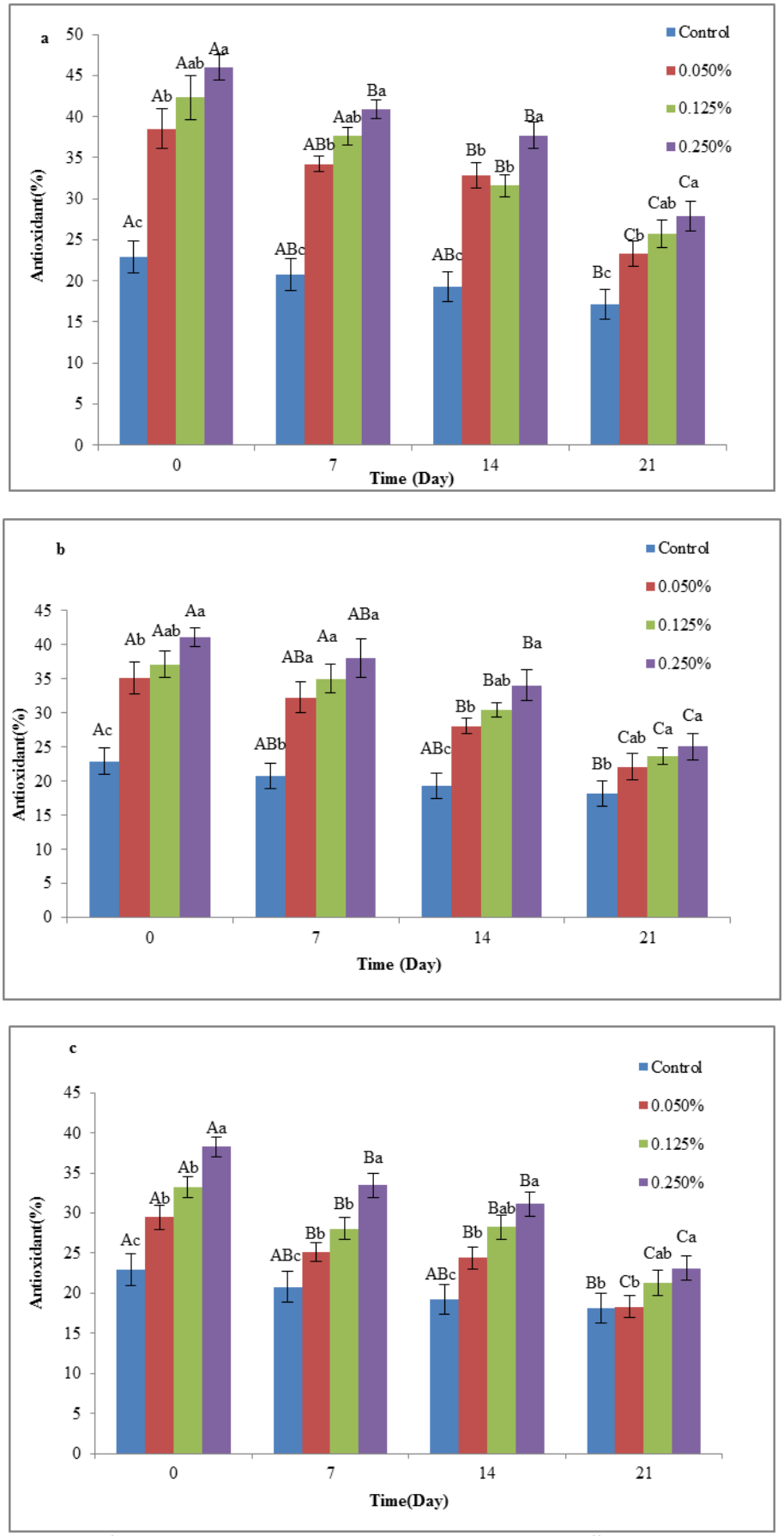

Figure 2. Antioxidant properties of sports drinks a) water b) hydroalcoholic and c) alcoholic extracts. Different small letters and capital letter show significant difference between means of different concentrations and one sample during storage respectively $P < 0.05$.

Total sugar

Figure 3 shows the effect of different concentrations of water, hydroalcoholic and alcoholic extracts spirulina during 21 of days storage on sports drinks. The use of spirulina had a significant effect on the total sugar of sports drinks ($p<0.05$).

In the aqueous extract, there was significant difference between 0.05, 0.125 and 0.25% extract in during storage ($p<0.05$). In the hydroalcoholic extract, sample containing 0.25% spirulina had the highest total sugar.

Recent reports have shown that the growth of bacteria in drinks containing fruit or vegetables reduces the product's sugar content [42].

The chemical properties of spirulina showed that the carbohydrate levels in *Spirulina platensis* and *Spirulina maxima* were 16 and 12.6, respectively. Glucose, rhamnose, ribose, and galactose were prominent among spirulina sugars [43]. Therefore, the effects of spirulina extract on total sugar for the sports drink in this research are consistent with other study, as the addition of 10% spirulina extract into nectar increased the total sugar of the beverage from 17.51 to 21.14% [44].

Another study was performed on the chemical properties of cookies containing 1 and 2% spirulina biomass. The results showed that addition of spirulina to cookies resulted in carbohydrate content increase [45].

Chaiklahan et al. (2013) studied optimizing the extraction of polysaccharides from spirulina. These authors demonstrated the presence of six natural sugars in spirulina after hydrolysis of polysaccharides. Their main constituents include rhamnose (about 53%), glucose (14%), and ribose (10%), while galactose, xylose, and mannose are measured for less than 5% of sugars[39].

The mentioned studies reported that spirulina extracts contain a variety of sugars, such as glucose, rhamnose, mannose, xylose, and galactose, which increase the total sugar content of the end product.

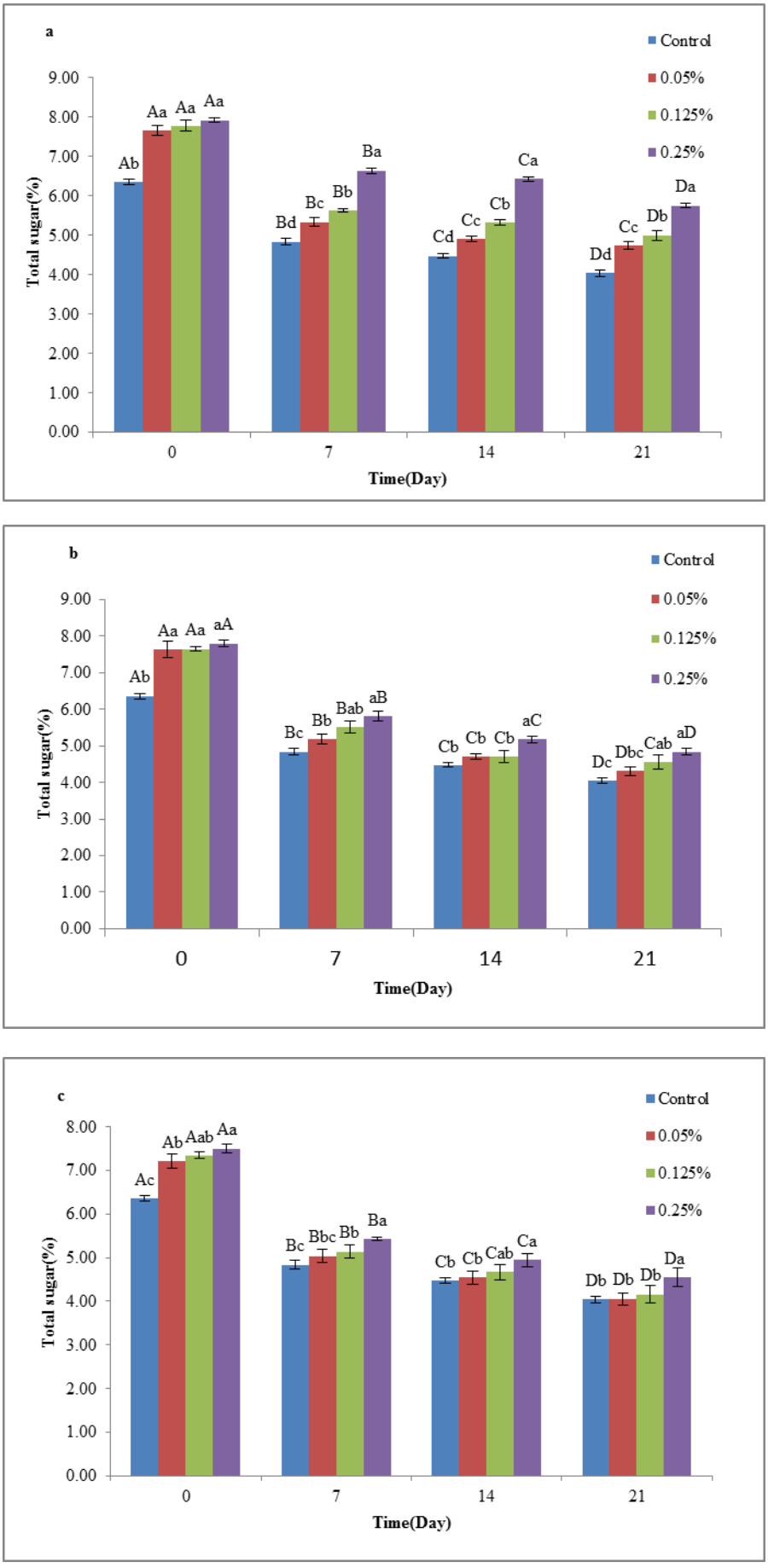

Figure 3. Total sugar content of sports drinks a) water b) hydroalcoholic and c) alcoholic extracts. Different small letters and capital letter show significant difference between means of different concentrations and one sample during storage respectively $P < 0.05$.

CONCLUSIONS

Based on the obtained results, it is concluded that the addition of spirulina extract to sports drinks helped improve the product's nutritional value because of its high ascorbic acid concentration. The results showed that the addition of different levels of spirulina to sports drink significantly affects its total sugar content than in the control sports drink sample**.** Also, using spirulina at different concentrations to manufacture sports drinks considerably improved the final products' antioxidant activity**.** These findings could be applied to develop a novel sports drink enriched with spirulina as a good source of bioactive compounds.

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Conflict of interests

The authors declare that there is no conflict of interest.

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