Determination of the phenolic content, antioxidant activity and total anthocyanins of a beverage based on aloe vera and Jamaican sorrel (*Hibiscus sabdariffa*)

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Abstract

Among the particularities of a food are beneficial properties such as polyphenols, antioxidant activity and anthocyanins. In fact, recent research has shown that the biological activity of these compounds reduces cellular ageing, thus helping to prevent and enhance the therapeutic effects. In order to develop an aloe vera and Jamaican sorrel (*Hibiscus sabdariffa*) beverage, nine treatments were compared with three concentrations of aloe vera (2, 5, 7%) and three concentrations of Jamaican sorrel (0.5, 0.7, 0.9%). Therefore, we determined the total phenolic content by applying the Folin Ciocalteu method, the antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl free radical capture (DPPH) and total anthocyanins by differential pH. The best beverage formulation: 5% aloe vera and 0.9% Jamaica, had a total phenol content of 714.86 mgAGE / 100ml, antioxidant activity 1858.56 mg Trolox/100ml, total anthocyanins 5587.99 mg C-3GE / 100ml, °Brix 7.5, pH 3.8 and acidity 0.38%; it was evident that the formulations made of this beverage contain high potential amounts of natural active compounds, which may be used and beneficial for human health due to their antioxidant and nutritional properties.

Introduction

Healthy beverages are considered functional drinks [1], which require an industrial production process, mainly a controlled pasteurisation process in order to keep favourable nutrients such as phenols, antioxidants, anthocyanins and among others. This important process ensures quality and safe products [2]; however, inadequate pasteurisation may lead to significant bioactive properties alterations due to denaturation [3]

Jamaican sorrel extracts composed of flavonoids and antioxidants have been proven to protect human body cells from free radicals [4]; the Mexican beverage industry has widely used Jamaican sorrel for its diuretic properties, stomach disorders, fevers, liver failure, hypertension and hypercholesterolemia [5]. Moreover, Aloe gel reduces cell ageing due to its high content of antioxidants and anti-inflammatory properties as well as there is evidence that the fibre content in aloe beverages helps to enhance the therapeutic effects, it is usually consumed either directly or mixed with fruit juices and natural essences [6].

Although the aloin content of aloe vera products in European countries is 0.1 ppm in food and beverages, the International Aloe vera Science Council (IASC) recommends an aloin concentration of less than 10 ppm in a 0.5% Aloe vera solids solution for consumption [7]. On the other hand, studies show that the daily consumption of Jamaican sorrel tea is 2 g in 250 ml of hot water, steeping for 20-30 minutes as the best extraction of essence and colour. Recently, freeze-dried Jamaican sorrel powders represent a solution in the food industry as they contribute a pleasant colour and flavour to beverages [8].

Therefore, in order to determine the phenolic content, antioxidant activity and total anthocyanins, a formulation of an aloe vera and Jamaican sorrel beverage was developed.

Materials And Methods

A completely randomised design with a factorial arrangement (3A*3B) was used, with factor A: aloe vera concentration (a1=2, a2=5, a3=7%) and factor B: jamaican sorrel concentration (b1=0.5, b2=0.7, b3=0.9%) with four replicates, thus giving a total of 36 experimental units.

Raw material procurement and initial treatments

Samples were taken from aloe vera (*Aloe vera*) leaves bought from the central market in Chachapoyas-Amazonas, Peru and from jamaican sorrel (*Hibiscus sabdariffa*) calyxes bought in Moyobamba-San Martin, Peru. The samples were taken to the engineering laboratory of the National University Toribio Rodríguez de Mendoza de Amazonas. The jamaican calyxes were freshly acquired and dried at 40°C in an oven at the laboratory up to a humidity of 12%, then they were ground in a mortar in order to reduce the particle size and conserved in an airtight bag at room temperature; the aloe leaves were cut at the base and edges, and were left to rest for 12 hours in cold water to reduce the content of aloin.

Obtaining the beverages

Subsequent to the initial aloe leaf treatments, aloe vera crystal cubes were obtained to be mixed with Jamaican sorrel essence, at 50°C for 15 minutes; sugar and 0.01% food grade potassium sorbate were added, then the beverage was pasteurised at 60°C for 15 minutes; it was stored at 6°C in refrigeration.

Determination of the content of total phenolic compounds

It was determined by the Folin-Ciocalteu spectrophotometric method as described by Singleton et al. [9] for which a 100 mg/L stock solution of gallic acid (Sigma Aldrich, Germany) was prepared with serial dilutions of 10 mL at increasing concentrations from 0 to 16 ppm using water to obtain the standard curve. It was then added 0.050 µL of the beverage sample, plus 0.45 µL ultrapure water, 2.5 mL of Folin-Ciocalteu reagent (Merck, Germany) diluted 1:10; in the same manner, it was added 2 mL of 7.5% sodium carbonate solution and incubated for 5 minutes at 50°C. Absorbances were measured in spectrophotometer (UV-Visible S2100 UVTE, ÚNICO) at 760 nm; results were expressed as milligram gallic acid equivalents (mg GAE) / 100 mL of the beverage [10].

Determination of antioxidant activity

For the determination of the antioxidant capacity, we used the method of sequestration of the free radical 2,2-Diphenyl-1-picrylhydrazyl (DPPH), as described by Brand-Williams [11] the calibration curve was formed with a trolox standard solution (Merck, Germany) at a concentration of 2000 µM; with concentrations from 100 to 2000 µM. Once the solutions were obtained, they were placed in a dark environment for 30 minutes. To analyze the samples, 30 µL of the beverage sample was added and mixed with 3.0 mL of the DPPH free radical solution and agitated in the vortex for 10 seconds; once the procedure was completed, ethyl alcohol was used as a blank to calibrate the spectrophotometer and a reading was taken at 517nm. The results were plotted in trolox concentrations (M) on the X-axis and

inhibition on the Y-axis. Afterwards, we used the formula [12] to calculate the antioxidant activity value in percentage in the equation of the line:

$$AA(\%) = 100 - \left(\frac{Ae - Ab}{Ac} \right) * 100$$

Where: Ae = absorbance of the extract with DPPH, Ab = white absorbance (tube B, in which the DPPH solution was replaced by the extraction solvent), Ac = control absorbance (tube C as DPPH solution)

Determination of total anthocyanins

It was performed following the differential pH method, where potassium chloride (KCl) 0.025M/HCl buffer solutions at pH 1 and sodium acetate (CH₃CO₂Na) 0.4M/HCl) buffer solutions at pH 4.5 were prepared. Had obtained the buffers, a 0.3 mL aliquot of the beverage sample was added and mixed with pH_{1.0} (potassium chloride buffer, 9.7 mL) and then pH_{4.5} (sodium acetate buffer, 9.7 mL) solutions, respectively, as well as it was equilibrated during 30 min at room temperature, in the dark [13]. The absorbances of each sample were measured at 510 nm and 700 nm. The final absorbance was calculated with the following equation:

$$A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5}$$

To obtain the concentration, the calculated absorbance value was replaced in the equation:

$$Anthocyanins \left(\frac{mg}{L} \right) = (A * MW * DF * 1000) / (\epsilon * 1)$$

Where MW and ϵ , stand for the molecular weight and molar absorptivity of the predominant anthocyanin (cyanidin-3-glucoside, MW: 449.2 g / mol and ϵ : 26.900) and DF is the di-lution factor (total volume / extraction volume). The result was expressed as the milligram equivalent of cyanidin-3-glucoside in 100 g cc (mg C-3GE / 100 g cc) [14].

Statistical Analysis

An analysis of variance or F test was performed to determine the statistical difference of the effect of the treatments, identifying its components which were the main effects, the effect of the elaboration of the aloe vera and hibiscus drink was also analyzed using the Duncan test at the p<0.05 level of significance to identify the differences between treatments and choose the best, the results were expressed with their standard deviation. For the evaluation of physicochemical data, a parametric test analysis was used under a Completely Randomized Design scheme and a correlation test between treatments for phenolic compound content, antioxidant activity and anthocyanins.

Results And Discussion

Chemical properties content of the beverage.

Table 1, shows that the content of phenols and anthocyanins in all treatments are directly proportional to the antioxidant activity. It is also observed that the treatments with higher concentrations obtained higher values of bioactive properties.

Table 1. Information matrix of the content of total phenols, antioxidant capacity and anthocyanins content in the formulated beverage.

% Aloe Vera	% Jamaican sorrel	Total phenols mgAGE/100ml		Antioxidant Activity mgTrolox/100ml		Anthocyanins mgC-3GE/100ml	
2	0.5	455.019	±0.617	774.396	±0.298	2690.669	±0.660
2	0.7	560.664	±0.615	1015.555	±0.791	2906.241	±0.350
2	0.9	565.743	±0.557	1502.465	±0.880	4455.069	±0.926
5	0.5	595.205	±0.363	995.764	±0.591	2532.319	±0.750
5	0.7	490.742	±0.734	1314.355	±0.669	2912.365	±0.600
5	0.9	714.857	±0.316	1858.556	±0.896	5587.992	±0.837
7	0.5	644.041	±0.340	1280.122	±0.335	3541.384	±0.755
7	0.7	840.303	±0.903	1850.705	±0.712	5027.707	±0.818
7	0.9	687.451	±0.180	1892.575	±0.626	5190.391	±0.861

The antioxidant activity of phenolic compounds, anthocyanins and others is of particular interest from a technological and nutritional approach, since they positively affect the health on the consume; thus obtaining and producing foods with a high antioxidant content is becoming increasingly demanded by today's society [15].

The results showed statistical differences between the beverages in all the analyses performed for phenolic compounds, antioxidant activity and total anthocyanins ($p < 0.05$). The performance of these is due to the concentrations of each treatment. The treatment with 5% aloe vera, 0.9% Jamaican sorrel, presented better characteristics in total phenols 714.86 mgAGE /100g, antioxidant activity 1858.56 mg Trolox/100mL and total anthocyanins (5587.99 mg C-3GE / 100 ml). If we compared the total phenol content results with the research carried out by Sayago-ayerdi and Goñi [16], they obtained 217.05 mgAGE /100ml in jamican sorrel. Here, we found a significant difference as our results obtained are higher. This may be due to the extraction method applied and the different treatments to which the

samples were subjected; moreover, we should take into account the large variety of phenolic compounds that are more soluble in mixtures of solvents than in pure aqueous solvents.

Total phenol content of the formulated beverages

Fig. 1 shows the phenol content in each treatment. Of course, there is a significant difference between them: the treatments with 2%, 5% aloe vera and 0.5%, 0.7% of Jamaican sorrel have a lower content in comparison to the treatments with 5% aloe vera and 0.9% of Jamaican sorrel, and 7% aloe vera and 0.7% of Jamaican sorrel, showing a higher amount of phenols. In fact, Jamaican sorrel has a direct influence on the difference in the concentration of total phenols in the beverage formula.

On the other hand, Pacheco [17] when comparing the content of total phenolic compounds in three brands of *H. calyx* beverages, found that the concentration of total phenols in the aloe vera and Jamaican sorrel beverage is due to the fact that aloe vera contains phenols, as well as the processing factors that directly influence its quality. [18] indicate that the concentrations of compounds such as anthocyanins in Jamaican sorrel beverages are due to genetic factors, maturity index, variety; a beverage made from Jamaican sorrel presented (3000.80 mg C-3GE /100ml) [16] while in this study results of anthocyanin content were obtained (5587.99 mg C-3GE / 100 ml); the differences are attributed to the processing conditions, extraction temperature and time.

Antioxidant capacity of formulated beverages

Fig. 2 shows that the treatments with: 5% aloe vera and 0.9% Jamaican sorrel; 7% aloe vera and 0.7% Jamaican sorrel; and 7% aloe vera and 0.9% Jamaican sorrel, had a higher antioxidant capacity than the others treatments. This is due to a direct influence of the aloe vera and Jamaican sorrel content on the chemical characteristics of the beverages with different concentrations.

Total anthocyanin content of the formulated beverages

Fig. 3 shows that the treatments with 2% aloe vera and 0.9% jamaicas sorrel, 5% aloe vera and 0.9% Jamaican sorrel and 7% aloe vera and 0.9% Jamaican sorrel had higher anthocyanin contents compared to the others treatments. This is explained by the fact that aloe vera has no anthocyanin content; therefore, the treatments with the highest concentrations of Jamaican sorrel had the highest anthocyanin content in the beverages.

Dornier [19] indicates that the higher the extraction or pasteurisation temperature applied to a food, the higher by 30% the presence of phenolics, anthocyanins, etc. The calyxes of Jamaican sorrel and aloe vera provide an important source of bioactive compounds [20]; the results of antioxidant capacity found in this study, the treatment with 5% aloe vera, 0.9% Jamaican sorrel achieved 1858.556 mg Trolox/100ml; evidence to further promote the consumption of natural beverages with a high content of antioxidant compounds that favour the health of human beings. The antioxidant activity of a food of plant origin depends on its concentration and structure[15]; not to mention the state of maturity or the variety of the plant, which are factors that directly influence antioxidant activity.

Conclusions

The beverage formulated with aloe vera and Jamaican sorrel had a high content of bioactive compounds. Based on the chemical analysis, it was found that treatment six (5% aloe, 0.9% Jamaican sorrel) showed the best total phenolic content (714.86 mgA-GE /100g), antioxidant activity (1858.56 mg Trolox/100ml) and anthocyanins (5587.99 mg C-3GE / 100 ml); and it also had °Brix 7.5, pH 3.8, acidity 0.38%, being the best beverage treatment for people demanding a healthier diet.

Abbreviations

DPPH 2,2-Diphenyl-1-picrylhydrazyl

mgAGE milligram gallic acid equivalents

mgC-3GE milligram equivalent of cyanidin-3-glucoside

Declarations

data availability statement: The version contains supplementary material available at (MS excel) files.

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Conflicts of Interest

The authors declare that they not have conflicts of interest.

Author Contributions

Conceptualization: Diner Mori-Mestanza, Robert Javier Cruzalegui Fernandez and Segundo Grimaldo Chavez Quintana; methodology: Diner Mori-Mestanza, Segundo Grimaldo Chavez Quintana, Darwin Perez-Ruiz, Veronica Zuta-Chamoli; supervision: Guillermo Idrogo-Vasquez; statistics and analysis: Lucas Dalvil Muñoz-Astecker, Segundo Grimaldo Chavez Quintana, Armstrong Barnard Fernández Jeri; chemical research: Diner Mori-Mestanza, Segundo Grimaldo Chavez Quintana Darwin Perez-Ruiz, Veronica Zuta-Chamoli; data conservation, Diner Mori-Mestanza, Segundo Grimaldo Chavez Quintana Darwin Perez-Ruiz, Veronica Zuta-Chamoli; writing - original draft: Diner Mori-Mestanza, Segundo Grimaldo Chavez Quintana Darwin Perez-Ruiz, Veronica Zuta-Chamoli and all authors have read and accepted the manuscript version.

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Supplementary

Supplementary Material MS excel files are not available with this version

Figures

Figure 1

Total phenol content of the formulated beverages

Figure 2

Antioxidant capacity of the formulated beverages

Figure 3

Total anthocyanin content of the formulated beverages