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Article

Physicochemical and sensory characteristics of a Chagalapoli fruit (*Ardisia compressa*) beverage fermented using *Saccharomyces cerevisiae.*

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Abstract: Chagalapoli fruit *(Ardisia compressa)* is similar to *Vaccinium myrtillus* (berries) with high-polyphenol content. The objective of this study was to evaluate the physicochemical properties of Chagalapoli fruit and to determine the conditions for the preparation of a fermented beverage using *Saccharomyces cerevisiae* yeast, evaluating the impact on sensory properties. The fermentation process lasted 4 days at 27 °C, with absence of light and a fixed pH of 3.8. The phenolic contents obtained in samples according to chromatograms were 1.27 mg(EPI)/mL in filtered juice, 1.59 mg(EPI)/mL in filtered fermented beverage, 1.91 mg(EPI)/mL in partially filtered juice and 3.19 mg(EPI)/mL in partially filtered fermented beverage. An affective test was carried out to determine the sensory acceptability of the final product, evaluating the flavor, color and aroma parameters. The fermented beverage with the greatest preference on color and flavor attributes was the partially filtered fermented beverage.

Keywords: Chagalapoli (*Ardisia compressa*); fermented beverage; phenolic compounds

1. Introduction

Chagalapoli (*Ardisia compressa subsp. Myrsinaceae*) belongs to the *Myrsinaceae* family, an extensive family of trees with approximately 500 species [1]. The chagalapoli fruit has a round shape of approximately 12 mm in diameter, with a purple red color that turns black once it reaches maturity; its shell is smooth and delicate, its seed represents up to 50% of its total weight, and the ripe fruit has a bittersweet, mildly astringent taste [2]. In Mexico it is distributed in the states of Chihuahua, Chiapas, San Luis Potosí, Tlaxcala, Hidalgo, Aguascalientes, Puebla, Tamaulipas and Veracruz [3]. Chagalapoli fruit has a similar composition to common berries with a high total polyphenol content; that is the reason for the high interest on this fruit from a health point of view [4]. The fruit is used to make food products such as juices, jams, and liqueurs, considered to possess antioxidant and antimicrobial properties. In the state of Veracruz the fruit is used against digestive diseases [5]. Chandra & Mejia [6] quantified the total polyphenols of the *Ardisia compressa* leaf extract, obtaining values of 0.58 mgEAG/mL DL. They identified the presence of gallic acid, catechin, epicatechin gallate, ardisin and kaempferol. Heredia-Vasquez [3] determined the total polyphenol content of chagalapoli fruit and obtained 1.74 mgEAG/mL in *Ardisia compressa kunth*; Joaquín-Cruz, et al., [4] obtained 1.051±0.43 mgEAG/g and identified the polyphenols of flavonols, flavan-3-ols (catechin and proantocyanidin dimers) and hydroxycinnamoyl derivatives. Jácome-Hernandez [2] obtained 1,638.12±74.98 mgEAG/kg in dry weight (DW). Fermented beverages, such as grape wines, have been associated with health benefits due to the presence of large amounts of phenolic compounds, which are claimed to possess antioxidant properties and have an important role in the prevention of deleterious processes such as ageing, diabetes, cancer, neurological disorders, atherosclerosis and cardiovascular diseases. Moreover, several studies have shown that these beverages induce relaxation in isolated vessels, which makes them important allies for cardiovascular protection [7,8]. This means that it is necessary to carry out research studies focused on the physicochemical characterization of the fruit and the process of making a fermented beverage that will promote the potential of the chagalapoli fruit. For this reason, the aim of this work was to evaluate the physicochemical properties of the chagalapoli (*Ardisia compressa)* fruit and determine the conditions for the preparation of a fermented beverage using *Saccharomyces cerevisiae*, evaluating the impact on the physicochemical and sensory properties of the final product.

2. Material and methods

2.1. Materials

Acetonitrile and formic acid were high-performance liquid chromatography grade. Glucose, Epicatechin, Kaempferol, Quercetin, Catechin, Chlorogenic and Folin-Ciocalteu reagents were provided by Sigma-Aldrich. Milli-Q water was produced using an Elix Millipore water purification system.

2.2. Raw material

Chagalapoli *(Ardisia compressa)* fruit was collected in Aguascalientes, Mexico. 20 Kg were manually selected and washed with chlorine (0.05 mL/L). Samples were stored at 4 °C for further analysis.

2.3. Characterization of raw material

Proximal analysis of chagalapoli (*Ardisia compressa*) was determined according to the AOAC methods; protein (976.05) – determined by Micro Kjeldahl (Nx6.25) –, moisture (934.06), ash (942.05), fat (920.39), crude fiber (962.09) and carbohydrates.

2.4. Must preparation

Fruit was crushed in a destoner (Bertuzzi, Brugherio, Milan, Italy) with a 0.2 sieve separating the seed. The experiment was divided into two batches: Filtered juice (all crushed fruit was filtered with linen), and partially filtered juice (75% filtered crushed fruit and 25% unfiltered). A 615 ml sample was obtained, which pH was adjusted to 3.8 with tartaric acid, and 61.5 mg of potassium metabisulfite was added (0.1 mL/L).

2.5. Yeast strains

Commercial *Saccharomyces cerevisiae* yeast was used (Red Star brand, provided by Maltas e Insumos Cerveceros S.A. de C.V.). 0.173 g of dried yeast was added to 173 mL of warm water with 1.73 g of sugar at 35-38°C for 20 min, it was left to cool for 10 min.

2.6. Fermentation conditions

All fermentations were carried out in a 1L bioreactor (BioBundle, Applikon Biotechnology, Netherlands) at 27 °C. Fermentation process was evaluated every 24 h and considered complete when the relative density was stable. At the end of the fermentation, the beverage was transferred to a glass bottle and stored at 10 °C. After 24 h, the beverage was transferred to a new bottle and after 10 days the beverage was filtered and stored at 5 °C in 350 mL glass bottles filled to the top to avoid oxygen entrance [9].

2.7. Analysis of juice and fermented beverage

Titratable acidity (TAC), relative density, pH, cell counts (Neubauer chamber, Celeromics France), total soluble solids (TSS), probable alcoholic degree (ABV) were determined according to the usual enology analysis techniques of Panreac Química S.A. After fermentation and storage, sulfur dioxide and alcoholic degree method were determined. Color was measured with a colorimeter model CR400 (Konica Minolta sensing NJ USA).

2.8. Determination of reducing sugar

The reducing sugar was obtained using the method of Miller [10]. Briefly, 0.5 mL of sample was added with 0.5 mL of DNS reagent, kept in a boiling water bath for 5 minutes, then the reaction was stopped by placing the test tubes in a cold water bath, 5 mL of distilled water was added and it was left to rest for 15 minutes. Absorbance was determined at 540 nm in an Absorbance microplate reader model ELx808 (Biotek VT USA). All the experiments were performed in triplicate. The reducing sugar content was calculated based on a standard glucose curve.

2.9. Determination of total sugar

The total sugar content was determined using the method of Dubois [11] and Chow & Landhausser [12]. 2 mL of sample was diluted in 2 mL of 5% phenol solution; it was boiled in a water bath for 5 min and then cooled with ice. Then 5 mL of H_2SO_4 was added and it was stirred, then it was left to rest during 30 min. Absorbance was determined at 490 nm in an Absorbance microplate reader. All experiments were performed in triplicate.

2.10. Total phenolic content

The total phenolic content was determined using the method of Folin-Ciocalteu [13]. 30 μL of sample was diluted in 3 mL of water and 200 μL of Folin-Ciocalteu reagent and 600 μL of a sodium carbonate solution was added. Afterwards this sample was warmed up at 40 °C for 20 min, then it was cooled to room temperature and after 15 min the absorbance was measured at 760 nm. Total phenolic content was calculated as gallic acid equivalent based on a standard gallic acid curve. All the experiments were performed in triplicate.

2.11. HPLC-DAD analysis

Samples were filtered through a 0.45 μm membrane filter (Millipore Corporation). Samples were analyzed on an Agilent 1100 HPLC (Agilent, Technologies CA USA) equipped with a diode detector, with wavelengths at 280 nm, 320 nm and 360 nm. A C18 column (Phenomenex) was used for the stationary phase. The mobile phase consisted in two solvents: (A) water/formic acid (99:1; v/v), and (B) acetonitrile (100%). The gradient employed was: isocratic 0% B for 8 min, 10% B for 2 min, 20% B for 13 min, 30% B for 7 min, 40% B for 15 min, 80% B for 5 min, 100% B for 5 min. Flow rate was set at 1 mL/min and a temperature of 25 °C. Phenolic identities were assigned based on their retention characteristics and UV-visible spectra. For the identification and quantification of total phenolics compounds external standard calibration curves of the reported compounds were used (Epicatechin, Kaempferol, Quercetin, Catechin, Chlorogenic).

2.12. Sensory analysis

Data for all of the measurements were obtained in triplicate and expressed as mean ± standard deviation. Statistical analyses during fermentation were performed with a one-way analysis of variance and means comparison (Tukey). Fermented beverages (filtered and partially filtered beverages) were compared by means of a Student's test to determine the significant difference between samples. A significance level $p<0.05$ was adopted. Different letters were used to label significantly different values. This statical treatment was carried out using Minitab 17 Statistical Software.

3. Results and discussion

3.1. Physicochemical characteristics of Ardissia compressa

Results obtained in the proximal composition are shown in Table 1. The water content of the fruit was 80.5%, which is similar to values reported by Joaquin-Cruz, et al., [4] who reported 86.8% in *Ardisia compressa* from Veracruz. The Chagalapoli fruit used in this research had a pH level of 4.2±0.1. This result is higher than values reported by Jacome-Hernandez [2] (2.91) and Joaquin-Cruz, et al.,[4] (2.73). Likewise, total soluble solids were different from the reported by Jacome-Hernandez [2] (10.5), while our results were 17.7±0.05. This could be attributed to the fact that each fruit comes from different locations, weather conditions, harvest season, storage, etc. that affect the characteristics of each fruit. Low pH can contribute to a decrease of general sensory quality of the beverage to an unacceptable level [14].

Table 1. Proximal composition and physicochemical characteristics of chagalapoli (*Ardisia compressa*) fruit.

Components[a]	Value
Water (%)	80.52 ± 0.8
Ether extract (%)	0.55 ± 0.05
Ash (%)	0.66 ± 0.01
Protein (%)	8.58 ± 0.4
Crude Fiber (%)	3.58 ± 0.005
Carbohydrates (%)	11.9
Water activity	0.97 ± 0.001
TSS[b] (%)	17.7 ± 0.05
pH	4.2 ± 0.1

Results are expressed as mean ± SD (n=3). [a]Proximal components are expressed in FW [b]Total soluble solids (°Brix).

3.2.Analysis during fermentation

Table 2 shows the results obtained of pH, relative density, alcohol by volume (ABV%), total soluble solids (TSS) and total titratable acidity (TAC). It was observed that pH values of the beverages did not have a significant difference during the fermentation process, in fermented filtered chagalapoli the change was from 3.84±0.04 to 3.62±0.09 and in fermented partially filtered it was from 3.87±0.2 to 3.54±0.1. The TAC did not exhibit significant difference in neither of the batches (8.9 g/L to 8.6 g/L and 8.9 g/L to 9.2 g/L tartaric acid); in fermented fruit the limit values are 5.5 g/L to 9 g/L (Norma Oficial Mexicana PROY-NOM-199-SCFI-2015) which indicates that TAC obtained in samples is within the permitted limits. Relative density showed significant difference between samples, which shows a tendency to decrease with time; batches had the same value at the end of fermentation. ABV showed significant difference until the third day. ABV is inversely proportional to values of TSS, as ABV increases, TSS values decrease. During alcoholic fermentation of both batches of *Ardisia compressa*, initial value of yeast was $1.9 \times 10^6 \pm 0.2$. Number of microorganisms increases due to environmental conditions and sugar content decreases rapidly. Maximum value of yeasts was obtained on the second day with an amount of $4.1 \times 10^7 \pm 0.1$; this is relevant because it suggests that at this point the yeast is in optimal conditions that favor the production of alcohol. A decrease was observed after the third day and fermentation concluded without presenting a significant difference on the fourth day with a value of $4.4 \times 10^6 \pm 0.2$.

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Table 2. Physicochemical properties of chagalapoli (*Ardisia compressa*) fruit filtered and partially filtered beverage during fermentation.

	Filtered fermented beverage					Partially filtered fermented beverage				
Days	0	1	2	3	4	0	1	2	3	4
pH	3.84±0.04^{a}	3.75±0.06^{ab}	3.65±0.07^{ab}	3.61±0.09^{b}	3.62±0.09^{b}	3.87±0.2^{a}	3.74±0.07^{a}	3.5±0.1^{a}	3.51±0.1^{a}	3.54±0.1^{a}
SP. Gr[a]	1.072±0.005^{a}	1.048±0.007^{b}	1.028±0.001^{c}	1.009±0.001^{d}	1.004±0^{d}	1.074±0^{a}	1.044±0.002^{b}	1.030±0.003^{c}	1.006±0.001^{d}	1.004±0.001^{d}
ABV[b] (%)	0	3.69±0.1^{c}	5.77±0.2^{b}	8.26±0.1^{a}	8.95±0.08^{a}	0	3.89±0.2^{c}	5.64±0.4^{b}	8.82±0.1^{a}	9.08±0.1^{a}
TSS[c] (%)	17.5±0.1^{a}	11.16±0.2^{b}	7.09±0.3^{c}	2.34±0.2^{d}	1.04±0^{d}	17.9±0^{a}	11.03±0.4^{b}	7.74±0.8^{c}	1.7±0.2^{d}	1.2±0.2^{d}
TAc[d] (g/L tartaric acid)	8.9±0.1^{a}	8.6±0.2^{a}	8.6±0.08^{a}	8.6±0.1^{a}	8.6±0.08^{a}	8.9±0.1^{a}	9.1±0.2^{a}	9.3±0.05^{a}	9.2±0.1^{a}	9.2±0.2^{a}

Results are expressed as mean ± SD (n=3). Values with same letter are not statically different ($p<0.05$). [a] Specific Gravity. [b] Alcohol by Volume. [c] Total soluble solids . [d] Titratable acidity.

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3.3.Color parameters

Table 3 shows the values obtained from the parameter determined by CIELab. In L* parameter the values were of 27.5±0.005 to 31.09±0.09 which indicates that samples tend more towards to darkness presenting significant difference between all samples. For the a* parameter there were significant differences between samples and values with a trend towards red color. The b* parameter indicates color yellow to blue, thus samples had a tendency towards to yellow, showing no significant difference between fermented samples. Angle of hue and chromaticity defined the sample within the red color, presenting significant differences in all samples. Heredia-Vasquez [3] reported different values in L*, a* and b* parameters, obtained low luminosity and more tendency towards red color. Differences can be justified by the type of fruit used as well as by climatic condition, region, harvest time, etc.

Table 3. Chagalapoli (*Ardisia compressa*) juice and fermented beverage color analysis.

	L*	**a***	**b***	**Croma**	**Angle of hue (h°)**
Filtered juice	27.50 ± 0.005[d]	8.87 ± 0.02[a]	5.63 ± 0.02[c]	14.50± 0.01[d]	0.56 ± 0.002[d]
Filtered fermented beverage	30.34 ± 0.01[c]	8.42 ± 0.02[b]	9.07 ± 0.02[b]	17.50 ± 0.02[a]	0.79 ± 0.002[c]
Partially filtered juice	30.75 ± 0.04[b]	7.15 ± 0.03[c]	9.44 ± 0.02[a]	16.59 ± 0.03[b]	0.87 ± 0.002[b]
Partially filtered fermented beverage	31.09 ± 0.09[a]	6.92 ± 0.06[d]	9.36 ± 0.09[a]	16.28 ± 0.03[c]	0.87 ± 0.006[a]

Results are expressed as mean ± SD (n=5). Values with same letter are not statically different ($p<0.05$).

3.4.Alcoholic grade

Fermented filtered and partially filtered beverage of chagalapoli (*Ardisia compressa*) fruit obtained a similar result in alcoholic degree method, 6% and 6.4% respectively. Official Mexican Standard NOM-199-SCFI-2017 specifies the limit values for fermented fruit beverages from 6% to 12%; the values obtained from chagalapoli (*Ardisia compressa*) fermented beverage are within the established range. Chowdhury & Ray [15] reported a 6% of alcohol content in a fermented jamun berry (*Syzygium cumini L.*) beverage.

3.5.Reducing sugar

There was no significant difference in filtered juice and partially filtered juice with values of 132.08±1 and 96.63±2 mg/mL, respectively. For filtered and partially filtered fermented beverage, there is no significant difference observed with values of 3.42±0.3 mg/mL and 3.58±0.3 mg/mL, respectively. Results are shown in Table 4. Similar results were reported by Oliveira et al., [9] where values of 1.2 g/L and 2.4 g/L of reducing sugars were obtained in Cagaita fruit wine.

Table 4. Reducing sugar, total sugar and total polyphenol contents of Chagalapoli (*Ardisia compressa*) fruit juice and filtered and partially filtered fermented beverage.

	Reducing sugar mg (glucose)/mL		Total sugar mg (glucose)/mL		Total polyphenols			
					Folin-Ciocalteu mg (EAG)/mL		HPLC-DAD mg (EPI)/mL	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Chagalapoli filtered	132.08±1[a]	3.42±0.3[b]	137.82±0.9[a]	3.74±0.1[b]	1.8±0.03[a]	1.11±0.02[b]	1.07±0.04	1.47±0.09
Chagalapoli partially filtered	96.63±2[a]	3.58±0.3[b]	83.04±0.6[a]	3.27±0.03[b]	1.75±0.1[a]	1.59±0.19[b]	1.43±0.1	2.86±0.01

Results are expressed as mean SD (n=3). Values with same letter are not statically different (p<0.05).

3.6.Total sugar contents

Total sugar contents results are shown in Table 4. Filtered juice presented more total sugars than partially filtered juice with 137.83±0.9 mg/mL and 83.04±0.6 mg/mL, respectively. These results may be compared to the report by Kosseva, et al., [16] where blueberry presented values of 155.2 g/kg to 164.7 g/kg, while in red grapes it was around 200 g/kg or more at maturity. Comparing these results with reported values of blueberry, chagalapoli (*Ardisia compressa*) sugars are lower. There was a reduction during the fermentation, and at the end of samples 3.74±0.1 mg/mL and 3.27±0.03 mg/mL of total sugars were obtained in final products of fermented beverage and partially filtered fermented beverage. Kelebek et al., [17] reported 120.19 g/L in orange juice and 48 g/L in fermented orange juice. These results could differ since the orange is considered a citrus fruit and because the sugars present in each fruit are different.

3.7.Analysis of phenolic compounds

3.7.1. Folin-Ciocalteu method

The total polyphenol content by the Folin-Ciocalteu method in chagalapoli (*Ardisia compressa*) juices was 1.8 mgEAG/mL for filtered juice and 1.75 mgEAG/mL for partially filtered juice. Similar values were previously reported by Heredia-Vásquez [3], who obtained values of 1.74 mgEAG/mL in *Ardisia compressa kunth*; Joaquín-Cruz, et al., [4] obtained 1.051±43.5 mgEAG/g in chagalapoli fruit. In filtered and partially filtered fermented beverage the values obtained were 1.11±0.02 mgEAG/g and 1.59±0.19 mgEAG/g, respectively. Martins de Sá, et al., [7] reported 1,105±57 μgEAG/mL in a fermented jabuticaba (*Myrciaria jaboticaba*) beverage. Johnson, et al., [17] reported a total polyphenol content of 375.4 μgEAG/mL to 657.1 μgEAG/mL in blackberry (*Vaccinium sps)* fermented beverage.

3.7.2. HPLC-DAD analysis

An increase in the total polyphenols was observed in both samples of chagalapoli (*Ardisia compressa*) fermented beverages. At the beginning of the fermentation, the value was 1.07±0.04 mgEPI/mL, with increases towards the end of fermentation with a final value of 1.47±0.09 mgEPI/mL. The partially filtered chagalapoli sample initially presented 1.43±0.1 mgEPI/mL and at the end of fermentation it increased to 2.86 ± 0.01 mgEPI/mL. Total polyphenols are shown in Table 4. Recent studies by Zhang, et al.,[19] demonstrated the same behavior in fermentations in different grape varieties. In the fermentation process, several enzymes are excreted during the metabolism of the yeast, and these enzymes could act on the conjugated phenolic compounds to release free phenolic compounds, and thus change their composition. Figure 1 show the compounds identified in *Ardisia compressa* samples at 280 nm wavelength. Peaks 1 and 2 correspond to catechin and epicatechin; catechin was found at 7.12 min up to 7.45 min, epicatechin was found at 8.2 min up to 8.54 min in the first chromatograms, while in the chromatogram of the partially filtered fermented beverage it was observed at 11.24 min. Figure 2 shows the juices and fermented beverage chromatograms at 320 nm wavelength. In partially filtered fermented beverage, a major peak is shown, which was identified as chlorogenic at 12.48 min, 14.49 min and 13.73 min for filtered beverages and at 14.52 min in partially filtered juice. Figure 3 shows compounds identified in *Ardisia compressa* beverages at 360 nm wavelength. Different derivatives of quercetin were identified between 24.65 min and 24.81 min for filtered beverages (juice and fermented beverages) and for partially filtered beverages they were identified at 23.23 min up to 23.92 min. Kaempferol was identified at 26.6 min in the filtered samples and in partially filtered samples it was found in 25.72 min and 24.48 min. Joaquín-Cruz, et al. [4] identified 6 derivatives of quercetin with retention times of 18.61 min, 19.49 min, 19.92 min, 20.21 min, 22.98 min and 23.72 min. For the identification of kaempferol, retention times of 16.94 min and 17.24 min were obtained.

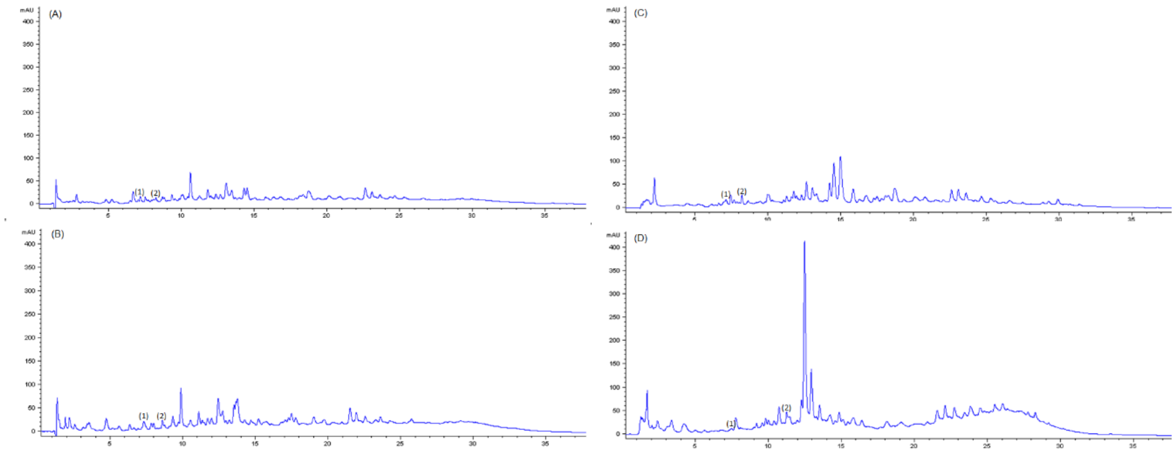

Figure 1 Chagalapoli (*Ardisia compressa)* HPLC Chromatograms at 280 nm: (A) filtered juice, (B) partially filtered juice, (C) filtered fermented beverage, (D) partially filtered fermented beverage.

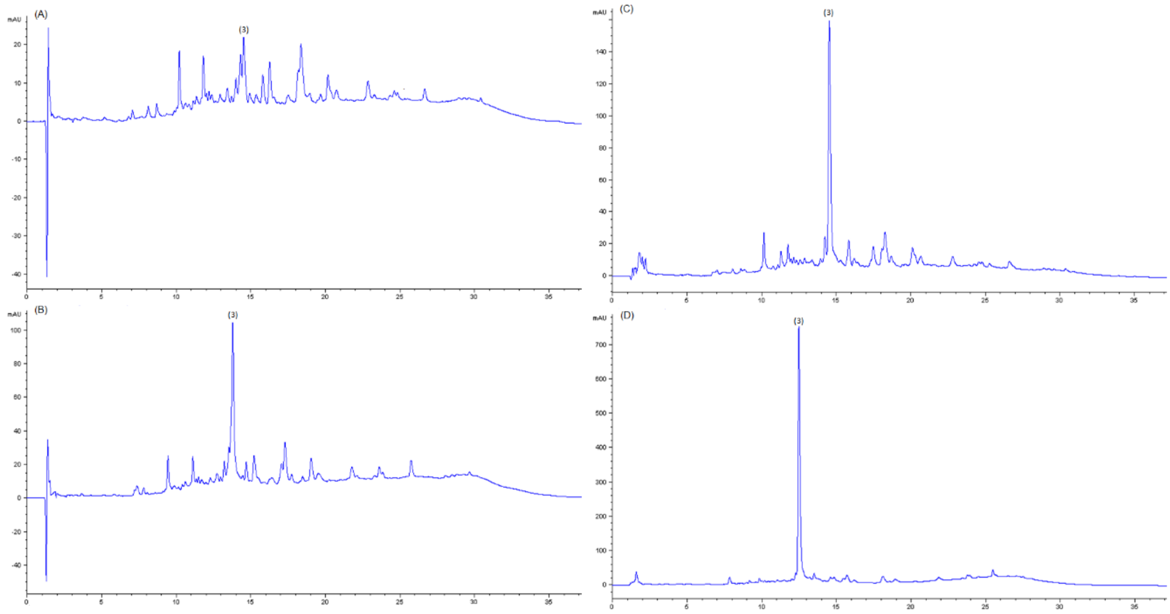

Figure 2 Chagalapoli (*Ardisia compressa*) HPLC chromatograms at 320 nm: (A) filtered juice, (B) partially filtered juice, (C) filtered fermented beverage, (D) partially filtered fermented beverage.

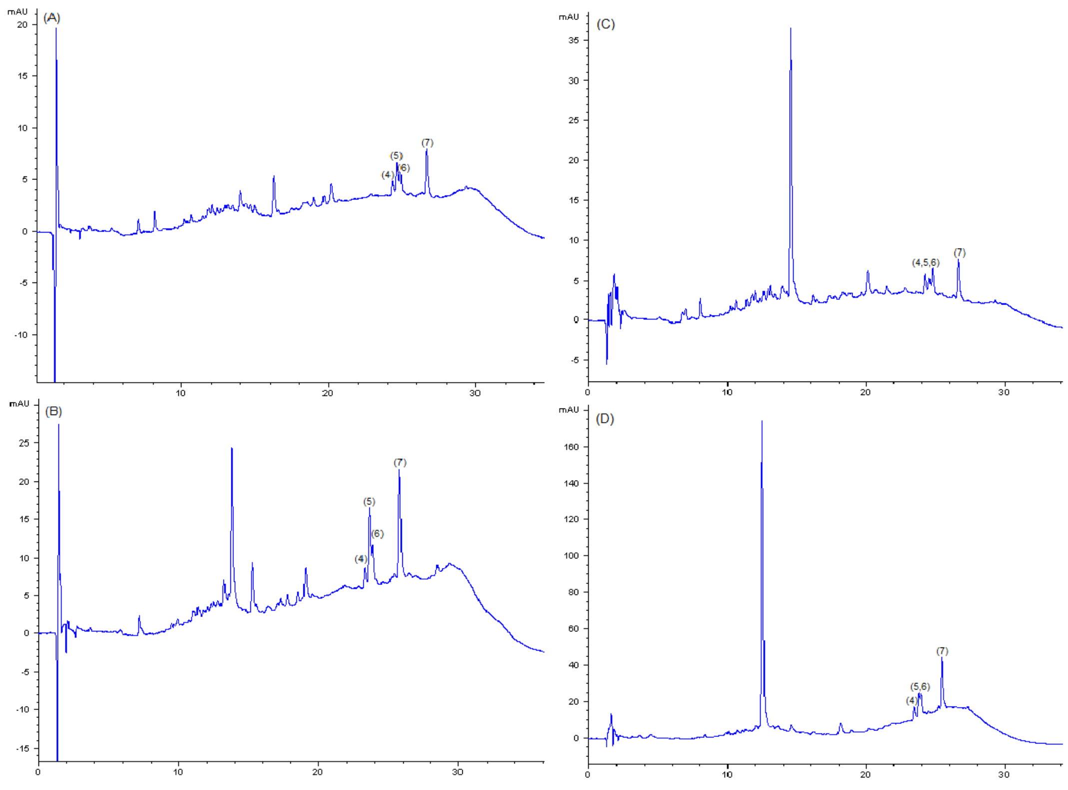

Figure 3 Chagalapoli (*Ardisia compressa*) HPLC chromatograms at 360 nm (A) filtered juice, (B) partially filtered juice, (C) filtered fermented beverage, (D) partially filtered fermented beverage.

3.8. Sensory evaluation

Fermented beverages from both batches were subjected to a sensory analysis to evaluate the degree of satisfaction of the final product, carried out with a panel of 32 untrained tasters older than 18 years. Table 5 shows the results of acceptance of each sample, it was observed that the partially filtered beverage obtained a higher acceptance value in color and flavor parameters with a difference of 0.87 and 1.41, respectively. Aroma did not present a significant difference between samples; this means that there was not a preference for any of the two fermented beverages.

Table 5. Sensory analysis of chagalapoli (*Ardisia compressa*) fruit filtered and partially filtered fermented beverages.

	Filtered fermented beverage	Partially filtered fermented beverage
Color	3.47 ± 0.8^{b}	4.34 ± 0.6^{a}
Aroma	3.69 ± 0.9^{a}	3.72 ± 1.2^{a}
Flavour	2.9 ± 0.1^{b}	4.31 ± 0.8^{a}

Results are expressed as mean SD (n=32). Values with same letter are not statically different (p<0.05).

4. Conclusions

The results of the present study revealed that chagalapoli fermented beverages represent a rich source of phenolic compounds and due to their phenolic composition they may be compared to other fruit fermented beverages. It was also demonstrated that chagalapoli fruit has the potential to be used to produce fermented beverages. This beverage can be considered as a dry spirit since the content of reducing sugars was lower than 4 mg/mL. Based on the characteristics of the produced chagalapoli fermented beverages, it was concluded there is a huge room for development and there may be extensive research since chagalapoli fruit presents a high market potential.

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Conflicts of Interest: The authors declare no conflict of interest.

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