

Physicochemical and microbiological characterization of blackberry (*Rubus glaucus Benth*) wine, El Hobo (Huila)

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Abstract

The department of Huila is a producer of Castilla blackberry (*Rubus glaucus Benth*), a perishable fruit in post-harvest, making it necessary to develop processing alternatives in order to extend its shelf life and improve its market price. It was therefore proposed to elaborate a fermented blackberry drink using clarification, maturation, and aging processes. The physicochemical and microbiological parameters were characterized in the different phases. The inoculated yeast generated changes during the fermentation of the blackberry juice reducing the soluble solids to 6.9°Brix and viscosity to 1.5 cP. The alcohol content at the end of the process was of 14.4 alcohol by volume (ABV).

Keywords: viscosity; fermentation; *Saccharomyces cerevisiae* var. *Bayanus*; enology;

Caracterización fisicoquímica y microbiológica del vino de mora (*Rubus glaucus Benth*), El Hobo (Huila)

Resumen

El departamento del Huila es productor de mora de Castilla (*Rubus glaucus Benth*), un fruto muy perecedero en poscosecha por lo que se hace necesario desarrollar alternativas de procesamiento para prolongar su vida útil y alcanzar mejores precios en el mercado. Así, se propuso elaborar una bebida fermentada a partir de la mora, implementando los procesos de clarificación, maduración y añejamiento. En las diferentes etapas se caracterizaron los parámetros fisicoquímicos y microbiológicos. La levadura inoculada generó cambios durante la fermentación del zumo de mora disminuyendo los sólidos solubles a 6.9°Brix y la viscosidad a 1.5 cP, al final la bebida tuvo un contenido de 14.4 alcohol por volumen (ABV).

Palabras clave: viscosidad; fermentación; *Saccharomyces cerevisiae* var. *Bayanus*; enología.

1. Introduction

The blackberry, originated in the tropical zones of the American continent, is highly sought after because of its smell, taste, color, and high nutritional value. It can be used in numerous food products such as juices, ice cream, and marmalades and it has nutraceutical applications [1].

One of the agroindustrial transformation processes for blackberry is to produce wine by subjecting the fruit to a normal alcoholic fermentation process in accordance with [2] the Colombia Technical Standard NTC 708.

Moderate red wine consumption has beneficial effects on health given its content of antioxidants, originating mainly from its phenolic compounds [3].

Blackberry wine contains some of the compounds that are part of this group of bioactive substances, such as bioactive flavonoids, to which anthocyanins belong. The latter are antioxidant compounds that can reduce pro-oxidant substances, even in low concentrations and they promote the formation of low toxicity products [4]. Their associated benefits include the protection of the circulatory system and the prevention of neurodegenerative diseases and cancer, among others [5,6].

The color of the wine is also due to its chemical composition, mainly due to the fruit's phenol content, responsible for developing the color during the winemaking process [7,8].

The flavonoid compounds include phenol acids, tannins, anthocyanins, anthocyanins, etc., found in the seeds, in the vacuoles of the plant cells and in the fruit pulp. These contain

aromatic rings responsible for the aroma and smell of the wine, which develop during the winemaking process [9].

Anthocyanins and tannins give the wine its characteristic color. Anthocyanins are natural pigments present all over the plant and in the ripe fruit, which can be red, violet and blue [10]. Tannins have a Flavan-3-ols structure, which belongs to the flavonoid family, responsible for the bitter, astringent taste, body and the wine's aging capacity [11].

The correct selection of strains of yeast is fundamental to guaranteeing good fermentation and, therefore, a quality wine [12]. As such, a proposal was made to make blackberry wine, monitoring the physicochemical and microbiological parameters throughout the process involving alcoholic fermentation, clarification, maturation and aging.

2. Methods and material

The study was carried out in the *Cesurcafe* Laboratory and the *Microbiología de Alimentos* Laboratory at the School of Engineering at Universidad Surcolombiana – Neiva.

2.1. Harvest

The ripe fruit was harvested in the department of Huila, El Hobo municipality, El Batán, Finca Nazareth, at an altitude of 1850 m.a.s.l.

2.2. Juice extraction equipment

A particular type of equipment was manufactured to extract only the juice of the blackberry drupes. The equipment was made in stainless steel (5mm caliber), it was 0.41m high, and had a 0.22m diameter, and was supported by a tripod. An acrylic strainer was installed inside the extractor with 4.5 mm holes (Fig.1). The fruit was placed in the top section, and then a piston was installed and together with a 3-ton jack, pressure was exerted on the fruit allowing out the juice contained in the drupes, which poured out of conical lower section of the equipment.

2.3. Blackberry juice

Castilla (*Rubus glaucus Benth*) blackberries were selected and introduced into the juice extractor equipment. The juice of 72 kg of drupes (40 L) poured out of the lower section free of seeds. The equipment can process 0.5Kg in 15 min.

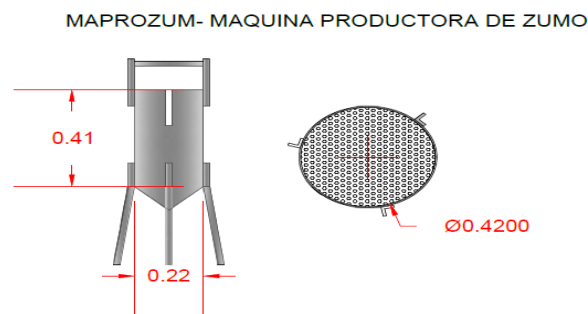


Figure 1. Machinery sketch by Manuel Oviedo-Jennifer Lozano-2016. Source: Oviedo M., Lozano J., 2016.

2.4. Fermentation

The blackberry juice was distributed between two 20 L tanks, and a hole was placed in each lid to allow CO₂ to escape and stop oxygen from entering. *S. Cerevisiae* var. *Bayanus* (Oenoferm) was inoculated and activated in a portion of the juice at 30°C for five days. It was then poured into the respective tank. Fermentation was at 30°C for five days, and a submersible motor pump (Squash) was inserted to aid homogenization.

2.5. Clarification

This was carried out using 4 g of unflavored gelatin (*Frutiño*) per tank letting the suspension rest for 3 days. Following this, the liquid and solid phases of the fermented juice were separated.

2.6. Maturation in casks

The fermented and clarified juice was placed in 15 L Colombian white oak barrels (Barriles de Colombia) for two months (day 76).

2.7. Bottle ageing

Once the bottles had been sterilized by autoclave (SciFinetech) at 180°C for 60 minutes, the wine was then bottled in 750 mL brown bottles and sealed with a cork. The bottles were stored at an angle for 2 months (day 135), making sure the cork was in contact with the wine to avoid any oxygen entering.

2.8. Physicochemical analysis

2.8.1. Color

To measure the color, a sample of 40 mL of the wine fermented to the different times stipulated measuring coordinates L*, a*, b* of the CIE (Commission Internationale Eclairage) system. The Konica Minolta Colorimeter CR-410 N.J.USA was used with illuminant D65 and an angle of observation of 10°.

The chromatic coordinate a* varies from red when the value is positive to green when the value is negative. Chromatic coordinate b* is yellow when the value is positive and blue when negative. Parameter L* indicates luminosity, and depending on the location of parameters a*, b* and L* gives the color a lighter tone, until reaching white (L*=100), or a darker color until black (L*=0).

The CIELAB parameters of c* (Chroma) (1), H* (tone) (2) and color difference ΔE (3) were calculated based on chromatic coordinates a* and b*. When the value of H*=0 is red, as the tone increases (H*) or as it moves away from 0, it changes to red-orange (H*=45), yellow (H*=90), green (H*=180) and blue (H*=270) tones.

$$c^* = \{(a^*)^2 + (b^*)^2\}^{1/2} \quad (1)$$

$$H^* = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

$$\Delta E = \sqrt{(L^* - L_{ref}^*)^2 + (a^* - a_{ref}^*)^2 + (b^* - b_{ref}^*)^2} \quad (3)$$

2.8.2. Viscosity:

Viscosity was measured using the Brookfield DV3T Rheometer, with 10 mL samples at 150 RPM with a ULA needle, and an average torque of 75.32%.

2.8.3. Soluble solids:

The soluble solids content (°Brix), for the samples of blackberry wine was determined using the (ATAGO DIGITAL PR-201α).

2.8.4. Potential of hydrogen (pH):

The pH was measured using a potentiometer (OHAUS 5000), previously calibrated according to [13] standard AOAC-981.12 (1997). The value was obtained by introducing the electrode directly into the sample.

2.8.5. Titratable acidity:

The titratable acidity was measured using the potentiometer method and expressed as the percentage of malic acid [14] (AOAC-942.15).

2.8.6. Determination of the percentage of alcohol

This was measured using a hydrometer, calculating the percentage of alcohol per density, for which two measurements were obtained, one prior to the fermentation process and the other at the end of the bottle ageing process, where the following equation was subsequently used (4).

$$ABV = (Starting\ SG - Final\ SG)/7.36 \quad (4)$$

2.9. Microbiological analyses

These were carried out according to [15] the Colombia Technical Standard 404 de 1998, undertaking a fungi and yeast count (cfu/mL) which were read on 0-4 days.

2.10. Sensorial analysis

The sensorial analysis was carried out at the CESURCAFE laboratory, with the participation of 15 non-experienced individuals, whereby they initially characterized the alcoholic drink on a hedonic scale. The participants were provided with a glass of blackberry wine each so that they could appreciate the color and clarity by holding the glass up to the light. They were then asked to make circular movements with the wine holding the glass from the base in order not to warm the drink, and then identify its aroma. They were then asked to take a sip, allowing the wine to cover the tongue from the tip to the

back, leaving it in their mouth for a few seconds so that the alcohol could rise to the palate. Finally, the participants were asked to identify the final sensation that the wine left in their mouth. A nine-point survey was applied in order to find out the degree of their acceptance of the product through pairing with fresh cheese, mature cheese, ham and cherries. In between each type of food, the participants were given saltine crackers in order to avoid taste saturation.

The participants made a quantitative assessment of the acceptability of the product according to its color, smell, sweetness, sensation, degree of alcohol, acidity, astringency, body and flavor. Each characteristic was given a 0-9 score, where zero was not acceptable and nine was acceptable.

2.10. Statistical analysis

The data obtained were managed statistically using (ANOVA) variance analysis at a 0.05% level of reliability. The StatGraphics (Centurion XVI) statistics program test was used.

3. Results

By using the extractor, it was possible to obtain a wine-colored juice given that the pressure exerted on the fruits allowed the extraction of the juice in the drupes and did not affect the fleshy receptacle, which was white.

A dry red wine was obtained with a pH=3.26; % malic acid = 0.17, °Brix=6.93; % Alcohol=14.4; Tone=0.11; Chroma=11.90; Viscosity=1.46 cP (Table 1 and 2)

The most highly assessed characteristic by the tasters was the color, with a score of 9, where it was possible to see that the pigment that give blackberries their color passed to the wine, and improved during clarification and maturation in casks, and were preserved as much as possible during the bottle ageing process. The smell was the second best assessed characteristic with a score of 8. The rest of the characteristics fell within the product acceptability range and did not present statistically significant differences ($p > 0.05$).

For the pairing process, the best products to accompany the wine were cherry and ham.

Tables 1 and 2 present the data obtained for each of the parameters measured.

4. Discussion

The blackberry must had a soluble solids content of 7.2, which made it necessary to ameliorate or chaptalize the juice in order to aid the metabolic activity of the *S. cerevisiae* var. *Bayanus* strain and, thus, the fermentation process. The pH and acidity as a percentage of malic acid presented statistically significant differences ($p < 0.05$) between days 0 and 135 (Table 1). During fermentation the soluble solids diminished due to the metabolic activity of the different strains of yeast, whose initial population was of 5.55 ± 0.07 Log cfu/mL (Table 1). This grew in the nutritional conditions of the chaptalized blackberry must until reaching a population of 8.04 ± 0.07 Log cfu/mL on day 2.

Table 1.
Physical-chemical characterization of blackberry wine (135 days).

Phase	Time (day)	pH	%Acidity	Soluble solids	Viscosity (cP)	Yeast (Log cfu/mL)
Fermentation (0-15)	0	3.16±0.00	0.16±0.01	21.68±0.21	3.58±0.23	5.55±0.07
	1	3.18±0.10	0.15±0.01	14.59±0.30	3.03±0.45	7.30±0.14
	2	3.24±0.04	0.14±0.00	12.82±0.02	2.95±0.45	8.40±0.07
	3	3.31±0.01	0.13±0.00	9.80±0.71	2.89±0.41	8.37±0.18
	4	3.32±0.01	0.13±0.00	6.97±0.05	2.74±0.23	7.82±0.32
	5	3.27±0.01	0.13±0.00	7.00±0.04	2.64±0.24	-
	7	3.28±0.01	0.15±0.01	6.97±0.05	2.57±0.29	-
	8	3.27±0.01	0.16±0.01	6.95±0.07	2.33±0.29	-
14	3.28±0.01	0.16±0.01	6.97±0.05	2.15±0.25	-	
Maturation in casks (15-76)	76	3.28±0.01	0.17±0.01	6.92±0.02	1.47±0.01	-
Bottle ageing (76-135)	135	3.28±0.01	0.17±0.00	6.93±0.00	1.46±0.00	-

Source: Adapted from Oviedo M., Lozano J., 2016.

Table 2.
Color. Characterization of the blackberry wine during the process.

Phase	Time (day)	a*	b*	L	Tone	Chroma
Fermentation (0-15)	0	13.90±0.62	2.86±0.24	26.02±0.08	0.21±0.01	14.20±0.66
	1	14.46±0.95	2.86±0.24	26.02±0.08	0.21±0.01	14.20±0.66
	2	15.40±3.63	5.42±0.33	27.44±0.23	0.28±0.00	19.47±1.07
	3	14.50±5.94	5.69±0.12	27.94±0.26	0.30±0.00	19.36±0.30
	4	12.78±8.27	5.05±0.33	27.16±0.54	0.27±0.01	18.93±0.61
	5	12.56±7.90	4.79±0.32	27.59±0.05	0.26±0.01	18.52±0.42
	7	12.40±7.63	4.50±0.22	27.23±0.47	0.26±0.01	17.98±0.57
	8	10.97±5.75	3.39±0.04	26.23±0.78	0.23±0.01	15.33±0.11
14	10.57±5.14	2.42±0.01	25.08±0.43	0.17±0.00	14.44±0.04	
Maturation in casks (15- 76)	76	9.42±3.51	1.42±0.01	24.72±0.00	0.11±0.01	12.99±1.41
Bottle ageing (76-135)	135	9.38±3.46	1.32±0.05	24.46±0.11	0.11±0.00	11.91±0.01

Source: Adapted from Oviedo M., Lozano J., 2016.

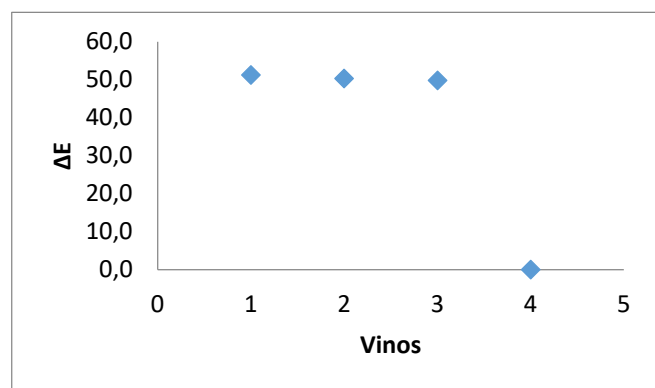


Figure 2. Color difference between blackberry wine and grape wine. 1: Pre-ferm cold maceration. 2: Post-ferm hot maceration. 3: classical maceration. 4: Blackberry wine.

Source: Adapted from Oviedo M., Lozano J., 2016.

The stationary phase continued to day 4 and from day 5, there was no growth of viable yeasts, which may be attributed to the production of ethanol. It is also worth noting that the soluble solids became established as from day 4. The viscosity of the blackberry wine reduced during fermentation due to alcohol production greater than the values obtained by [16].

In the fermentation process, the coordinate a* is constant on days one and two, and increases on day three, favoring the red tone. On day 8, there is a reduction showing statistically significant differences between the beginning and end of the fermentation phase (Table 2) [17]. During the first five days, the tanks were homogenized allowing us to take advantage of the anthocyanins and other phenolic compounds for their diffusion [7]. In the cask process, a* continues to decrease with significant differences (8-76 days), which is attributed to pigments transferred from the wood to the wine [7]. In the bottling process, it became evident that the amber-colored bottles kept the color stable [17] (Table 2).

The b* coordinate displayed constant behavior during days 1 and 2, subsequently increasing up to day 4, due to the homogenization of the treatments, which lasted until day 5. The phenolic compounds are dissolved during fermentation making this the phase that shows the highest value for chromatic coordinate b* [7]. Following this, there is a decrease with significant differences on days 8 to 76, modifying the tonality of the wine in the yellow chromatic coordinate [18], most likely due to the reduction of the polyphenol content over time.

From the beginning of the maturation in casks process (day 15-76), there is a decline due to oxidation because of cask porosity.

When aging the wine in amber bottles (day 76-135), there is no significant differences in the wine for coordinate b*, stabilizing due to the fact that the presence of the reaction's phenolic compounds is lower and does not alter the color of the wine and its reddish tone given by anthocyanins.

In the fermentation process, for coordinate L*, we can see that on days 1 and 2, it is stable, but presents significant differences by the end of the process. During the fermentation process (days 1-15), its value increases due to the presence and reaction of anthocyanins and polyphenols, which are reduced in concentration over time. It is worth mentioning that the remontage process to maintain the homogeneity of the juice in the fermentation tanks increased the values of luminosity. During the maturation in casks process (days 15-76), luminosity reduced with significant differences as the polyphenols are oxidized due to the redox potential. The porosity of the wood together with the presence of elagitannins in the casks may have contributed to the evolution of the color through co-pigmentation and its antioxidant action [7]. The blackberry wine presented values of 11.91 and 0.11 of chroma and tone respectively. According to [19], chroma values close to or greater than 50 indicate vivid colors which does not correspond to this study. Values for tones of between -10 and 10 correspond to red-purple, which, in this case, applies for blackberry wine. By comparing the ΔE of blackberry wine with grape wine, we can observe differences greater than 2.7 CIELab units. This demonstrates that blackberry wine presents chromatic characteristics that are different to grape wine and that can be perceived by the human eye (Fig. 2) [19].

Aging in amber bottles (days 76-135) demonstrated significant differences given the light (UV rays) present in the laboratory, which modifies the color of the wine. This process was implemented given that, according to [20] it improves the stability of a number of different parameters. As the wine is not in contact with oxygen, the components react due to biochemical processes, in particular the phenolic compounds (tannins), responsible for the wine's longevity. The wine's aroma and bouquet also improve as they depend mainly on the amount of polyphenols, such as esters, developed through the action of the acids on the alcohols. This is a slow and limited reaction in which volatile acids are formed (ethyl tartrate, diethyl succinate), which can be perceived after several years, and reaching their limit by 50 years. According to the results of the sensorial analysis, we can see that the effect of not having placed the must in the casks for maturation straight after the fermentation process (first five days) meant that the yeast in the must, immediately began the degradation process, increasing acidity, which, in turn, affects the wine's astringency, sweetness and body [20].

5. Conclusion

A dry blackberry wine was obtained with a red tonality, 6.9 °Brix, 14.4 degrees of alcohol, which are the values established in the NTC 708, recommended for pairing with ham and cheese.

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