

WILD BARBERRY FRUIT (*Berberis microphylla* G. Forst.) AS A NATURAL INGREDIENT FOR BEER BREWING

María Laura Lemoine^{1a*}, Ulises Fontana^{1b}, Jazmín B. Hurtado^{1b}, Federico M. Pintos^{1c}, Miriam E. Arena^{1d}, Ariel R. Vicente^{1e}, and Luis M. Rodoni^{1f}

^{1a} Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Cs. Agrarias y Forestales, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
<https://orcid.org/0000-0002-8870-8366>

^{1b} Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Cs. Agrarias y Forestales, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina

^{1c} Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Cs. Agrarias y Forestales, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

^{1d} Universidad de Morón, Laboratorio de Fisiología Vegetal, Morón, Buenos Aires, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

^{1e} Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Cs. Agrarias y Forestales, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
<https://orcid.org/0000-0003-1289-9554>

^{1f} Laboratorio de Investigación en Productos Agroindustriales (LIPA), Facultad de Cs. Agrarias y Forestales, Universidad Nacional de La Plata (UNLP), La Plata, Buenos Aires, Argentina. Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
<https://orcid.org/0000-0003-4409-6211>

* Corresponding Author: E-mail: mllemoine@agro.unlp.edu.ar

ABSTRACT

Barberry (*Berberis microphylla*) is a wild berry endemic to the Andean-Patagonian zone of Argentina and Chile. Even though it is not widely used or consumed, it has great potential for coloring and enrichment of different food matrices due to its high anthocyanin and antioxidant contents. The objective of this study was to evaluate the impact of barberry addition on quality parameters and stability of sour beer. Nutritional, physicochemical and sensory characteristics of the beer were analyzed. Firstly, different amounts of freeze-dried barberry were added to sour beer (0, 2.5, 5, and 10 g L⁻¹) after primary fermentation. In the next experiment, beers brewed with 0 (control) and 5 g L⁻¹ of barberry were stored at 5°C for up to 90 days and evaluated on a monthly basis. As demonstrated by the CIE Lab and Abs_{520nm} parameters, barberry addition resulted in a marked color shift towards ruby red tones. It also increased TEAC and phenolic contents by 2-4 times, without affecting beer pH, acidity, or density. Furthermore, it had a positive impact on overall impression, appearance, aroma, flavor and balanced scores in a trained sensorial panel. Beer total anthocyanins varied between 30 and 100 mg D3G L⁻¹. The beers brewed with barberry fruit presented stable antioxidant capacity, total anthocyanin content and both anthocyanin ionization and polymerization degree during storage. The results show that barberry can be used as an ingredient to make red and antioxidant-enriched sour beers with good stability during storage.

Key words: "calafate", Andean, red-beverage, berry, antioxidants.

INTRODUCTION

Berberis microphylla G. Forst. is an evergreen non-timber species naturally growing in the Argentine-Chilean Andean-Patagonian forest. The spiny shrub yields tiny purple berries (barberry-fruit), which are several-fold richer in anthocyanins and antioxidants than many of the so-called “super fruits”, such as blueberry (*Vaccinium corymbosum*) (Rodoni et al., 2014) and murtila (*Ugni molinae*) (Ruiz et al., 2010). Traditionally, barberry has been used for jams and infusions by local populations, and for cosmetics by the industry. Due to its colorant and antioxidant properties, it can be an attractive food ingredient but evaluation is required in each particular food matrix.

Beer is the most widely consumed alcoholic beverage in the world, and the third-most-popular drink after water and tea (Guido, 2019). Microbreweries have been one of the highest growth drivers for the beer market, gaining momentum in local markets and international trade share (Yeo and Liu, 2014). Though still dwarfed by India Pale Ale (IPA), sour beers such as Belgian Lambic, Flanders and German Berliner Weisse are quickly becoming one of the most produced beer styles (Strong and England, 2015; Chervina et al., 2019). Their clean lactic acidity is achieved through lactic-fermented wort, with specific lactic acid bacteria culture added before primary fermentation (Dysvik et al., 2019). The low bitterness and alcohol content of many of these styles contribute to their refreshing profile (Strong and England, 2015).

Many brewers have experimented with new ingredients in their formulations, leading to an innovative social process in the beer and craft-beer industry (Forde, 2017). In fact, the use of raw materials other than malt, hops, yeast and water has been a common approach for novel beer formulations (Schuina et al., 2020). Fruits, along with vegetables, spices and flowers, are the most frequently used ingredients for that purpose (Strong and England, 2015). While fruits can be added to almost any beer style, brewers usually prefer to add them to sour beer because fruit flavors taste more natural (Tonsmeire, 2014). Besides, a lot of fruits are an antioxidant source (Jin et al., 2014; Martínez et al., 2017a), and thus fruit addition can increase beer added-value, bioactive compounds and antioxidant power. Furthermore, a high antioxidant activity may be favorable to reduce oxidative reactions, preserving sensory characteristics of beer during storage (Caballero et al., 2012).

Some studies have evaluated the impact of brewing with fruits (Martínez et al., 2017b; Kawa-

Rygielska et al., 2019) and vegetables (Horincar et al., 2020) on beer physicochemical characteristics and antioxidant capacity. However, there is little information on the impact of fruit addition on the quality and antioxidant properties of sour beer. Therefore, the objective of this study was to evaluate the impact of barberry addition on the quality parameters and stability of a novel sour ale beer. Physicochemical properties, anthocyanin levels, antioxidant capacity and sensory properties were analyzed.

MATERIALS AND METHODS

Plant material

Barberry fruit (*Berberis microphylla*) was harvested from a wild population located near Ushuaia city (54°48' S.; 68°19' W., Tierra del Fuego, Argentina) in early 2019. The fruit was refrigerated and transported to the laboratory for analysis. The fresh fruits had 20°Brix, pH of 3.4, acidity of 17 g kg⁻¹ (as malic acid equivalents) and a water content of 720 g kg⁻¹ (fresh wet basis). At the laboratory, the fruit was washed with sodium hypochlorite (150 mg kg⁻¹, pH 6.5 for 1 min), and then rinsed three times with tap water, frozen in liquid nitrogen, crushed in a mill and frozen at -80 °C. The obtained powder was freeze-dried (RIFICOR, L-A-B3, Bs. As. Argentina) at 35 °C and 2.13 mbar for 48 h and stored at -20 °C until use. The samples were dried at 105 °C to constant mass (AOAC, 2012) and dry matter content was determined. The moisture content in the final product was 170 g H₂O kg⁻¹ wet basis. Freeze-dried barberry fruit was used for the experiments.

Beer manufacturing

A sour beer (Berliner Weisse) was brewed at LAURUS® craft beer company located in La Plata, Argentina. Ten kilograms of a mixture containing 50% Pilsner barley malt (Sacha Maltería Platense, Argentina) and 50% wheat malt (Maltear, Argentina) were ground. Mashing water was filtered through a reverse osmosis system (Romin Ingeniería, Serie Riepro 800, Argentina). The following compounds were added to osmosis water (55 L): 0.054 g L⁻¹ of CaCl₂, 0.018 g L⁻¹ of CaSO₄, 0.013 g L⁻¹ of CaCO₃, 0.013 g L⁻¹ of MgSO₄ and 0.27 g L⁻¹ of phosphoric acid. Mashing was conducted at 67°C for 90 min, adjusting the pH to 5.5 and obtaining a specific gravity of 1.048. The grain was washed with 25 L osmosis water at 78°C to remove residual sugar; the final volume of the batch was 80 L. The wort was boiled for 15 min to eliminate the microbial flora and quickly cooled to 35°C. For kettle souring, a culture of *Lactobacillus plantarum* (Wild Brew™ Sour Pitch Lallemand, Canada) was used. The wort was

rehydrated with sterile osmosis water at 25°C, and then the culture was added at 35°C. The wort initial pH was 5.4 at 35°C dropping to 3.4 after 24 h of incubation. Subsequently, the wort was boiled for 60 min, adding 30 g of hop pellets (Crystal variety, United States) and yeast nutrients (Servomyces, Lallemand Brewing, Canada) 15 minutes before boiling ended. An original gravity of 1.037 was obtained. After boiling, the whirlpool was conducted. For primary fermentation, the wort was cooled quickly to 18 °C, poured into fermenters as described below and inoculated with *Saccharomyces cerevisiae* yeast (Ale Nottingham, Lallemand, Canada) at a rate of 0.8 g L⁻¹ after its activation in sterile water at 25 °C for 30 minutes. Two independent experiments were conducted.

Fruit addition

Once primary fermentation was completed (final gravity 1.010), the beer was split into 4 groups of 5 L fermenters each and freeze-dried barberry was added at 0 (control), 2.5, 5, and 10 g L⁻¹. CO₂ was bubbled through the beers for 10 min to ensure correct distribution and favor fruit component extraction, avoiding O₂ incorporation and oxidation. The beers were maintained at 10 °C for five days, and then transferred and kept at 5 °C for 2 d for clarification. Three replicates were used for each treatment. After clarification, the beers were carbonated with CO₂ and bottled under pressure in 340 mL caramel-colored glass bottles. After 2 d at 5 °C, samples were taken and evaluated.

Beer storage

A sour beer brewed with 5 g L⁻¹ of freeze-dried barberry powder was prepared as described above. The bottled product was stored at 5 °C in darkness for 0, 30, 60 and 90 d. At each storage time, beer was sampled and the following parameters were evaluated: color, pH, acidity, density, contents of anthocyanin and phenolic compounds, and antioxidant activity using Trolox equivalent antioxidant capacity (TEAC). A sensory analysis was also performed.

Quality assessments

Sample degassing. Samples were frozen and conserved at 20 °C until use. For color, pH, acidity, soluble solids, density, total phenolic content, anthocyanin content, ionization, browning and polymerization, and antioxidant capacity determinations, the samples were thawed and vortex-shaken for 10 min to expel the remainder CO₂. For each assessment, three measurements were made for each beer replicate and the values were averaged.

European Brewery Convention (EBC) color,

red color component and color density. One milliliter of degassed beer was poured into 1 cm glass cuvette. The sample was analyzed spectrophotometrically (Numak, Model 721, China). Absorbance was measured at 420, 430, 520 and 700 nm using distilled water as reference. Color was determined using the European Brewery Convention (EBC) method. Estimations were made as follows:

$$EBC\ color = (Abs_{430} - Abs_{700}) \times 25$$

$$Red\ color = (Abs_{520} - Abs_{700})$$

$$Color\ density = (Abs_{420} - Abs_{700}) + (Abs_{520} - Abs_{700})$$

Color. Beer color was determined with a colorimeter (Minolta, Model CR-300, Osaka, Japan) equipped with an immersion head. A volume of 10 milliliters of the degassed sample was poured in a 2 cm diameter and 2 cm high cylindrical opaque cuvette, where the colorimeter head was immersed. The CIE Lab color parameters (*L**, *a** and *b**) were recorded. Three measurements were conducted for each replicate and the values obtained were averaged.

pH, acidity, °Brix and density. Samples of degassed beer were used. The pH was determined using a pHmeter (Numak, PHS-3E, China) (AOAC, 981.12 method, 2012). A digital refractometer (Milwaukee, MA871) was used for °Brix measurements. For acidity evaluation, 10 mL of beer were brought to 100 mL with distilled water and titrated with NaOH 0.1 mol L⁻¹ until reaching pH: 8.2. Acidity was expressed in g of lactic acid equivalents [H⁺] per liter. Density was measured using a densimeter and expressed as specific gravity. All measurements were made in triplicate.

Anthocyanins. Anthocyanin content was measured by the differential pH method (Horincar et al., 2020). Degassed beer samples were diluted with KCl buffer (0.025M, pH: 1) or with sodium acetate buffer (0.4 mol L⁻¹, pH: 4.5). The absorbance of both solutions was measured at 520 nm (Numak, Model 721, China) in a 1 cm cuvette. Anthocyanin content was calculated using extinction coefficient for delphinidin-3-glucoside, $\epsilon=0.051\ L\ mg^{-1}\ cm^{-1}$. Anthocyanin content was expressed as mg of delphinidin-3-glucoside (D3G L⁻¹):

$$D3G = (A \times DF - B \times DF) \times \epsilon^{-1} \times 1\ cm^{-1}$$

where:

A: ($Abs_{520, pH:1} - Abs_{700, pH:1}$); B: ($Abs_{520, pH:4.5} - Abs_{700, pH:4.5}$); DF: dilution factor.

Anthocyanin ionization, polymeric color and browning index. The absorbance of an aliquot portion of degassed beer was measured at 520 nm (Numak, Model 721, China). A second aliquot portion was diluted with KCl buffer, at pH<1, and the absorbance was measured at 520 nm. The proportion of anthocyanin in its ionized flavylum form was estimated reading the absorbance of beer (pH≈3.4) and acidified beer (pH<1) The result was expressed as a percentage of ionized anthocyanin (%ionized anthocyanin) and was calculated as follow:

$$\%ionized\ anthocyanin = 100 \times A \times (B \times DF)^{-1}$$

where:

A: ($Abs_{520; pH\ of\ beer} - Abs_{700; pH\ of\ beer}$); B: ($Abs_{520; pH<1} - Abs_{700; pH<1}$); DF: dilution factor

Anthocyanin degradation products were measured by polymeric color assay. The degassed beer sample was diluted with distilled water. A 2.8 mL-aliquot portion of the dilution was placed in a test tube and 0.2 mL of $K_2S_2O_5$ (20% p/v, freshly prepared) was added. The solution was mixed in a vortex and, absorbance was measured after 15 minutes at 420 and 520 nm. Polymerization index (PI) was calculated as follow:

$$PI = (Abs_{420} \times DF + Abs_{520} \times DF)$$

where DF is the dilution factor.

Based on an aliquot portion of the degassed beer, browning was calculated as the ratio between the absorbance measured at wavelengths of 430 and 520 nm corresponding to the browning index (BI):

$$BI = \left(\frac{Abs_{430}}{Abs_{520}} \right)$$

Trolox equivalent antioxidant capacity (TEAC). Measurements were made according to Gómez-García et al. (2021). Absorbance at 734 nm was measured using a spectrophotometer (Numak, Model 721, China) after 6 min of incubation. Trolox was used as a standard and the results were expressed as Trolox equivalent (mg TE L^{-1}) on a fresh weight basis.

Phenolic compounds. Total phenolic content was determined by Folin-Ciocalteu reagent (Petrón et al., 2021) using gallic acid as a standard; absorbance was read at 760 nm (Numak, Model 721, China). The results were expressed as gallic acid equivalents (mg GAE L^{-1}) on a fresh weight basis.

Sensory analysis. The beers brewed with different amounts of barberry fruit were sensory

evaluated by a panel of four judges certified by the Beer Judge Certification Program (BJCP). Overall impression, appearance, aroma, flavor, sourness, and balanced attributes were evaluated using a 10-point hedonic scale. The control and barberry enriched (5 g L^{-1}) beers were evaluated after 0 and 90 d storage.

Statistical analysis

A factorial design was used. For the selection between beers brewed with barberry, the factor was the amount of fruit added. For the storage assay, the factors were the amount of fruit added and storage time. The statistical unit was each fermenter ($n=3$).

Mean values (of three replicates) and standard deviation were calculated. The means were compared using an analysis of variance (ANOVA) and Tukey test at a level of significance of $p<0.05$. The linear relationship strength between two variables was calculated by the Pearson correlation.

RESULTS AND DISCUSSION

Influence of barberry addition on beer physicochemical and sensory properties

Barberry is an excellent ingredient for anthocyanin enrichment of beverages due to its exceptionally high anthocyanin content. In the present study, anthocyanin reached levels of 11.0 ± 0.1 ($n=4$) and 30.0 ± 1.1 ($n=4$) g D3G kg^{-1} on a fresh weight basis in fresh and lyophilized fruit, respectively. These values are 10 and 15 times higher than those reported for blueberry and eggplant (*Solanum melongena*), which are recognized as anthocyanin-rich sources (Sadowska et al., 2017; Horincar et al., 2020), and comparable with other so-called super-fruits like maqui (*Aristotelia chilensis*), black chokeberry (*Aronia melanocarpa*) and bilberry (*Vaccinium myrtillus* L.) (Ruiz et al., 2010; Sadowska et al., 2017).

As expected, barberry addition resulted in a noticeable color shift (from golden to red hues) in the beers, which varied depending on the amount of fruit added (Fig. 1A). The high anthocyanin content in barberry powder caused an extensive color change with relatively low fruit addition. The beer color for the control (no fruit added) and for the treatments with 2.5, 5 and 10 g L^{-1} of fruit added corresponded to 6 (the red color component was almost nil), 10.3, 13.5 and 23.5 EBC units, respectively (Fig. 1B and 1C). Furthermore, the addition of barberry resulted in an increase in EBC units, probably explained by the broad anthocyanin absorbance range at wavelengths less than 430 nm (Ahliha et al.,

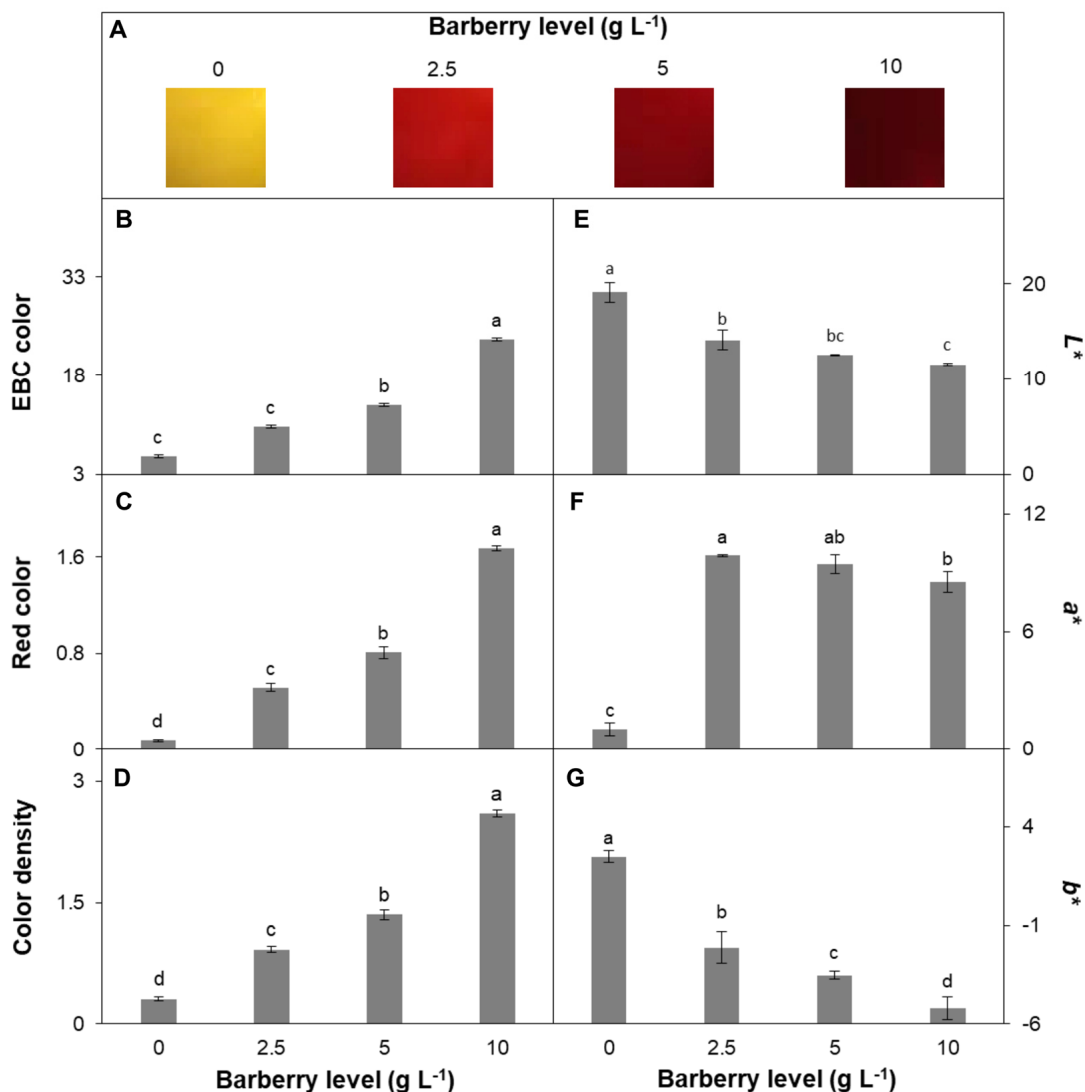


Fig. 1. A. Visual appearance; B. European Brewing Color (EBC color); C. Red color component; D. Color density; E. Lightness (L^*); F. a^* and G. b^* color values of sour beer enriched with 0 (control), 2.5, 5 and 10 g L⁻¹ of freeze-dried barberry fruit. Means \pm standard errors are shown. Letters indicate significant differences based on a Tukey test at a significance level of $p < 0.05$.

2018). In a previous study, quince fruit (*Cydonia oblonga* Miller) addition to American amber ale beer slightly increased EBC color (Zapata et al., 2019). In the present study, barberry addition caused a significant increase in both the red color component and color density in direct association with barberry level ($R=0.998$; $p=0.000$) (Fig. 1C and 1D). A marked drop in beer L^* from 19 to 11 was found with 10 g L⁻¹ of barberry (Fig. 1E). Barberry caused opposite trends in a^* and b^* color beer parameters, which was in line with an increment in red and blue components, respectively (Fig. 1F and 1G). The highest Δa^* was observed in the beer with the lowest amount of fruit added (2.5

g L⁻¹), without further relevant increases with higher fruit levels. In contrast, the b^* component was reduced when fruit level increased ($R=0.913$; $p=0.001$).

Horincar et al. (2020) also observed a decrease in L^* and an increase in a^* in lager beer enriched with eggplant skin powder. However, the authors also reported an increase in b^* . This difference may be due to the presence of anthocyanins in the yellow chalcone form related to the high beer pH (~ 4.5) used in the study. Barberry addition leads to novel hue varieties that can be selected by controlling the amount of fruit added in the brewing process, becoming a new and interesting

ingredient for beer brewing.

The final pH was around 3.4 and did not vary with barberry addition (Table 1). This may be due to the close pH values of barberry fruit and sour beer. Contrarily, organic acids for *Hibiscus sabdariffa* flowers (Martínez et al., 2017a) or cornelian cherry (*Cornus mas* L.) (Kawa-Rygielska et al., 2019) dropped the beer pH. Increases in acidity, °Brix and density were observed with 10 g L⁻¹ of fruit added (Table 1), which is in agreement with previous studies (Ulloa et al., 2017; Horincar et al., 2020). The additional sugar incorporated with the fruit can lead to over-carbonation of beer, especially with high fruit levels and when stored above refrigeration temperatures (Wray, 2020).

Barberry allowed obtaining beers with 30-100 mg D3G L⁻¹ of anthocyanins, varying with the amount of fruit added ($R=0.997$; $p=0.000$) (Fig. 2A). Similar anthocyanin concentration was achieved in beer with dried eggplant skin added (Horincar et al., 2020). In barberry fruit, anthocyanin/total solid ratio is close to 40 g kg⁻¹, which is higher than that of other fruits like raspberry (*Rubus idaeus* L.), blueberry or blackberry (*Rubus fruticosus* L.), with values of 4.2; 12 and 21, respectively (Sadowska et al., 2017). This allowed increasing anthocyanin levels, without adding a high load of other solids that could affect beer characteristics.

Antioxidants like phenolic acids, flavonoids and proanthocyanidins are naturally present in beer (Wannenmacher et al., 2018). The use of fruit, herbs, spices and other unconventional ingredients in beer brewing comes from the search for new beers styles, with novel colors and flavors (Tonsmeire, 2014). In fact, antioxidant-rich fruit can add natural antioxidants that may contribute to protect beer quality, hindering undesirable oxidative reactions (Martínez et al., 2017a). In this sense, there is an increasing interest in beer enrichment with healthy biological active compounds (Ulloa et al., 2017; Horincar et al., 2020). A study conducted by Kawa-Rygielska et

al. (2019) revealed that cornelian-fruit resulted in beers with higher phenolic compounds (70%) and antioxidant activity when added after rather than before primary fermentation. Based on this, barberry fruit was added after primary fermentation to obtain the highest possible value added.

In terms of antioxidant capacity, the barberry beer with 2.5 g L⁻¹ fruit added was 2-fold higher than the control (Fig. 2B). Higher fruit addition caused lower, but still significant increases, which indicates that there is some interaction between fruit antioxidants and some beer components. Despite the nature and extent of such interactions, which prevented a proportional increase in antioxidant capacity with the level of fruit incorporation, the beer with 10 g L⁻¹ of barberry added recorded an antioxidant level that was four times higher than that of the control (Fig. 2B). The increase in beer antioxidant capacity correlated with the level of anthocyanins ($R=0.951$; $P=0.000$) (Fig. 2A and 2B).

Levels of total phenolic compounds vary with the ingredients and brewing practices used. Depending on the beer style, 70-80% of them derive from malt and the rest from hops (Wannenmacher et al., 2018). The literature has already described that fruit addition can be a relevant source of phenolic compounds in fruit-beer styles (Kawa-Rygielska et al., 2019). In commercial beer, levels vary greatly from 22 to 300 mg L⁻¹ depending on the beer type (Zhao et al., 2010; Patraşcu et al., 2018), but the presence of synthetic antioxidants may contribute to these differences. In the present study, the finished beer with 0 (control beer), 2.5, 5 and 10 g L⁻¹ of barberry recorded total phenolic compounds of 152, 276, 300 and 360 mg L⁻¹, respectively (Fig. 2C). These results show that low levels of barberry added could be used to yield red-colored antioxidant-rich beer, without affecting product density or pH. In terms of anthocyanins, levels were high and comparable with those commonly found in

Table 1. pH, titratable acidity and density of sour beer enriched with 0 (control), 2.5, 5 and 10 g L⁻¹ of freeze-dried barberry fruit.

Parameter	Barberry added (g L ⁻¹)			
	0	2.5	5	10
pH	3.44±0.03a	3.44±0.02a	3.45±0.01a	3.41±0.01a
Acidity (g L ⁻¹)	5.7±0.5b	6.0±0.1b	6.1±0.5b	7.1±0.1a
°Brix	5.3±4x10 ⁻² c	5.4±5x10 ⁻² b	5.5±1x10 ⁻² b	5.7±1x10 ⁻² a
Specific gravity	1.010±7x10 ⁻⁴ b	1.011±2x10 ⁻⁴ ab	1.011±3x10 ⁻⁴ ab	1.012±1x10 ⁻⁴ a

The means ± standard errors are shown ($n=3$). Letters indicate significant differences based on a Tukey test at a significance level of $p<0.05$.

Se muestran las medias ± error estándar ($n=3$). Valores con letras distintas indican diferencias según test de Tukey con un nivel de significancia $p<0.05$.

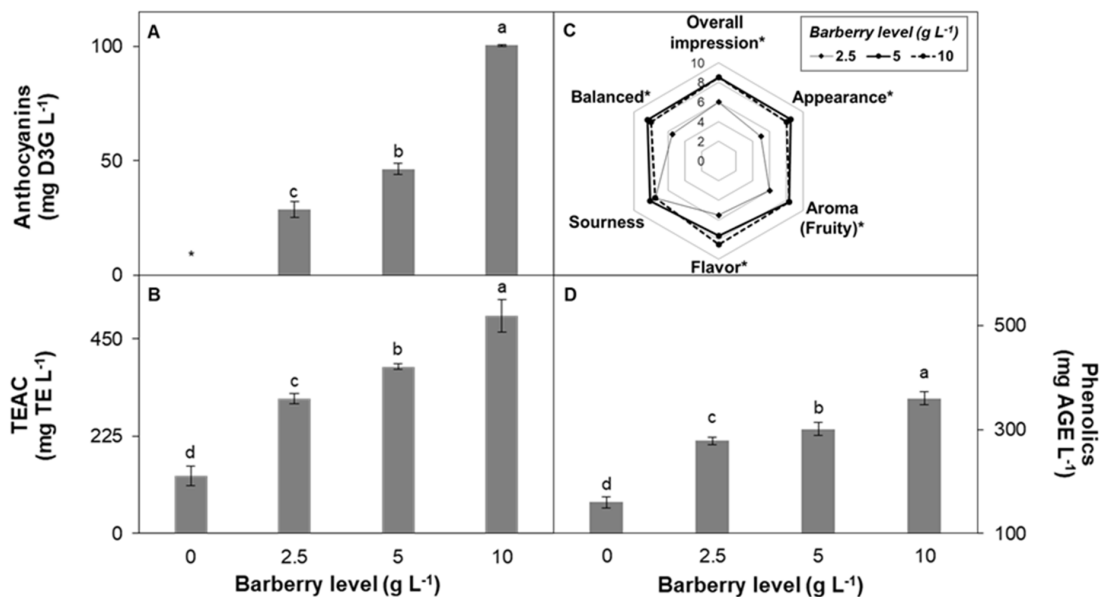


Fig. 2. A. Anthocyanins; B. Antioxidant capacity (TEAC); C. Sensory scores of barberry beer enriched with 2.5, 5 and 10 g L⁻¹ of freeze-dried barberry fruit; D. Phenolic compounds of sour beer enriched with 0 (control), 2.5, 5 and 10 g L⁻¹ of freeze-dried barberry fruit. Means \pm standard errors and means are shown in the bar and spider graphs, respectively. Letters or asterisks indicate significant differences based on a Tukey test at a significance level of $p < 0.05$.

grape juices (Muche et al., 2018).

Brewers need to get unbiased feedback on their beers. Given the hundreds of beer styles available at present, there is a tendency to evaluate beers within certain criteria or styles (Fitzpatrick et al., 2017). The Beer Judge Certification Program (BJCP)-authorized competitions currently include the 'fruit-beer' style (Tonsmeire, 2014; Strong and England, 2015). When barberry beers were evaluated accordingly, the beers with 5 and 10 g L⁻¹ of barberry added recorded the highest scores (Fig. 2D). The 5 g L⁻¹ barberry-fruit level resulted in antioxidant-enriched beers with high attributes within the fruit beer style, and thus it was chosen for subsequent analyses.

Changes in beer quality during storage

Increasing consumer concern about the use of synthetic additives has led to the search for natural pigments as coloring agents for different food matrices. Anthocyanins have been a promising alternative due to their high water solubility, dyeing capacity, antioxidant activity and health benefits (Horincar et al., 2020; Jin et al., 2014). However, they have shown lower stability compared to synthetic additives (Ertan et al., 2020), pH dependence on both color and stability (West and Mauer et al., 2013), and potential undesirable interactions with other food components. The most widely used colorant is caramel, mainly

for minor adjustments in the brewing process or ingredients. In general, the use of other pigments other than caramel is related to fruit-beer styles. To our knowledge, few studies have evaluated anthocyanin stability in beer, while there is no information on the quality of sour beers during storage. In this sense, a trend to aging and color degradation has been reported (Martínez et al., 2017a).

In the present study, the beers with 0 (control) and 5 g L⁻¹ of barberry added were bottled and stored at 5°C for 3 months and sampled on a monthly basis. Beer quality during storage was evaluated focusing on anthocyanin stability and antioxidant capacity. The pH values of both beers was around 3.4 and did not vary during storage. As pH is closely related to physicochemical and microbiological beer stability, it is desirable to be constant during storage. The acidity was 6.3-7.0 g L⁻¹ and the specific gravity ranged between 1.009 and 1.011. The EBC color of the control and barberry beers was 6 and 11 before storage, respectively (Fig. 3A). During storage, a slight increase in the EBC color was observed. There were no relevant changes in either the red color component or color density of the control beer during storage (Fig. 3B and 3C). However, a reduction of 15% in barberry beer red color was observed after 30 d storage (Fig. 3B), while no further changes were detected afterwards.

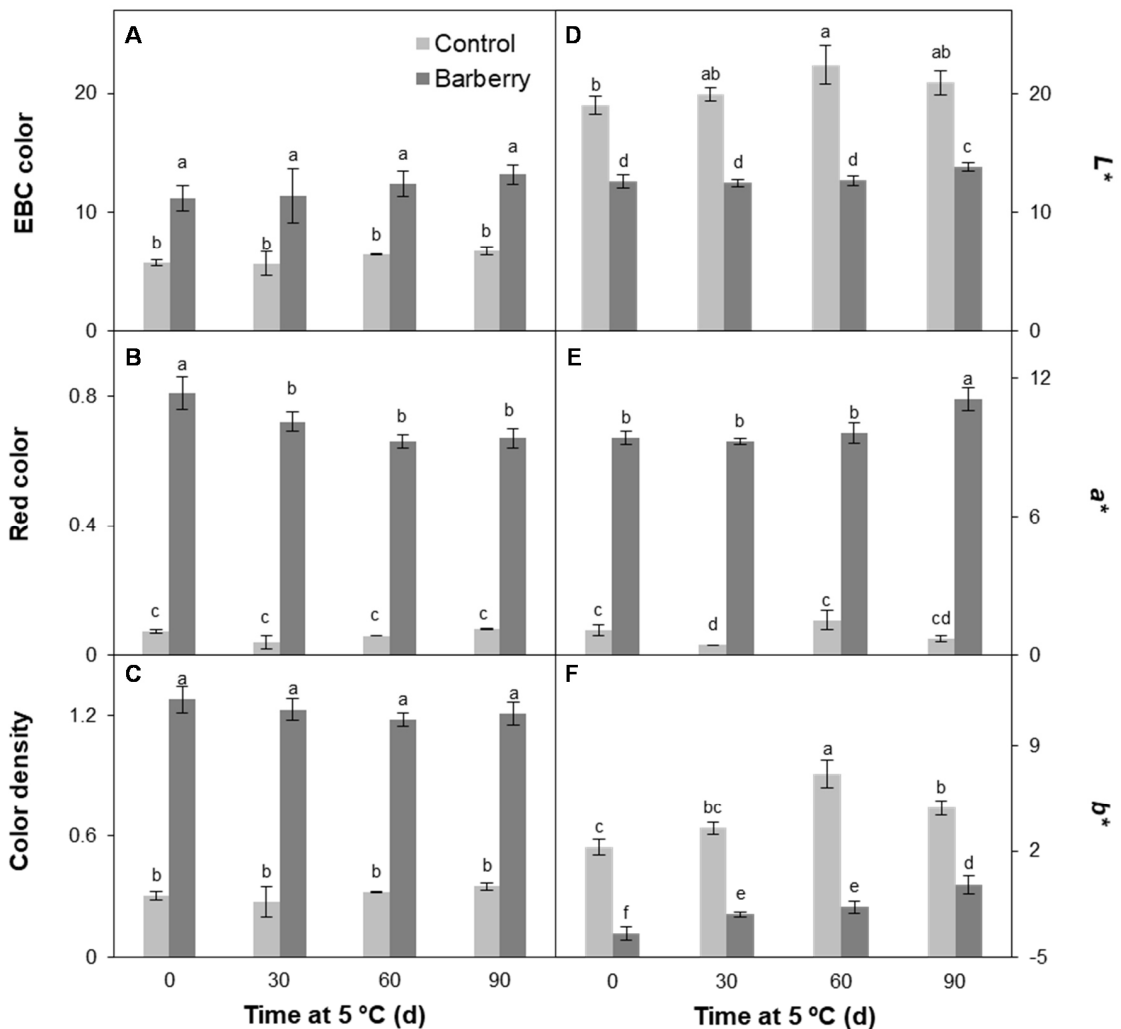


Fig. 3. A. European Brewing Color (EBC color); B. Red color component; C. Color density; D. Lightness (L^*); E. a^* and F. b^* color values of control and barberry (5 g L^{-1}) beers after bottle storage at 5°C for 0, 30, 60 and 90 d. Mean \pm standard errors are shown. Letters indicate significant differences based on a Tukey test at a significance level of $p < 0.05$.

A similar trend was observed in terms of color density (Fig. 3C).

Initial luminosity (L^*) was 19 and 12.5 for the control and barberry beer, respectively. Beer luminosity tended to increase by 1-2 units, with a more noticeable increase in the control (Fig. 3D). The control showed an increasing trend in yellow (b^*) component until 60 d with a subsequent drop until 90 d (Fig. 3F). A study on Pilsen beer enriched with eggplant skin reported that both a^* and b^* parameters decreased as storage time increased, which was attributed to low anthocyanin stability (Horincar et al., 2020). In contrast, we observed an increasing trend in a^* and b^* in the barberry beer (Fig. 3E and 3F). In addition, anthocyanin concentration was *c.a.* 45 mg D3G L^{-1} , without significant changes during

storage (Fig. 4A). These results do not agree with other studies that have described anthocyanin instability during beer storage. For example, Martínez et al. (2017a) found that beers brewed with 5 and 20 g L^{-1} of hibiscus recorded 40 and 13% decreases in total monomeric anthocyanins, respectively, after accelerated storage (7 days at 45°C). Another study in beer brewed with 5 g L^{-1} of eggplant peels and kept refrigerated at 5°C failed to prevent anthocyanin degradation, decreasing from 44 to 30 mg L^{-1} after 21 d storage (Horincar et al., 2020). The molecular structure of anthocyanins influences their chemical stability, with mono-glycosylated forms being less stable than poly-glycosylated and acetylated forms (Ertan et al., 2020). In barberry fruit, anthocyanins are not the most stable compounds in terms

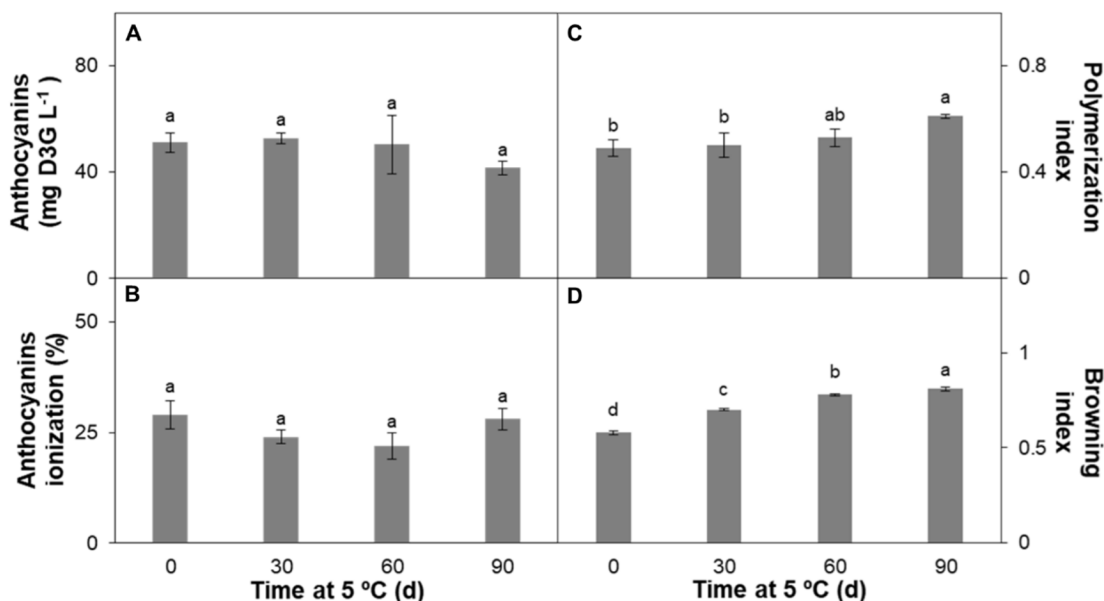


Fig. 4. A. Anthocyanins; B. Anthocyanin ionization (%); C. Polymerization index and D. Browning index of barberry beers (5 g L⁻¹) after bottle storage at 5°C for 0, 30, 60 and 90 d. Mean \pm standard errors are shown. Letters indicate significant differences based on a Tukey test at a significance level of $p < 0.05$.

of their glycosylation and acetylation degree (Ruiz et al., 2010). However, color and stability depend on the matrix where it is present, which is more important than anthocyanin structure (West and Mauer, 2013). Low molecular weight phenolic compounds, which are able to form a co-pigmentation complex through π - π orbital interactions, may considerably increase anthocyanin stability (Ertan et al., 2020). Some phenolic compounds, commonly found in cereal grains like p-coumaric, synaptic and ferulic acids, may interact with anthocyanins in a beer matrix (Piazzon et al., 2010). Interestingly, ferulic acid, which has been found at higher levels in sour beers compared with other beer styles, has been especially effective as a stabilizer (Fan et al., 2019). However, pH level is definitely the most important factor affecting anthocyanin stability in foods (West and Mauer, 2013). In fact, it influences the equilibrium between different anthocyanin molecular forms. Such effect results from the relative level of the quinoidal, carbinol/chalcone and flavylium ionic forms, with the latter being by far the more stable and prevalent at low pH values (West and Mauer, 2013). Remarkably, 20-30% of the anthocyanin contents were in flavylium ionic forms (Fig. 4B), exceeding the levels reported in some wines (Tavares et al., 2017). During storage, the ionization degree (ID) decreased between 30 and 60 d and then increased at 90 d. The low pH values of sour beer favored

high ID levels. Furthermore, the polymerization index (PI) was determined as an estimation of the abundance of brown polymers resistant to SO₂ bleaching from anthocyanin degradation (Aleixandre-Tudo et al., 2017). No changes in the PI were recorded until 60 d (Fig. 4C). After that, an increase in PI was detected. The browning index (BI) increased during storage (Fig. 4D). A high correlation between BI and *b** CIELab color parameter was found ($R=0.910$; $P=0.000$).

The antioxidant activity of beer was monitored by the ABTS^{•+} cation method and phenols by Folin-Ciocalteu molybdenum-tungsten reagent. Both methods showed the same trend for the control and barberry beer (Fig. 5A and 5B), indicating that phenolic compounds represent the most relevant antioxidant source in sour beer. The antioxidant capacity and phenols in the control beer increased *c.a.* 30% after 30 d, and then remained constant until 90 d. This contrasts with the reduction of phenolic compounds during storage reported in a previous study (Wannenmacher et al., 2018). This increase may be related to the formation of new high molecular weight phenolic compounds with higher antioxidant activity than single counterparts (Callemien and Collin, 2010). The antioxidant capacity of barberry beer was two-fold higher than the control and remained unchanged throughout storage (Fig. 5A). Sensory analyses of the control and barberry beers are shown in Fig. 5C and 5D, respectively. Sourness

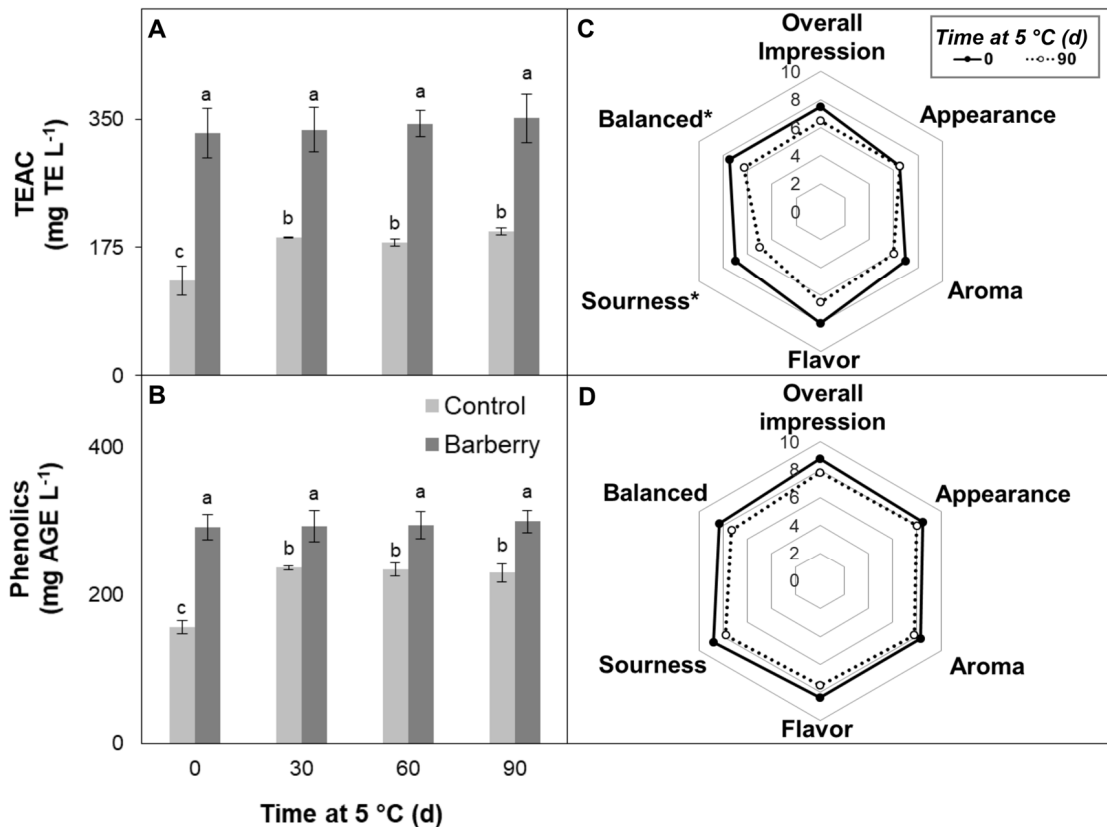


Fig. 5. A. Antioxidant capacity (TEAC); B. Phenolic compounds after bottle storage at 5°C for 0, 30, 60 and 90 d; and C-D. Sensory scores of the control (C) and barberry beers (D) after bottle storage at 5°C for 0 and 90 d. Mean \pm standard errors and means are shown in bar and spider graph, respectively. Letters or asterisks indicate significant differences based on a Tukey test at a level of significance of $p < 0.05$.

and balanced attributes decreased in the control after 90 d storage. No changes in the attributes of barberry beer were detected.

CONCLUSIONS

Freeze-dried barberry powder added in the range of 2.5-10 g per liter caused a marked increase in red color and antioxidant capacity of sour beer. The addition of 5 g L⁻¹ of barberry resulted in a red ruby beer with high fruit-like attributes, showing a two-fold increase in antioxidant capacity without relevant changes in pH, acidity, or density. The barberry beer proved stable after 90 d storage at 5°C, with no changes in beer color, anthocyanin content, antioxidant capacity, pH, acidity, or density. Color stability was related to the relatively low pH level of the sour beer analyzed, which allowed for a high content of anthocyanins in their stable ionized

flavylium form. The results show that barberry can add value to beer. Brewing with barberry resulted in a ruby-red color, antioxidant and anthocyanin concentrated sour-beer with high stability.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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