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Determination of anthocyanins content and antioxidant activity of beer from Chicha Morada obtained of the purple corn (*Zea mays* L.)

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ABSTRACT - Beer is the most preferred alcoholic drink in Latin America. Craft-beer adds novel ingredients such as spices, cereals, fruits or herbs. Purple corn (*Zea mays* L.) grown in Andes peruvian is the main ingredient used for made a typical non-alcoholic drink called "Chicha Morada". Anthocyanins are responsible of purple coloration and have healthy properties for human acting as bioactive compounds. The current study determined total anthocyanins content (TAC) and antioxidant activity by pH-differential method and DPPH assay, respectively from seeds and cob of purple corn, and chicha morada beer. The total anthocyanin content decreased in the order to cob, seeds and beer. The antioxidant activities decreased in the order to cob, beer and seeds. Chicha Morada beer remain have antioxidant activity and bioactives compounds that could affect to health positively.

Keywords: Anthocyanins, antioxidant activity, purple corn, Chicha Morada beer

INTRODUCTION

In Latin American, beer is the most preferred alcoholic drink in 11 out of 23 countries (Toro-Gonzalez, 2017). Moderate consumption of alcoholic beverages leads to improved lipid metabolism and to increased antioxidant and anticoagulant activity (Buemann, Dyerberg, & Astrup, 2002; Gorinstein et al., 1997) on the basis of beer polyphenols, which act as antioxidants (Gorinstein et al., 2007). Beer contains vitamins, minerals, antioxidants (phenolic compounds and melanoidins); malted grains and adjuncts are sources of nitrogenous compounds, carbohydrates, dietary fibre (Bertuzzi et al., 2020). Hops confer to beer a range of phenolic compounds and bitter resins (Fumi, Galli, Lambri, Donadini, & De Faveri, 2011).

Nonetheless, craft-beer producers add unusual ingredients such as cereals, herbs, fruit and other spices looking for innovative taste. Thats additives modify the chemical composition of beer, including the raw materials and the brewing process (Almeida et al., 2006). Thus, the ingredients also provide to beer bioactive compounds that increasing antioxidant activity. Many authors reported that the antioxidant activity increasing in differents styles of beer (Bertuzzi et al., 2020; Piazzon, Forte, & Nardini, 2010), fruit beers (Nardini & Garaguso, 2020) and herbs-beers (Djordjevic et al., 2016).

Purple corn (*Zea mays* L.), grown in Andean Region from Peru, is the main ingredient with other spices such as cinnamon, cloves and fruit peels (Salvador-Reyes & Clerici, 2020); to obtention a traditional non-alcoholic drink called chicha morada. Purple corn is an important source of anthocyanins. Anthocyanins already had been characterized in purple corn cobs and seeds include cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their respective malonated counterparts (Aoki, Kuze, & Kato, 2002; Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2002; Pedreschi & Cisneros-Zevallos, 2007).

Anthocyanins are phenolic compounds with bioactivity that bring health benefits such as anticarcinogenic (Pu Jing et al., 2008; Long et al., 2013; Pedreschi & Cisneros-Zevallos, 2006), antioxidant (Pedreschi & Cisneros-Zevallos, 2006; Yang & Zhai, 2010), anti-inflammatory, antihemorrhagic (Einbond, Reynertson, Luo, Basile, & Kennelly, 2004) and improve visual acuity (Liu, Xiao, Chen, Xu, & Wu, 2004; Mazza & Miniati, 2018).

The aim of the present study was determinated of anthocyanins content and antioxidant activity from seeds and cob of purple corn (*Zea mays* L.), and antioxidant activity of beer from Chicha Morada obtained of the purple corn.

MATERIAL AND METHODS

2.1. Obtaining of raw materials

Five kg of purple corn were obtained in local market from Arequipa, Peru; 500 g were destinated for determinarion of anthocyanins content and antioxidant activity and stored in dark bags at 4 °C until analysis. The restant samples were utilized to prepare of beer from Chicha Morada in Machay brewery (Industrial Research and Development Services Machay S.R.L., Arequipa, Peru) undername "Loba Special beer". On this study, the beer process will not be described due to brewery patent issues. Raw materials are shown in Fig 1. Purple Corns were pre-treated separating seeds purple corn from cob. Cob purple corn was cut off in small pieces by mill (Solab SL-30). Corn seed and corn cob were manipulated independently.

In this study, the beer process will not be described due to brewery patent issues.

The methanol used was of Merck analytical grade. The 2,2 diphenyl-1-picryl-hydrazil (DPPH) reagents were purchased from Sigma-Aldrich.

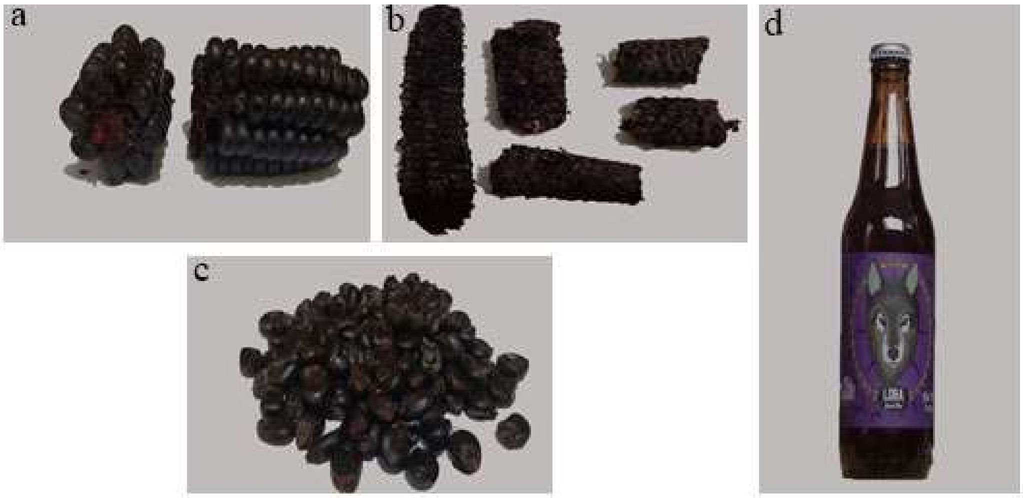

Figure 1. Raw materials. a) purple corn, b) purple corn cobs, c) purple corn seeds and d) chicha morada beer

2.2. Preparation of extracts

Seed (100 g) and cob (100 g) were macerated with 400 ml methanol for 24 h in the dark at 4 °C, respectively. Both crude extracts obtained were filtered through filter paper and the remaining residues were washed with 200 ml of methanol, and then collected the crude extract filtrate. The methanol in the crude extract was evaporated by the rotary evaporator (0.1 MPa, 35 °C) (Yang & Zhai, 2010). Beer only was disgasified and used. The extracts were then subjected to the measurement of TAC and antioxidant activity.

2.3. Determination of total anthocyanin content (TAC)

The TAC was determined according the pH-differential method (Giusti & Wrolstad, 2001), with modification as decribed below.

An aliquot of the sample (0.2 mL) was placed into 25ml volumetric flask and added 1.8 mL with potassium chloride pH 1.0 buffer. Another 0.2 mL of the sample was also placed into a 25ml volumetric flask, added 1.8 mL with sodium acetate pH 4.5 buffer. Absorbance was measured by a spectrophotometer (Biochrom, Libra S60PC) at 510 and 700 nm, respectively. Absorbance was calculated using Eq.1

$$Abs = (A_{510nm} - A_{700nm0})_{pH\ ,0} - (A_{510nm} - A_{700nm})_{pH4,0} \quad (1)$$

TAC was calculated using the following Eq 2.

$$TAC(mgL^{-1}) = \frac{Abs\ x\ MW\ x\ D\ x\ 1000}{\varepsilon\ x\ 1} \quad (2)$$

Where: *Abs* is absorbance; *MW* is the molecular weight of cyanidin-3-glucoside (449.2 Da); *D* is the dilution factor; ε is a molar extinction coefficient for cyanidin-3-glucoside of 26 900. All TCA shown was made as cyanidin-3-glucoside equivalents.

2.4. *Antioxidant activity determination*

Antioxidant activity was determinated by DPPH assay described by Rufino et al. (2007). Briefly, a 60 µM solution of methanolic DPPH solution was prepared. The initial absorbance of the DPPH in methanol was measured at 515 nm. An aliquot (0.1 mL) of each sample was added to 3.9 mL of methanolic DPPH solution and were measured at 515 nm. Antioxidant activity was calculated with Eq 3.

$$y = -ax + b \quad (3)$$

Where: y is initial absorbance / 2; x is IC_{50}. IC_{50} values calculated denote the concentration of a sample required to decrease the absorbance at 515 nm by 50%.

2.5. Statistical analysis

All results were realized in triplicate. The results were analyzed statistically by the analysis of variance using the software Statistica® 7.0 (StatSoft, Inc., Tulsa, USA). Mean separation was determined using the Tukey's test at $p \leq 0.05$.

RESULTS

3.1. Determination of total anthocyanin content (TAC)

As it can be observed in Table 1, the TAC measured in cob, seeds and beer were calculated to be 252.12 ± 0.36 mg C3G 100 g^{-1}; 57.89 ± 0.18 mg C3G 100 g^{-1} and 3.34 ± 0.04 mg C3G 100 g^{-1}, expressed in cyanidin-3-glucoside, respectively.

3.2. Antioxidant activity determination

As it can be observed in Table 1, the antioxidant activity measured by DPPH assay and expressed in IC_{50} where the higher the antioxidant activity, the lower is the value of IC_{50} (Molyneux P, 2004). In this study, showed values of 30.00 ± 0.48; 18.97 ± 0.15 g mL^{-1} and 23.93 g mL^{-1} ± 0.46; for seeds, cob and beer repectively.

Table 1. TAC and antioxidant activity of different samples

Sample	TAC (mg C3G 100 g^{-1})	Antioxidant activity (IC50 gmL^{-1})
Seeds	57.89 ± 0.18^{b}	30.00 ± 0.48^{a}
Cob	252.15 ± 0.36^{a}	18.97 ± 0.15^{c}
Beer	3.34 ± 0.04^{c}	23.93 ± 0.46^{b}

4. DISCUSSION

4.1. Determination of total anthocyanin content (TAC)

According to results, purple corn showed more anthocyanins content in cobs than seeds with 252.12 ± 0.36 mg C3G 100 g^{-1} and 57.89 ± 0.18 mg C3G 100 g^{-1}, respectively. Yang and Zhai (2010) found 55.8±1.5 and 92.3±2.1 mg C3G 100 g^{-1} for seeds and cob respectively. Monroy, Rodrigues, Sartoratto and Cabral (2016) found 63.79 and 63.09 mg C3G g^{-1} for cob and pericarp respectively. Aguilar-Hernández et al. (2019) found 496.3 and 967.9 mg C3G 100 g^{-1} for seeds and cob respectively. Correspondingly, the highest values of anthocyanins contents are found in cob that could explain why into cob are present purple coloring, instead seed the colour purple is prensent only in pericarp of seeds. On the other hand, the values respect to anthocyanins contents from purple corn could be influenced for growth conditions (Aguilar-Hernández et al., 2019; Yang & Zhai, 2010) and diferrents cultivars of purple corn (Harakotr, Suriharn, Tangwongchai, Scott, & Lertrat, 2014b;

Salinas-Moreno, Sanchez, Hernandez, & Lobato, 2005).

On the other side, beer showed anthocyanins content of 3.34±0.04 mg C3G 100 g^{-1}, presenting lower level of anthocyanins than cob and seeds. Anthocyanins degradation is influenced by many factors, one of these is termal treatment (Silva, Costa, Calhau, Morais, & Pintado, 2017), and during process of preparation of Chicha morada, purple corn and spices (cinnamon, cloves and fruit peels) are boiled at 100 °C for approximately 20 minutes (Salvador-Reyes & Clerici, 2020). Yang et al. (2008), reported that degradation rate of anthocyanins content on purple corn increase according temperature increasing. Harakotr et al. (2014b), found that anthocyanins content of purple waxy corn decrease until 50 % when its cooking at boiling point. Jing and Giusti (2007) reported that the loss of anthocyanins in purple maize water extracts is due to the denaturation of proteins at 100 °C, leading to a reduction of anthocyanins content in the drinks and desserts. Consequently, beer showed lowest level of anthocyanins compared to cob and seed because the boiling point is reached in the Chicha Morada preparation process.

4.2. Antioxidant activity determination

According to results, the antioxidant activity is higher in cob than seeds. Yang and Zhai (2010) found IC_{50} values of 40.1±0.8 e 48.5±0.5 µg mL^{-1} for cob ans seeds respectively. Monroy et al. (2016) found IC_{50} values of 35.0 µg mL^{-1} e 37.5 µg mL^{-1} for seed and cob rescpetively. Correspondingly, antioxidant activity is mainly higher in cob than seeds, this could be related to the anthocyanin content.

On the other hand, beer showed IC_{50} value of 23.93 g mL^{-1}, showing a higher antioxidant activity than seed of purple corn but lower than cob. The antioxidant activity in beer is influenced for raw materials such as malt and hops (Bertuzzi et al., 2020). In addition, to prepare chicha morada; cinnamon, cloves and fruit peels are used apart from purple corn (Salvador-Reyes & Clerici, 2020). Thus, additives modify the chemical composition of beer (Almeida et al., 2006). Other works who used DPPH assay and diferent methods to assay the antioxidant activity reported that the presence of different ingredients in beer increase the antioxidant activity such as beer enriched with eggplant peel extract reached until values of 1.333 mmol TE mL^{-1} (Horincar, Enachi, Bolea, Râpeanu, & Aprodu, 2020). Djordjevic et al. (2016) reported the increment of antioxidant activity in presence of distinct medical plants where *Thymi herba* showed a highest antioxidant activity with 3.72 mM TE. Fruit beers also showed an increment in antioxidant activity, showing 2.05 mmol TE L^{-1} by adding mango juice (Gasiński et al., 2020) and cherry beer showed values of 3.53 mM TE using ABTS assay (Nardini & Garaguso, 2020). Consequently, craft-beers show that higher values of antioxidant activity by adding unusual ingredients. Moreover, antioxidant activity on beer is affected by preparation process such as filtration, clarification, boiling, fermentation and maturation that affect polyphenols contents consequently antioxidant activity also is influenced (Gorjanović, Novaković, Potkonjak, Ida, & Sužnievič, 2010).

CONCLUSION

In conclusion, the total anthocyanin content from purple corn seed, purple corn cob and Chicha Morada beer were determined by pH-differential methods and its antioxidant activities were determined by DPPH assay. Form the data obtained, the total anthocyanin content decreased in the order to cob, seeds and beer. The degradation of anthocianins on beer is related to thermal process. The antioxidant activities decreased in the order to cob, beer and seeds. The antioxidant activities of purple corn may be related to its anthocyanin substrates and beer related to ingredients used. The results provide that Chicha Morada beer remaining antioxidant activity and bioactives compounds that could affect to health positively.

Authors contributions

FAE and NTF made experimental and analysis part, JSPM provided production beer and facilities, SSVH wrote this article, SP assisted in the preparation of the scientific article and WRCV adviced and supported during whole time dedicated to this work.

Conflicts of interest

The authors declare no conflict of interest.

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