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Determination of antioxidant activity and phenolic compounds in different Mexican craft beers

Determinación de la actividad antioxidante y compuestos fenólicos en diferentes cervezas artesanales mexicanas

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Resumen El objetivo del presente estudio fue evaluar y comparar el contenido fenólico (fenoles y flavonoides totales) y la capacidad antioxidante de diecisiete cervezas artesanales producidas en México. Los resultados mostraron una cantidad considerable de contenido de fenoles totales y flavonoides en los estilos Coffee Stout Imperial (362 mg GAE/L y 75 mg QE/L) y Stout Ale (299 mg GAE/L y 40,92 mg QE/L), seguidos por American Brown Ale (271 mg GAE/L y 27,35 mg QE/L), Brown Ale (211 mg GAE/L y 24,20 mg QE/L) y Dark (328 mg GAE/L y 20,94 mg QE/L). En contraste, la American Wheat Ale (65 mg GAE/L y 2,91 mg QE/L), Lager (102 mg GAE/L y 3,56 mg QE/L) e IPA (103,73 mg GAE/L y 3,75 mg QE/L) mostraron el menor contenido. La capacidad antioxidante se detectó en todas las cervezas evaluadas, principalmente en la de estilo Coffee Stout Imperial (80 %). En este estudio se observaron diferentes contenidos de carbohidratos totales según el tipo de cerveza, donde las Coffee Stout Imperial, American Brown Ale y Brown fueron las que presentaron el mayor contenido de carbohidratos (24,25, 15,55 y 15,36 g/L, respectivamente), seguidas del tipo Pilsner. (13,60g/L). Finalmente, nuestro estudio mostró que los diferentes estilos de cervezas en México presentaron diferentes contenidos de polifenoles (fenoles y flavonoides), capacidad antioxidante y carbohidratos. No obstante, las cervezas estilo Coffee Stout Imperial mostraron tener los mayores valores de los parámetros antes mencionados. Futuros estudios deben ser realizados en la evaluación de la actividad biológica de los diferentes estilos de cervezas sobre parámetros de nutrición en la población mexicana.

Palabras clave: antioxidantes; fenoles; flavonoides; México, cerveza artesanal.

Abstract The objective of the present study was to evaluate and compare the phenolic content (total phenols and flavonoids) and the antioxidant capacity of seventeen craft beers produced in Mexico. The results showed a considerable amount of content of total phenols and flavonoids in the styles Coffee Stout Imperial (362 mg GAE/L and 75 mg QE/L) and Stout Ale (299 mg GAE/L and 40.92 mg QE/L), followed by American Brown Ale (271 mg GAE/L and 27.35 mg QE/L), Brown Ale (211 mg GAE/L and 24.20 mg QE/L), and Dark (328 mg GAE/L and 20.94 mg QE/L). In contrast, the American Wheat Ale (65 mg GAE/L and 2.91 mg QE/L), Lager (102 mg GAE/L and 3.56 mg QE/L), and IPA (103.73 mg GAE/L and 3.75 mg QE/L) showed the lowest content of phenols and flavonoids. The antioxidant capacity is detected in all the beers evaluated, mainly in the Coffee Stout Imperial style beer (80%). In this study, different contents of total carbohydrates were observed according to the type of beer, with Coffee Stout Imperial, American Brown Ale, and Brown being the ones that presented the highest content of carbohydrates (24.25, 15.55, and 15.36 g/L, respectively) followed by the type of Pilsner beer. (13.60g/L). Finally, our study showed that the different styles of beers in Mexico presented different contents of polyphenols (phenols and flavonoids), antioxidant capacity, and carbohydrates. However, the Coffee Stout Imperial style beer showed the highest values of the aforementioned parameters. Future studies should be carried out in the evaluation of the biological activity of the different styles of beers on nutritional parameters in the Mexican population..

Keywords: antioxidants; phenols; flavonoids; México, craft beer.

Introduction

Beer is a product recognized as a natural drink with great value, nutritionally rich in vitamins, minerals, amino acids, protein, and other bioactive compounds which are responsible for the many health benefits such as its antioxidant activity, anti-inflammatory, neuroprotective, and cardio-protective effects (Pandey & Rizvi, 2009; Sohrabvandi *et al.*, 2012; Di Domenico *et al.*, 2020; Ullah *et al.*, 2020). The history of beer reaches far back to the ancient times, when the yeast was probably the first microorganism to be exploited by Sumerians, Babylonians, and Egyptians in the production of brewed a very simple drink made of water, damp cereals left to germinate (Oliver, 1991; Habschied *et al.*, 2020a).

Beer contains a complex mixture of bioactive compounds derived from malt and hops, including hydroxycinnamic acids, such as ferulic and p-coumaric acids, flavonoids, tannins, coumarins, chalcones, and proanthocyanidins (Almeida *et al.*, 2017; Kawa-Rygielska *et al.*, 2019). The polyphenols provided from malt and hops are considered one of the quality indicators of beer processing (Mitić *et al.*, 2014). These contribute directly to the color, flavor, foam stability, shortening beer's shelf-life taste, and astringency characteristics of beer, and they also protect raw materials from oxidative degradation throughout the brewing process (Ambra *et al.*, 2021).

According to the information generated by the National Institute of Statistics and Geography (INEGI), beer represents the most consumed alcoholic beverage in Mexico and their annual per capita consumption is of 68 L/year. Mexico is the world's sixth-largest beer producer and 30th in the ranking of beer consumption worldwide (INEGI, 2021). Such a level of consumption has led some researchers to focus on the nutritional appropriateness of beer, considering the potential benefits that it may confer to the human body when taken in moderation (Granato *et al.*, 2011; Boronat *et al.*, 2020). For example, the moderate consumption of lager and dark beer (225 mL per day) has been reported to increase plasma antioxidant capacity and anticoagulant activities, inhibit atherosclerosis, and facilitate cholesterol efflux, which may prevent lipid deposition in the vessel wall (Vinson *et al.*, 2003; De Francesco *et al.*, 2020). Additionally, some studies mention that a moderate intake of beer does not exert vascular detrimental effects nor increases body weight in obese healthy individuals (Padro *et al.*, 2018).

In Mexico, several studies have investigated the malts and solid waste from the craft beer process (Medina-Saavedra *et al.*, 2018; Guzmán-Ortiz *et al.*, 2019). However, studies about the biochemical parameters of Mexican craft beer are scarce. Although some studies exist regarding the polyphenol composition and antioxidant capacities of beers, there are no studies related to the determination of these parameters in commercial craft beers in Mexico. Therefore, the aim of this work was to evaluate and compare the phenolic content (total phenols and total flavonoids) and the antioxidant capacity of seventeen craft beers produced in Mexico.

Materials and methods

Beer samples

A total of 17 different styles of beer produced in Mexico in 2019 were collected from different markets and stored in the dark at 5 °C (Table 1). The analysis was made within a few days from the purchase. All beers used in this study were analyzed before the expiration date (at least 6 months before). Beer bottles were analyzed immediately after opening. Samples of beer were degassed and centrifuged at 6000 rpm for 5 min, before their analysis.

Table 1

Commercial beer evaluated in the present study

Sample code	Beer type	Ethanol contents (%)	Origin
Beer-01	American Wheat Ale	4.3	Nuevo León
Beer-02	Mexican Pale Ale	7.0	Baja California
Beer-03	Lager Agave	4.2	Guanajuato
Beer-04	Lager	5.0	Baja California
Beer-05	IPA	4.5	Nuevo León
Beer-06	Ginger	5.5	Baja California
Beer-07	Imperial IPA	7.2	Baja California
Beer-08	Golden Ale	6.5	Nuevo León
Beer-09	Craft Pilsner	4.1	Nuevo León
Beer-10	Brown Ale	5.0	Guanajuato
Beer-11	Stout Ale	5.5	Baja California
Beer-12	Brown	5.5	Baja California
Beer-13	Pilsner	5.0	Baja California
Beer-14	Coffee Stout Imperial	10.0	Baja California Sur
Beer-15	Dark	4.8	Baja California
Beer-16	American Brown Ale	4.8	Baja California
Beer-17	Brown Ale	5.3	Baja California

Note. Own work.

Determination of total phenolic and flavonoids content

The total phenolic content (TPC) of the beer was determined using the Folin-Ciocalteu as described by Singleton *et al.* (1999) with minor modifications (Stratil *et al.*, 2006). A beer sample (500 µL) and Folin-Ciocalteu reagent (1.0 mL at 0.20 M) were pipetted into cuvettes. After 3 min, 1.0 mL of 20% (w/v) sodium carbonate was added and the mixtures were incubated in the dark at 25 °C for 60 min. The absorbance was measured using a spectrophotometer at the wavelength of 765 nm. All measurements were made in triplicates and the results were expressed as milligrams of gallic acid equivalents (GAE) per liter of beer (mg GAE/L).

The total flavonoid content (TFC) was determined using aluminum chloride colorimetric according to Hosu *et al.* (2014). Briefly, 50 µL of beer samples were diluted with distilled water to a final volume of 500 µL. Then 500 µL of 10% AlCl3 solution and 500 µL of 1M CH3COONa were added. The volume was adjusted to 2 mL with distilled water, mixed, and the absorbance at 430 nm was measured. The total flavonoid content was determined in triplicates and the results were expressed as quercetin equivalents in mg per liter of beer (mg QE/L).

Total carbohydrates in beer

Total carbohydrates were determined according to the Phenol-Sulfuric Acid method (Masuko *et al.*, 2005). Aliquots of 500 µL beer sample were transferred into a test tube that was mixed with 500 µL phenol solution and 7 mL concentrated sulfuric acid. After allowing, the test tubes are vortexed for 30 s and placed for 20 min in a water bath at room temperature. Absorbance was measured at 490 nm. The content of reducing sugars was determined in triplicates and the results were expressed as the sugar content in mg per liter of beer (mg sugar content/L).

Determination of antioxidant activity

The antioxidant activity of beer samples was determined according to Mendez-Trujillo and Gonzalez-Mendoza (2021). An aliquot of 1 mL of DPPH (1,1-diphenyl-2-picrylhydrazyl radical) in methanol (0.025 mg/100 mL) was mixed with 500 µL of beer sample. The mixture was incubated in the dark for 30 min at 25 °C and the absorbance was measured at 517 nm in a spectrophotometer. The antioxidant activity was determined in triplicates and report as DPPH radical inhibition (%) = [(AControl - ASample/AControl) × 100]. Where AControl is the absorbance of the DPPH reaction and ASample is the absorbance in the presence of beer.

Statistical analysis

Statistical analysis was performed using Statistical software ver. 10 (StatSoft, Inc., USA) for Windows. The data were processed using analysis of variance (ANOVA) and Turkey's multiple range tests ($p < 0.05$). The results were expressed as a mean of triplicates ± standard division (SD).

Results and discussion

In the present study, our results showed there are significant differences in phenolic and flavonoid content according to the beer type (e.g., black, dark, lager, pilsner). Table 2 represents the analytical data for phenolic and flavonoid content of the beers. Our results clearly show a considerable amount in beer-14 (Coffee Stout Imperial) and beer-11 (Stout Ale), followed by beer-16 (American Brown Ale), 17 (Brown Ale), 12 (Brown), and 15 (Dark).

In contrast, beer-01 (American Wheat Ale), beer-04 (Lager), and beer-05 (IPA) showed the lowest content of phenolic and flavonoid content. This is in accordance with the investigation made by Habschied *et al.* (2020b), who reported that black and dark beers are richer in polyphenolic content than the light (Lager and Pilsner) ones. Similar results were found by Sanna and Pretti (2015) in some Italian craft beers produced by aging in a wood barrel that previously contained red or white wines. These authors mention that the final concentration of phenolic compounds depends on the quantity and quality of raw materials, and on the brewing-aging processes. Socha *et al.* (2017), mention that the antioxidant activity and phenolic compounds in analyzed dark beers (e.g., hydroxycinnamic acids and hydroxybenzoic acids) are responsible for biological activities compared to other styles of beer.

Similar results were obtained by Gouvinhas *et al.* (2021) for 23 commercial beers in Portugal using the Folin-Ciocalteau method. The authors observed that higher values of phenolic and flavonoid content were also positively correlated to black and dark beers previously analyzed. On the other hand, beer contained a large variety of phenolic components derived from the biotechnological process that involves the fermentation of barley malt and hops that are responsible for the overall antioxidant activity (Chen *et al.*, 2014).

On the other hand, Zhao *et al.* (2012) reported that the phenolic compound group is by far the most antioxidative in 40 lager beers evaluated. In the present study, the antioxidant activity was detected in all said beers (Table 2), but a significantly higher value was noted in dark and brown, beer-14 (80%), and beer-17 (73%).

Similar results were found by Pai *et al*. (2015), during the characterization of Indian beers, where the DPPH radical scavenging activity was observed in the range of 68 to 89 % in different styles of Indian beer. In contrast, beer-01 (American Wheat Ale), beer-08 (Golden Ale), beer-03 (Lager Agave), and beer-04 (Lager) showed the lowest content of antioxidant activity. The results for antioxidative activity obtained in our research can be compared to the ones published by Habschied *et al*, (2020b), who observed a significant change in antioxidative activity in correlation with beer type.

Table 2

Total phenolic and flavonoid contents of selected commercial beer

Sample	Beer type	TPC (mg GAE/L)	TFC (mg QE/L)	DPPH radical inhibition (%)
Beer-01	American Wheat Ale	65.68±0.05	2.91±0.79	11.12±3.54
Beer-02	Mexican Pale Ale	154.20±0.23	5.92±7.61	63.30±1.37
Beer-03	Lager Agave	78.93±0.25	4.48±0.38	29.40±1.03
Beer-04	Lager	102.06±0.08	3.56±0.91	30.90±2.24
Beer-05	IPA	103.73±0.54	3.75±0.46	44.96±0.20
Beer-06	Ginger	122.92±0.04	4.48±0.62	59.68±1.06
Beer-07	Imperial IPA	164.23±0.84	7.16±0.14	63.88±1.73
Beer-08	Golden Ale	121.04±0.58	5.31±2.45	5.40±3.14
Beer-09	Craft Pilsner	109.47±0.86	4.05±2.09	39.74±4.40
Beer-10	Brown Ale	245.09±0.79	19.51±2.81	19.49±6.50
Beer-11	Stout Ale	299.11±0.06	40.92±0.51	55.30±1.93
Beer-12	Brown	288.58±0.33	34.18±2.67	64.48±2.48
Beer-13	Pilsner	148.01±0.22	4.58±2.10	55.21±2.34
Beer-14	Coffee Stout Imperial	362.05±0.86	75.20±3.90	80.85±0.78
Beer-15	Dark	328.29±4.80	20.94±3.75	52.16±3.07
Beer-16	American Brown Ale	271.41±1.03	27.35±2.40	61.19±3.55
Beer-17	Brown Ale	211.20±3.71	24.20±0.89	73.87±0.90

Note. Own work.

On the other hand, Gribkova *et al*. (2022) mention that genetic, technological, and agricultural factors in the raw material in the brewing process influence the content of natural antioxidants (polyphenols and melanoidins) in the beer. Therefore, further research is needed to elucidate the potential role of antioxidants in beer and their variations concerning to agricultural and technological factors in their elaboration. The carbohydrates in beer vary from 3 to 61 g/L and play an important role in the fermentation process (Li *et al*., 2020). In the present study, different contents of total carbohydrates were observed according to the beer type (Figure 1).

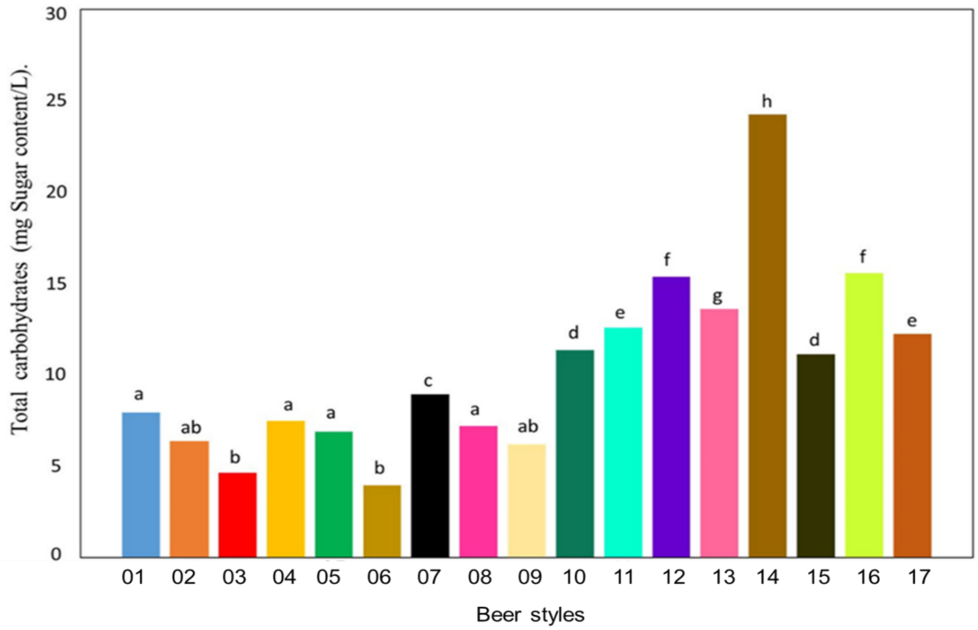

Figure 1

Total carbohydrates present in the different Mexican beer styles
Note. Own work.

Beers 14 (Coffee Stout Imperial), 16 (American Brown Ale), and 12 (Brown) presented the highest carbohydrate content (24.25, 15.55, and 15.36 g/L, respectively) followed by the beer type Pilsner (13.60 g/L). Brown Ale (beer-10 and 17), Stout Ale, and Dark showed values of 11.35, 11.12, 12.57, and 12.21 g/L total carbohydrates, respectively. On the other hand, beer types Imperial IPA (beer-07), American Wheat Ale (beer-01), Lager (beer-04), IPA (beer-05), Golden Ale (beer-08), Mexican Pale Ale (beer-02), and Craft Pilsner (beer-09) showed values of 8.92, 7.93, 7.48, 6.88, 7.18, 6.32, and 6.19 g/L total carbohydrates, respectively. Finally, beer types Lager Agave (beer-03) and Ginger (beer-06) showed the lowest content of total carbohydrates, 4.64 and 3.94 g/L among all beer samples analyzed.

According to Li *et al.* (2020), some brewing styles include fully attenuated low carbohydrate beers that contain amounts between (4-9 g/L) as a result of completely digested and fermented dextrins. On the other hand, Gąsior *et al.* (2020) mention that the use of dark malts and roasted cereal grains in the production of brewing wort influence the carbohydrate profile. This may indicate that the content of sugars in the darker worts may be less susceptible to fermentation, or that the substances produced as a result of the Maillard reaction have an inhibitory effect on yeast metabolism (Coghe *et al.*, 2005; Puligundla *et al.*, 2021). Finally, further research is needed on the use of different raw materials, yeast strains, and untypical malts in the production of a low carbohydrate beer.

Conclusions

The present study showed that the different styles of beers in Mexico presented different contents of polyphenols and carbohydrates. However, Stout was the principal style that proved to have the highest amount of polyphenols. Future studies should be supported to evaluate the different bioactive compounds present in Mexican beers.

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