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ORIGINAL RESEARCH PAPER

PHYSICOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF CRAFT AND COMMERCIAL BEERS MARKETED IN ROMANIA

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Abstract

Beer quality is determined by a multitude of physicochemical characteristics and bioactive properties, which influence not only the taste and appearance of the product, but also its functional potential on the consumer's health. The present study aimed to determine the physicochemical properties and *in vitro* antioxidant activity of a total of 21 samples of different beer types, such as Lager, Australian Pale Ale, Indian Pale Ale, Amber, Double Amber Dark Lager, Black, Stout, and Porter. The physicochemical characteristics considered in the study were EBC color, pH, total titratable acidity, soluble proteins, and viscosity. The biological activity of the beer was assessed by determining the antioxidant activity as DPPH and ABTS radicals scavenging activity and the total phenolic content. Both DPPH and ABTS-based methods indicated that wheat beers presented the lowest *in vitro* antioxidant activity among all tested samples, while the highest value was registered for black beer types. Results showed that antioxidant activity parameters were correlated with the original extract declared by the producers, which varied from 10.6 to 16.8 °P. Beer color varied from 8.5 EBC units (blonde Pilsner) to 250 - 253 EBC units (dark beers such as Amber, Black, and Porter), with no correlation between antioxidant activity and color. Other important correlations were found between original extract and energy value, alcohol content, viscosity or total phenolic content.

Keywords: Original extract, antioxidants, special malts, EBC color

Introduction

Beer is the most popular fermented beverage in the world. When consumed in moderation, it can provide significant health benefits, as it contains high amounts of nutrients and bioactive compounds such as vitamins and polyphenols (Quesada-

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Molina *et al*., 2019), compared to other low alcoholic beverages, including wine. Moreover, beer was stated to contain significantly higher amounts of proteins compared to wine (Blasco *et al*., 2011). Some beer-derived beverages, such as Radlers, were also found to have high contents of polyphenols (Patrascu *et al*., 2018). Phenolic compounds are also important for technological reasons, as they influence final quality, in terms of color, flavor, fragrance, stability, and clarity (Radonjić *et al*., 2020).

Characteristics such as antioxidant activity, primitive extract content, color, and viscosity provide relevant information about the chemical composition, degree of processing and functional potential of beer. The antioxidant activity is directly related to the presence of polyphenols and other bioactive compounds, while the original extract indicates the mass of soluble compounds in the wort, being directly correlated with the final body and alcohol content of beer. Color is an important technological and sensory parameter, influenced mainly by the type of malt used for preparing the wort, the intensity of the thermal processing, and the parameters of the fermentation (Eblinger and Narzib, 2000). Beer viscosity highly depends on the efficiency of the lautering and filtration steps (Blšáková *et al*., 2021). The main contributors to the viscosity of the final product are the polysaccharides, especially the high molecular weight β-glucans, which lower the extraction yield, interfere with beer clarification, and affect the final quality of the beer by increasing turbidity (Bogdan and Kordialik-Bogacka, 2017; Blšáková *et al*., 2021). Therefore, viscosity might provide information on the eventual colloidal nature and stability of the final product (Hollowood *et al*., 2002).

The worldwide demand for beverages is directed toward products with potential health benefits. In this context, the craft beer sector is constantly increasing, producers looking to add functional quality to their products (Giri *et al.*, 2023). Romanian consumers also show awareness in this direction as the trend is common to the entire European market (Mastanjević *et al.*, 2019; Gumienna *et al.*, 2024). Considering the continuous diversification of the Romanian beer market, with a growing number of industrial and artisanal brands available in the profile stores, the comparative evaluation of beer quality parameters becomes essential. Although there are international studies on these parameters, published data for commercial products present on the Romanian market are limited. Therefore, the present study aimed to contribute to the objective characterization of the quality of beers consumed on the local market, highlighting the differences between various types of beer (Lager, Porter, Stout, Ale, etc.), as perspective scientific indicators with practical relevance.

Materials and methods

A large variety of beer samples, including six lagers, three wheat beers, one Austrian and three India Pale Ales, four black beers, and one of each Amber, Porter and Stout types, purchased from local supermarkets (Galati, Romania) were considered in the study. Samples codification, the main ingredient declared on the label and the country of origin are presented in Table 1. Samples preparation prior to analyses consisted of degassing by intense shaking until all the CO_2 was released. The degassed beer samples were further sealed in plastic containers and stored under refrigeration conditions (+5°C) until analysis.

Table 1. Beer types considered in the study

Code no.	Beer brand	Beer type	Ingredients, other than water (according to the label)	Country of origin
S1	Bakalar Dry Hopped Lager	Lager	barley malt, hop products, hop cones	Czech Republic
S2	Holsten Pilsner	Lager	blond barley malt, barley, corn, malt extract, caramelized malt, hops	Ukraine
S3	The craftsman Manole craft blonde beer	Lager	barley malt, hops	Czech Republic
S4	The craftsman Manole craft blonde beer with extra hops	Lager	barley malt, hops	Czech Republic
S5	The craftsman Manole Ana's bouquet with hemp flowers	Lager	barley malt, hops, hemp flower extract	Czech Republic
S6	Primator Weizen	Lager-wheat	wheat malt, barley malt, hop products	Czech Republic
S7	Ursus wheat unfiltered	Lager-wheat	wheat malt, barley malt, hops	Romania
S8	Apostel Wiessbier unfiltered	Dark-wheat	wheat malt, barley malt, hop extract	Germany
S9	Volfas Engelmann APA	Austrian Pale Ale	barley malt, caramelized malt, hops	Lithuania
S10	Primator IPA	India Pale Ale	barley malt, wheat malt, hops, hop extract	Czech Republic
S11	Ursus IPA	India Pale Ale	barley malt, hops	Romania
S12	Steam Brew Imperial IPA	India Pale Ale	barley malt, hops, hops extracts	Germany
S13	The craftsman Manole amber craft beer	Amber	barley malt, hops	Czech Republic
S14	Grimberger	Double amber	barley malt, glucose syrup, sugar, caramel, hops	Poland
S15	Straopramen Dark	Dark lager	barley malt (Czech, Bavarian, caramel, colored), hops	Czech Republic
S16	Ursus Black	Black	barley malt, hops	Romania
S17	Silva Black premium	Black	barley malt, cornmeal, hop extract	Romania
S18	Leffe Brune monastic authentic	Black	barley malt, barley, corn, hops, sugar syrup	Belgium
S19	Bucur, dark beer	Black	barley malt, hops	Romania
S20	Lvivske Porter	Porter	water, pale barley malt, malt extract, caramel malt, barley, black malt, hop	Ukraine
S21	Primator Stout	Stout	barley malt, roasted barley, oats, hops, hop products	Czech Republic

Physicochemical analyses

The beer pH was measured directly on the degassed samples using a pH-meter (inoLab, pH7110, WTW). The total acidity was determined by titration with 0.1N NaOH solution, using phenolphthalein as an indicator, and reported as mL NaOH/100 mL.

The EBC color was determined according to the European Brewing Convention method as described by Patrascu *et al*. (2018). Absorbance was measured at 430nm and 700nm with a T80+UV/VIS Spectrometer (PG Instruments, UK). All samples presented $A_{700} \leq 0.039 \times A_{430}$ and were considered free of significant turbidity (Barth *et al*., 2020). Absorbance at 700 nm was included in the equation in order to eliminate any turbidity according to the method presented by Patrascu et al. (2018).

$$\text{Color EBC} = 25.5 \times (A_{430} - A_{700}) \quad (1)$$

Viscosity measurements

Beer viscosity was determined with a TA2000ex rheometer (TA Instruments Ltd., New Castle, DE, USA), using a double concentric cylinder geometry. The temperature was set at 20°C and controlled with a Peltier vane. The gap was set to 0.5mm. Viscosity (cP) was registered at a shear rate of 500 s^{-1} for three minutes. The viscosity value was determined as the average of 18 registered points.

Soluble protein content

Soluble proteins present in beers were determined using the Lowry method (Lowry *et al*., 1951). The method implies proteins reaction with alkaline copper sulfate in the presence of tartrate, followed by the addition of Folin – Ciocalteu reagent. The –CO–NH– (peptide bond) in polypeptide chain reacts with copper sulfate in an alkaline environment, to give a blue-colored complex. In addition, tyrosine and tryptophan residues of protein cause a reduction of the phosphomolybdate and phosphotungstate components of Folin – Ciocaulteu reagent to give a bluish product that enhances the sensitivity of this method. The quantity of proteins (mg/L) was determined using a T80+ UV/VIS Spectrometer (PG Instruments Ltd, UK) at a wavelength of 726 nm.

Total phenolic content determination

The total phenolic content was determined using the Folin - Ciocalteu method, as described by Patrascu *et al*. (2018). A volume of 0.2 ml sample was mixed with 1.5 mL of Folin - Ciocalteu reagent, previously diluted with distilled water (1:10, v/v). After 5 5-minute resting period, a volume of 1.5 mL of 60 g/L Na_2CO_3 was added. The admixture was allowed to rest for another 90 min, and the absorbance was further read at 725 nm, using a T80+ UV/VIS Spectrometer (PG Instruments Ltd, UK). The blank sample was obtained by mixing all reagents with distilled water instead of the beer sample. The total phenolic compounds concentration in beer samples was quantified using two different calibration curves, namely ferulic acid equivalents (FAE) and gallic acid equivalents (GAE) (g/L).

DPPH radical scavenging activity (DPPH-RSA)

The DPPH-RSA of beer samples was determined using the method of Martinez-Villaluenga *et al*. (2009). Prior to the experiment, a stock DPPH solution (10 mg DPPH in 25 mL 80% methanol) was diluted with 80% methanol until an absorbance of 1.00 was reached, at a wavelength of 515 nm (T80+ Spectrometer, PG Instruments Ltd, UK). A volume of 0.2 ml of sample was mixed with 4.5 ml of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical solution. After a resting period of 20 minutes in the dark, samples absorbance was read at 515 nm. The empty glass cuvette was used as a blank. The reading for the initial absorbance value (standard needed to determine the percentage of inhibition) was made by mixing 4.5 mL of DPPH working solution with 0.2 ml of 80% methanol. The absorbance of standards and samples was registered at a wavelength of 515 nm. The DPPH scavenging activity was expressed in terms of mmol Trolox equivalents/L.

ABTS radical scavenging activity (ABTS-RSA)

The antioxidant activity of the beer samples was determined as the capacity to reduce the $ABTS^{+}$ radical cation using the method described by Martinez-Villaluenga *et al*. (2009). A stock ABTS solution (7 mM in 80% methanol) was diluted with 80% methanol to prepare the working solution, until absorbance reached 0.700±0.20 at a wavelength of 734 nm. A volume of 40 µL of beer sample was mixed with 2.96 mL of ABTS (2,2′-Azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) solution. The absorbance was measured after 6 minutes at 734 nm. The reading for the initial absorbance value (needed to determine the percentage of inhibition) was made by mixing 2.96 mL ABTS with 40 µL of 80% methanol. A solution of 80% methanol was used as a blank. The ABTS radical scavenging activity was expressed as mmol Trolox equivalents/L.

Statistical Analysis

Statistical analysis was performed using Microsoft Office Excel predefined tools. All measurements were performed in duplicate, and the results were reported as mean values ± standard deviation. ANOVA single factor with a 95% confidence level and Tukey’s HSD analysis were used to determine the statistical differences between samples with a 99% confidence level.

Results and discussion

Physicochemical characterization of beers

The present study focused on a variety of 21 beer samples (Table 1), with color ranging from light straw, in the case of some blonde lager beers, to dark brown, specific to Stout. Five beers were from the blonde Lager type (S1 to S5), from which three were labeled as Craft beers (S3-S5), and two were considered commercial beers (S1-S2). Three beers were produced from wheat malt (S6-S8). From these, one was dark (S8). All three wheat beers had specified on the label the presence of barley malt; however, the percentage was not specified. Four beers (S9-S12) were Pale Ale type, and two were ambers (S13-S14). Beers numbered from S15 to S21 were of dark type (black, Porter, and Stout). The original extract of the studied beer samples varied from 10.6 to 16.8°P, while for three samples the values were not specified on the label (Table 2).

Table 2. Alcohol, original extract, and energy value of the beer samples, as declared on the label

Sample code	Alcohol content, %vol.	Extract, °P	Energy value, kcal
S1	5.2	12.6	45
S2	4.7	ND	39
S3	6.3	13.5	45
S4	5.3	11.5	41
S5	4.5	10.6	38
S6	4.8	11.5	45
S7	5.1	12.5	48
S8	5.3	11.8	43
S9	5.0	11.5	41
S10	6.5	11.5	45
S11	5.8	13.5	50
S12	7.8	16.8	62
S13	6.3	13.1	45
S14	6.5	ND	57
S15	4.4	11.3	42
S16	6.0	15.0	56
S17	7.0	16.0	58
S18	6.5	15.6	ND
S19	6.5	14.3	54
S20	8.0	ND	71
S21	4.7	11.5	45

The original extract is a very important parameter in brewing technology, consisting of the total nonvolatile fraction dissolved in the unfermented, cold pitching wort (Eblinger and Narzib, 2000). The main soluble compounds in the wort, contributing to the original extract, are the fermentable sugars (fructose, glucose, maltose, maltotriose), and dextrins (Willaert, 2007), which are metabolized to a great extent by the yeast during fermentation. Mainly, the remaining sugars, together with the alcohol formed during fermentation, account for the energy value of the final product. The mashing parameters determining the extract content in wort, will influence the peptide and amino acid profiles of the wort, the yeast nutrient concentration, and finally the quality of the beer through the alcohol content, concentration of unfermented sugars, buffering capacity and pH, β-glucan content, and some beer physical properties such as foam, color, and clarity (Willaert, 2007).

The alcohol content is also linked to the attenuation degree of the wort, which can vary between 15% in low alcohol beers and up to about 100% in dietetic or low carbohydrate beers (Eblinger and Narzib, 2000). However, it is less likely to find a high original extract value and a low alcohol content in commercial beers. As seen in Figure 1, extract value declared on the labels had a rather high correlation (R^2>0.6) with both the energy value and the alcohol content of beer.

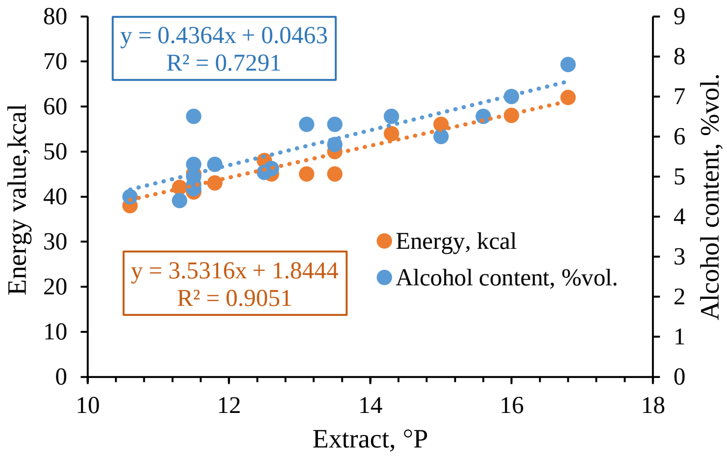

Figure 1. Correlations between alcohol content and energy value with the original extract of the studied beers, as reported on the label

Beer color mainly depends on the malt type used for brewing, wort boiling time (Eblinger and Narzib, 2000), the phenolic compounds (polyphenols) extracted from barley husks or hops, and the use of various additives such as caramel (Ualema *et al*., 2024). Malt color is formed during the drying after the grains have steeped and germinated. Depending on the desired malt color, the germinated grains can be subjected to drying at higher temperatures, which enables the formation of melanoidins, derived from the Maillard reaction. The color of the resulting special malts can vary between 50-120 EBC (caramel malt) to 1300-1600 EBC (roasted malt) (Gąsior *et al*., 2020). Melanoidins possess reducing properties and potentiate beer acidity. They also contribute to color development through polymerization and nonenzymatic oxidation (Eblinger and Narzib, 2000). Considering all these aspects, strong correlations are expected between color and other chemical properties of beer, like polyphenolic content. The EBC color units of the beer samples analyzed in the present study are presented in Table 3. The lightest beer was Holsten Pilsner, with only 8.45 EBC units, while the S13 to S19 dark beers had EBC color values of 252.09 to 253.24 (a homogeneous group with no statistical differences). The darkest color was registered for Leffe brune (S19). No correlation was observed between color and original extract values (R^2=0.002), polyphenol content (R^2=0.44), or antioxidant activity.

Table 3. Physicochemical characteristics of the studied beer samples

Sample code	pH	Titratable acidity, ml NaOH/100ml	EBC Color	Viscosity, cP
S1	4.65±0.05^{hij}	36.96±0.84^{efgh}	15.47±0.11^{e}	2.50±0.03^{ab}
S2	4.61±0.00^{gh}	31.08±0.84^{ab}	8.54±0.05^{a}	2.45±0.01^{a}
S3	4.76±0.02^{lm}	36.33±0.21d^{efg}	15.75±0.11^{e}	2.50±0.02^{abc}
S4	4.81±0.00^{mn}	28.35±0.21^{a}	11.86±0.03^{c}	2.52±0.01^{abc}
S5	4.74±0.01^{l}	31.71±0.21^{abc}	15.45±0.05^{e}	2.52±0.01^{abc}
S6	4.44±0.01^{c}	38.64±0.84^{ghi}	14.96±0.14^{e}	2.58±0.01^{a-e}
S7	4.50±0.00^{d}	36.33±0.63^{defg}	9.87±0.03^{b}	2.71±0.01^{a-d}
S8	4.56±0.00^{ef}	31.08±0.00^{ab}	65.62±0.22^{h}	2.87±0.01^{hi}
S9	4.67±0.00^{ij}	37.80±0.17^{fgh}	13.46±0.38^{d}	2.53±0.01^{abc}
S10	4.69±0.00^{jk}	39.99±0.34^{hi}	25.58±0.66^{f}	2.78±0.02^{f-i}
S11	4.57±0.00^{fg}	35.07±0.21^{cdef}	26.6±0.05^{f}	2.69±0.01^{c-h}
S12	4.64±0.00^{hi}	38.64±0.42^{ghi}	28.25±0.13^{g}	2.90±0.01^{i}
S13	4.73±0.01^{kl}	33.60±0.42^{bcde}	252.09±0.00^{kl}	2.66±0.02^{b-g}
S14	4.44±0.01^{c}	33.18±0.42^{bcd}	253.15±0.04^{l}	2.62±0.01^{a-f}
S15	4.45±0.01^{c}	37.17±0.63^{fhg}	252.46±0.01^{kl}	2.75±0.02^{e-i}
S16	4.57±0.01^{fg}	36.75±0.63^{efgh}	250.08±0.00^{j}	2.75±0.01^{d-i}
S17	4.62±0.00^{gh}	39.90±0.42^{hi}	253.08±0.00^{l}	2.83±0.01^{ghi}
S18	4.24±0.00^{a}	42.00±0.42^{i}	253.24±0.00^{l}	2.76±0.01^{e-i}
S19	4.48±0.01^{cd}	49.98±0.42^{j}	252.63±0.00^{l}	2.63±0.01^{a-f}
S20	4.38±0.00^{b}	37.38±0.42^{fgh}	251.25±0.05^{jk}	2.94±0.01^{i}
S21	4.52±0.00^{de}	37.80±0.84^{fgh}	246.70±0.0^{1i}	2.59±0.01^{a-f}

Data with the same superscript within a column are similar according to Tukey's honestly significant difference (HSD) method, with a confidence level of 99%

Beer viscosity is a significant factor that affects flavor perception. Hollowood *et al*. (2002) stated in this respect that the increase in the viscosity of a solution reduces the movement of the flavor molecules, therefore affecting their perception. The values of the apparent viscosity of the studied beers are reported in Table 3. The viscosity values ranged between 2.50 cP (for Lager types S1 and S3) to 2.90 - 2.94 cP (for S12 and S20 samples, IPA and Porter). The Stout type beer chosen in the present study registered only 2.59 cP, statistically similar to the blonde Lager and Pilsner beers. The viscosity values measured in the presented study are comparable to those reported in the literature. Langstaff and Lewis (1993) reported values between 1.26 and 2.2 cP at 20 °C (the type wasn't mentioned), while Severa and Los (2008) reported 2.53 cP for dark beer measured at 20 °C, with an inverse correlation between viscosity and beer temperature. Also, Langstaff and Lewis (1993) stated that sensory viscosity perception and instrumental viscosity can be correlated. The correlation between the measured beer viscosity and the original extract reported on the label (for 18 out of 21 samples) was also verified. Analyzing the results shown in Figure 2, one can observe a linear correlation between the reported original extract and the determined viscosity for 15 samples, with a correlation coefficient of R^2=0.7686.

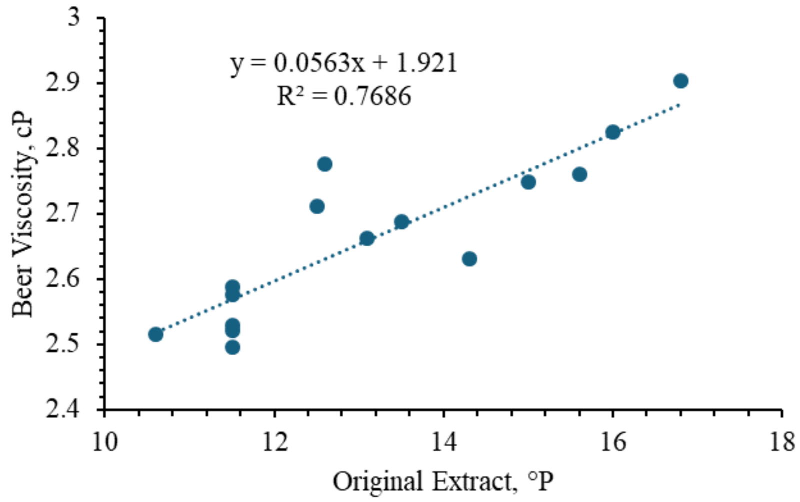

Figure 2. Correlation between viscosity values and the original extract of the studied beers.

The acidity of the studied beers varied between 31.08 and 49.98 ml NaOH/100ml ($p<0.05$), while pH varied between 4.24 and 4.81 ($p<0.05$), as reported in Table 3. No significant correlation was found between the two parameters. The highest pH value was recorded for S3 and S4 craft beer samples (Manole craft blonde beer and Manole craft blonde beer extra hops). With the exception of the S4 sample, the other three, which came from the same manufacturer (S3, S5, and S13), presented similar high pH values ($p>0.05$). The lowest pH values were registered for the dark beers, S18 and S20. Due to the use of roasted malts with higher amounts of melanoidins, dark beers are usually more acidic and have lower pH values compared to lighter beers (Eblinger and Narzib, 2000). Moreover, the larger range of organic acids formed during dark beer fermentation contributes to the pH drop. For instance, Godínez-Hernández *et al*. (2025) reported higher pH values for the blonde craft beer compared to Stout and American Pale. However, in the present study pH values did not show significant correlations with beer color ($R^2<0.2$), or original extract ($R^2<0.1$). In general, beer pH is influenced by the presence of organic acids such as acetate, malate, citrate, fumarate, formate, lactate, and succinate, among others (Li and Liu, 2015), together with melanoidins formed during malt roasting (Eblinger and Narzib, 2000). Some organic acids from beer, namely citric, malic, fumaric, and formic acids, originate from wort, and their quantities do not increase during fermentation, while lactic, acetic, and succinic acids are also formed during fermentation (Li and Liu, 2015).

The soluble protein contents registered for the investigated beers are shown in Table 4.

Table 4. Antioxidant activity and soluble proteins content of the studied beer samples.

Sample code	Total Polyphenols content		Soluble proteins, BSA mg/L	DPPH, mmol Trolox/L	ABTS, mmol Trolox/L
	Gallic acid, g/L	Ferulic acid, g/L			
S1	1.07±0.00^{c}	0.63±0.00^{c}	228.43±4.96^{def}	61.32±1.12^{cd}	71.01±0.28^{ghi}
S2	0.55±0.00^{a}	0.34±0.00^{a}	105.54±4.37^{a}	49.74±0.98^{b}	60.27±0.55^{d}
S3	1.26±0.01^{d}	0.74±0.01^{d}	185.55±5.36^{bc}	60.90±0.87^{c}	43.52±1.12^{b}
S4	0.90±0.01^{b}	0.53±0.01^{b}	122.81±2.18^{a}	60.49±0.87^{c}	42.33±1.68^{b}
S5	1.07±0.02^{c}	0.63±0.01^{c}	187.33±3.18^{bc}	66.73±0.14^{ef}	50.23±0.00^{c}
S6	0.81±0.00^{b}	0.48±0.00^{b}	179.00±6.76^{b}	51.42±0.28^{b}	77.84±1.93^{ijk}
S7	0.90±0.02^{b}	0.53±0.01^{b}	166.88±5.76^{b}	43.83±0.43^{a}	32.06±1.12^{a}
S8	0.84±0.00^{b}	0.50±0.00^{b}	172.05±4.57^{b}	49.34±0.42^{b}	77.84±2.21^{jk}
S9	1.02±0.01^{c}	0.60±0.01^{c}	222.67±3.10^{de}	64.78±1.82^{de}	70.22±1.93^{gh}
S10	1.23±0.01^{d}	0.72±0.01^{d}	212.35±3.97^{cd}	75.48±0.42^{hi}	79.21±1.38^{jk}
S11	1.49±0.01^{ef}	0.87±0.01^{ef}	262.38±5.95^{hij}	80.42±1.01^{jk}	67.62±0.56^{fgh}
S12	1.98±0.02^{h}	1.15±0.02^{h}	282.66±0.76^{ij}	78.17±0.14^{ij}	65.64±0.56^{def}
S13	1.48±0.01^{e}	0.87±0.01^{e}	253.05±3.38$^{f\text{-}i}$	82.06±0.43^{kl}	63.27±1.68^{de}
S14	1.25±0.03^{d}	0.73±0.02^{d}	237.76±3.57$^{d\text{-}g}$	77.85±0.98^{ij}	97.95±0.83^{lm}
S15	1.25±0.02^{d}	0.73±0.01^{d}	332.66±5.56^{l}	85.33±0.72^{l}	73.74±2.51^{hij}
S16	1.92±0.02^{h}	1.12±0.02^{h}	290.57±2.31^{jk}	105.97±0.14^{n}	93.10±0.28^{l}
S17	1.94±0.01^{h}	1.13±0.01^{h}	333.46±0.79^{l}	94.83±0.87^{m}	80.26±1.68^{k}
S18	1.66±0.00^{g}	0.96±0.00^{g}	255.43±3.38^{ghi}	72.45±0.43^{gh}	59.32±0.56^{d}
S19	1.59±0.01^{fg}	0.93±0.01^{fg}	318.57±3.38^{kl}	70.20±0.14^{fg}	59.71±1.12^{d}
S20	1.47±0.04^{e}	0.86±0.03^{e}	278.86±0.19^{ij}	73.20±0.28^{gh}	98.73±1.38^{lm}
S21	1.27±0.02^{d}	0.74±0.01^{d}	212.35±3.97^{hij}	71.02±1.40^{g}	103.03±0.83^{m}

Data with the same superscript within a column are similar according to Tukey's honestly significant difference (HSD) method, with a confidence level of 99%

Beer proteins are important for mouthfeel perception, as they form colloids which contribute to the "body" of beer, making it more "mellow", while low colloid content was associated with "body loss" (Langstaff and Lewis, 1993). Moreover, soluble proteins have a positive role in the formation and stabilization of the head foam (Blasco *et al*., 2011; Šibalić, *et al*., 2021). Barley is the most important source of beer proteins (Langstaff and Lewis, 1993; Gupta *et al*., 2010). In the present study, the correlation coefficient between soluble proteins and the original extract was 0.6 (data not shown). In most cases, proteins are declared zero on beer labels. The obtained results showed that indeed quantities are very low, as they varied from 0.17 to 0.33 g/L. However, other studies reported higher quantities. Gorinstein *et al*. (1999) reported protein concentrations ranging between 5.45 and 6.37 g/L, while Blasco *et al*. (2011) stated that beer contains approximately 500 mg protein/ml, mostly in the form of polypeptides with molecular masses of 5–100 kDa. The method used for soluble protein determination implies reaction with Folin - Ciocalteau reagent, which is common to the total phenolic content assay. This explains the high linear correlation between obtained values with R^2=0.8281 (data not shown).

Antioxidant activity of beers

The presence of antioxidant compounds in beers is important from two perspectives. First, there is the effect of preserving sensorial characteristics during storage, which might otherwise be affected by lipid oxidation (Klimczak *et al*., 2024). Then, there are the health benefits provided by beer antioxidants, together with other compounds like vitamins and minerals (Sohrabvandi *et al*., 2012; Martinez-Gomez *et al*., 2020). The main sources of antioxidant compounds present in beers are malts, responsible for 70 to 80% of the total phenolics from beer, and hops, contributing by 20 to 30 % (Martinez-Gomez *et al*., 2020). However, a lot of other factors are to be taken into account, such as barley variety, the malting processing parameters, temperature and pH during mashing, sparging and boiling, the variety of hops used and yeast fermentation, that influence the final content of these compounds in the product (Martinez-Gomez *et al*., 2020). In the case of ale and dark beer types, malt is the main source of functional compounds, with up to 90 % contribution, with a minimal effect of hop (Leitao *et al*., 2012).

In the present study the highest values of total polyphenol content were obtained for S12 - an IPA type, and black beers S16, S17 (Table 4). The total phenolic content was expressed as gallic acid equivalents (GAE) and ferulic acid equivalents (FAE), as both phenolic acids were reported among the most important phenolic constituents in beer, accounting for more than 50% of the total phenolic compounds (Zhao *et al*., 2010). The lowest registered total phenolic content was 0.55 g GAE/L or 0.34 g FAE/L for Holsten Pilsner, statistically different from all other samples ($p<0.01$). The highest values, ranging from 1.92 to 1.98 g GAE/L and 1.12 to 1.15 g FAE/L, forming a homogenous group ($p>0.01$), were registered for S12 (IPA), S16 (Ursus Black) and S17 (Silva Black) samples. Silva *et al*. (2022) reported similar values, with 0.48 g GAE/L for Lager and 2.17 g GAE/L for Imperial Stout. Wheat-type beers (S6, S7 and S8) formed another homogenous group ($p>0.01$) and registered rather high total phenolic content (0.81-0.90 g GAE/L). However, wheat beers recorded the lowest DPPH-RSA values (Table 4). The highest antioxidant activity (DPPH-RSA) was registered for Ursus Black (S16) with 106 mmol Trolox/L, significantly higher than all other studied beers ($p<0.01$).

In the present study, Turkey’s HSD test was used to discriminate among the means, distinguished eight homogeneous groups. However, it can be seen in Table 4 that, with the exception of the S3 sample, letters from “a” to “c” were attributed to blond beers, while letters from “d” to “h” were attributed to darker beers. The correlation between the total phenolic content and the original extract reported on the labels (18 samples) was checked, and a rather high linear correlation was observed with R^2=0.761, as shown in Figure 3. Among the methods applied for antioxidant capacity determination, only the total polyphenol content showed a rather good correlation with the original extract, while data obtained for DPPH and ABTS radical scavenging capacities did not show any linear tendency with regard to the original extract (R^2=0.012 for ABTS *vs*. OE and R^2=0.2501 for DPPH *vs*. OE). In addition, the total phenolic content showed no significant correlation with beer color (R^2=0.4428 when computing all 21 samples).

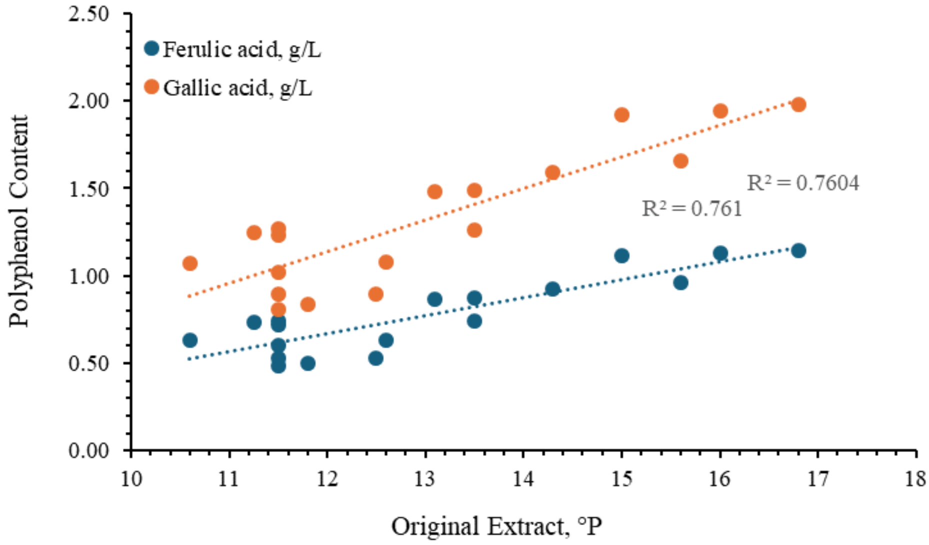

Figure 3. Correlations between polyphenol content and the original extract of the studied beers

Conclusions

The present study aimed to determine the physicochemical properties and antioxidant activity of some commercial beers from the Romanian market. A large variety of craft and commercial beer samples from different brands and different countries of origin were subjected to analysis. Overall, the results obtained in this study indicated good correlations between the original extract of the samples with other important characteristics of beers, such as energy value, alcohol content, viscosity, and total phenolic content. On the other hand, no correlation was found between antioxidant activity and beer color.

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