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ORIGINAL RESEARCH PAPER

QUALITY PARAMETERS OF FRUIT BEERS AVAILABLE ON ROMANIAN MARKET

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Abstract: Fruit flavored beers, also known as Radler, which are mixtures of beer and soft drinks, gained in the last years increasing popularity**.** The present study aimed at investigating the physical-chemical and sensory characteristics of different fruit beers commonly available on the Romanian market. The antioxidant properties of the fruit beers were investigated in terms of total phenolic contents and scavenging capacity against DPPH and ABTS radicals, such as to have an estimate of the degree of protection against oxidative damage associated to reactive oxygen species. All investigated commercial beers showed good antioxidant properties. Regardless of the flavor, rather large variations of the phenolic content and antioxidant activity were identified among beer samples. The results of the sensory analysis coupled with the physical-chemical and rheological properties indicated the good quality of the fruit beers available on Romanian market.

Keywords: *antioxidant properties, color, fruit beer, phenolic compounds, sensory analysis*

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INTRODUCTION

Beer is a popular low alcoholic drink which provides significant health promoting benefits when consumed with moderation [1, 2]. There are mainly four raw materials conventionally used for beer production: malted barley, hops, water and yeast. Compared to other alcoholic beverages, beer provides better nutritional and health benefits [3, 4]. It has important amounts of minerals and essential nutrients such as potassium, magnesium, calcium, phosphorus and a small amount of sodium [4, 5]. Beer is also a source of B complex vitamins (B_1, B_2, PP and B_{12}), originating from malt and yeast. Moreover, it contains higher amounts of proteins than wine, as well as important amounts of other biologically active compounds with antioxidant activity, such as phenolic compounds, Maillard reaction products, and sulfite, which were found responsible for flavor stability [4, 6]. These antioxidant compounds originate from malt, hops and eventually other cereals which are used as ingredients for beer production.
In the last years, manufacturers have expanded their product portfolios by adding a broad selection of fruit flavored beers. The so called Radler beer is a low-alcohol drink, made by mixing classic beer with juice, lemonade or aroma. This type of drink gained popularity mostly because of the rich fruity flavor and refreshing properties [7]. The proportion of ingredients in Radler beer varies, but common commercially available products often have rather low beer content. Because of the low alcohol contents, fruit beers can be consumed in larger amounts, being attractive especially for female consumers. As reported by Bamforth [8], consumers evaluate beer quality based on the following criteria: color, alcohol content, haze, foam (retention, cling), flavor profile and stability, together with CO_2 content. Among these characteristics, beer foaming properties appear to be the most important attribute. The existence of a stable head of foam impacts the way of perceiving the organoleptic properties of beers [9]. Particular attention should be given also to haze formation [10, 11] and foam stability [9], which are major quality characteristics directly related to the content and molecular weight of proteins present in beers. Several studies reported the antioxidant properties of different beers [4, 6, 12 – 14]. Many analytical methods have been employed in order to study the antioxidant properties of beers [4, 15]. The researches focused mainly on the relationship between antioxidant activity and the total phenolic content or the phenolic profiles of different types of beers [14], on hop [16, 17] or on malts [18]. It is difficult to keep a strict evidence of the quality characteristics of the fruit beers and, to the best of our knowledge, there are no reports available in the literature on the commercial brands. The objective of this study was to investigate and compare the most important quality characteristics of different fruit beers available on the Romanian market. The commercial beers were examined in terms of physical-chemical, rheological and sensory properties.

MATERIALS AND METHODS

Materials

Thirteen different fruit beers purchased from the local supermarkets (Galati, Romania) were considered for the present study (Table 1). The beer samples were divided in four groups based on the flavor or juice type used in the recipe: eight lemon juice beers (Ursus Cooler, Ciuc Radler, Beck's Lemon, Redd's Lemon, Lomza Lemonowe, Holsten Radler, Oettinger Radler and Bergenbier Lemon), two grapefruit juice beers (Bergenbier Grapefruit and Schofferhoffer Grapefruit), two beers with raspberries juice (St. Louis framboise and Belgian framboises), and one with cranberry juice (Redd's Cranberry). Some of the information labeled on the studied beer samples are listed in Table 1.

Except for CO_2 determination, prior to physical-chemical and rheological characterization the beer samples were degassed, sealed in plastic containers and kept in refrigeration conditions until analysis were performed.

Physical-chemical analyses

The content of sugars and density of studied beer samples were measured using the automatic FERMENTOSTAR analyzer (Funke Gerber, Germany).

The total acidity was determined through the titration of 25 mL of degassed beer with 0.1 N NaOH solution, in the presence of phenolphthalein as indicator. The total acidity of beer was calculated as follows:

$$Total\ acidity\ (mL/100\ mL) = 0.1 \cdot V \cdot 100/25 \qquad (1)$$

where: V is the volume of 0.1 N NaOH solution required for titration of 25 mL of beer.

Beer *p*H values were measured directly on the degassed filtered beer samples, using the 702SM Titrio *p*H-meter (Metrohm, Herisau, Switzerland) [19].

The content of CO_2 in the beer was determined through the SR 13355–8:2003 method [20]. The CO_2 from a volume of 25 mL beer sample was first absorbed in 50 mL of 0.2 N Na_2CO_3 solution, and further excess of alkalinity was titrated with 0.1 N HCl in the presence of phenolphthalein. An independent decarbonized beer sample was used for determining the existent acids by titration with 0.2 N Na_2CO_3. The CO_2 content was calculated as follows:

$$CO_2\ (g/100\ mL) = [(50 - V_1) - V_2] \times 4 \times 0.0044 \qquad (2)$$

where: V_1 is the volume of 0.1 N HCl used in the first titration in mL, V_2 is the volume of 0.2 N Na_2CO_3 used in the second titration in mL, and 0.0044 is the amount of CO_2 (in g) corresponding to 1 mL of 0.2 N Na_2CO_3.

The EBC color of the beer samples was determined by means of a spectrophotometric method, established by the European Brewing Convention, as described by Buckee [21]. The method is based on measuring the absorbance of the filtered and decarbonized beer samples at wavelengths of 430 nm (A_{430}) and 700 nm (A_{700}) against distilled water as control. The EBC color values are calculated by means of Equation 3, results being expressed as EBC units.

$$Color\ (EBC) = 25.5 \times (A_{430} - A_{700}) \qquad (3)$$

The CIELAB color parameters (L*, a* and b*) were measured using a CR300 Chroma Meter, (Konica Minolta) equipped with a D_{65} Illuminant. In order to be analyzed, all samples were placed in aluminum casings and the L*, a*, b* values were registered. The empty vat was used as blank for measuring the L*, a*, b* parameters. The real color characteristics of the beer samples were determined by subtracting the blank sample values from those of the beer samples.

Table 1. *Characteristics of the analyzed fruit beers*

Sample cod	Beer brand	Country of origin	Original extract [°P]	Alcohol [%, v/v]	Flavor	Juice	Artificial sweetener	Antioxidant	Acidifier	Other additives
B1	Holsten Radler	Germany	10.9	2.2	With	With	Without	Without	Without	With
B2	Ciuc Radler	Romania	9.3	1.9	With	With	Without	With	Without	With
B3	Oettinger	Germany	NS	2.5	With	Without	Without	Without	With	With
B4	Beck's Lemon	Romania	9.6	2.5	With	Without	Without	Without	With	With
B5	Ursus Cooler	Romania	10.1	1.9	With	With	Without	With	Without	With
B6	Lomza Lemonowe	Poland	NS	2.0	With	With	Without	Without	Without	With
B7	Redd's Lemon	Romania	11.9	4.0	With	With	With	Without	With	With
B8	Bergenbier Lemon	Romania	8.1	1.9	With	With	With	With	Without	With
B9	Schofferhoffer Grapefruit	Germany	NS	2.5	With	With	Without	With	With	With
B10	Bergenbier Grapefruit	Romania	8.1	1.9	With	With	With	With	With	With
B11	St. Louis framboise	Belgium	NS	2.8	With	With	With	Without	Without	Without
B12	Belgian framboises	Belgium	NS	3.5	Without	With	With	With	Without	Without
B13	Redd's Cranberry	Romania	11.7	4.0	With	With	With	Without	With	With

Determination of total phenolic content and antioxidant capacity

Total phenolic content. Beer samples were first diluted with acidified methanol (HCl : methanol : water = 1 : 80 : 20). A volume of 0.2 mL diluted sample was further allowed to react with 1.5 mL of Folin-Ciocalteu reagent (10 × dilution), while resting in the dark for 5 minutes. Afterwards, 1.5 mL of Na_2CO_3 (60 $g \cdot L^{-1}$) were added, and the samples were incubated for additional 90 minutes. Finally, the absorbance was measured at wavelength of 725 nm using a T80+ Spectrometer (PG Instruments Ltd). The concentration of phenolic compounds from beer samples was calculated using a calibration curve prepared with ferulic acid as the standard.
The 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (*DPPH-RSA*) of each sample was determined using the T80+ Spectrometer (PG Instruments Ltd) according to the method described by Zhao *et al.* [14]. An aliquot of 0.1 mL diluted beer sample (10µL beer and 90 µL methanol 80 %) was added to 3.9 mL DPPH solution (6×10^{-5} $mol \cdot L^{-1}$). The samples were allowed for 30 minutes under dark conditions and the absorbance was then recorded at wavelength of 515 nm. The antioxidant activity was calculated as:

$$\%\ DPPH\text{-}RSA = (1 - A_{30}/A_0) \times 100 \qquad (4)$$

where: A_{30} is the absorbance measured at λ of 515 nm after 30 minutes, and A_0 is the initial absorbance of a blank sample obtained by mixing the DPPH solution with 0.1 mL of methanol 80 %.
Trolox Equivalents Antioxidant Capacity (*TEAC)*. A volume of 40 µL of beer sample appropriately diluted with aqueous methanol (80 %) was added to 2.96 mL 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet +}$). The absorbance of the obtained solution was measured twice: immediately and after 6 minutes at 734 nm. A solution of 80 % methanol was used as blank. TEAC value was reported as µmol $Trolox \cdot L^{-1}$.

Rheological measurements

Rheological measurements on beer samples were performed by means of Rheotest-2 (VEB-MEDINGEN-R.D.G), which allowed measuring the shear stress (τ, [$N \cdot m^{-2}$]) while varying the shear rate ($\overset{o}{\gamma}$, [s^{-1}]). The apparent viscosity (η, [Pa·s]) was further determined as $\tau/\overset{o}{\gamma}$.

Sensory analysis

The sensory analysis of fruit beer samples was assessed by a group of eight trained panelists (18-20 year old). The evaluated attributes were general appearance, color, odor, flavor, CO_2 persistency, foam appearance and persistency. A five point scoring scale was considered as follows: 0 meaning unsatisfactory to 5 meaning highly appreciated. The resulted mean score for every attribute was multiplied by its importance coefficient thus obtaining the real score [22] based on the relevance to the global quality of the beer. The importance coefficients of the sensory attributes were: 0.6 for general appearance, 0.8 for color, 0.2 for odor, 1.4 for flavor, 0.6 for CO_2

persistency and 0.4 for foam appearance and persistency. The overall sensorial score was established for every fruit beer sample as the sum of all attributes` real scores.

Statistical analysis

Statistical analysis of the experimental results was performed using Microsoft Office Excel® predefined tools. The experimental measurements were performed in duplicate and the results were reported as mean values. Standard deviations were generally lower than 5 %. The results of sensory analysis were statistically processed using ANOVA single factor which allowed the analysis of variance, respectively analysis of variance of a variable in relation with the influencing factor.

RESULTS AND DISCUSSION

Physical-chemical characterization of fruit beers

Thirteen commercial fruit beers were considered in the study and some information available on the labels are presented in Table 1. No details on the brewing technological parameters were available. The original extract of beers was quite low, while the alcohol contents ranged from 1.9 to 4.0 % (v/v). Only four beer samples had the original extract over 10 °P, while for other five samples the values were not specified on the label (Table 1). Although fruit beers should contain low alcohol levels, some of the samples considered in the study had rather high contents of alcohol. Therefore, consumers that normally choose this kind of drink looking for low alcohol volumes, should consider carefully reading the label information first. Important health benefits were related to the moderate consumption of alcohol, namely lower incidence of coronary heart disease and developing neurodegenerative disease [3, 23].

In order to estimate the quality of the fruit beer samples, physical-chemical properties and sensory attributes were determined. The chemical composition and properties of beer are directly related to quality of all ingredients and brewing parameters. When referring to sugar contents, no significant differences were found among samples of the beer groups defined based on the fruit flavor (lemon, grapefruit, raspberry and cranberry) ($p>0.05$). The sugar content of the investigated fruit beer samples was rather high, ranging from 6.33 to 9.19 % (Table 2). Most of the sugar content came from the fruit juice. Based on the ingredients list declared on the label, all investigated samples, except for B3, included sucrose, fructose or honey used for the preparation of fruit juice added to the beer, eventually in addition to one or more sugar substitutes such as aspartame, acesulfame K, cyclamate and saccharin.

No significant variation of the titratable acidity was found between samples with lemon or grapefruit flavor, when the acidity values ranged between 2.40 and 4.64 mL 0.1 N NaOH/100 mL (Table 2). On the other hand, the raspberry flavored beers presented significant differences in terms of acidity ($p<0.05$). When compared to the blond beers, the high acidity values, recorded especially in case of the Framboise beers, can be explained by the high acidity of the juice or the use of acidifiers for preparing the fruit flavored beers considered in the study. Accordingly, *p*H values ranging from 2.84 to 3.55 were recorded for all investigated fruit beers, lower compared to the *p*H of 4.3 - 4.6

normally reported for beer [24]. As in case of the total acidity, no significant differences were found between lemon and grapefruit flavored beers samples in terms of *p*H values (p>0.05).

Table 2. *Physical-chemical characteristics of the commercial fruit beers*

Sample	Density [g·mL^{-1}]	Sugars [%]	CO_2 [g/100 mL]	Total acidity [mL 0.1N NaOH/100 mL]	*p*H	Viscosity [Pa·s]
B1	1.03	8.91	0.52	4.24	3.03	1.79
B2	1.02	8.96	0.52	3.36	3.34	2.33
B3	1.02	7.91	0.55	2.40	3.55	1.97
B4	1.01	7.98	0.45	3.04	3.28	1.79
B5	1.03	9.19	0.5	3.60	3.19	1.97
B6	1.03	9.08	0.48	4.00	3.09	1.97
B7	1.02	6.26	0.55	4.64	2.85	2.24
B8	1.01	7.94	0.52	4.32	3.04	1.79
B9	1.02	7.80	0.52	4.00	3.27	1.79
B10	1.01	7.53	0.52	4.40	3.49	1.79
B11	1.03	6.70	0.55	11.40	2.84	2.15
B12	1.02	6.38	0.65	4.24	3.50	2.15
B13	1.02	6.33	0.55	3.76	2.91	1.79

The level of CO_2 dissolved in beer is an important quality parameter, influencing to high extent how the tangy taste of the products is perceived. Regardless of the fruit flavor, the CO_2 content of the samples fell within the range of 0.45-0.60 % indicated as normal for the bottom fermentation beers by Kunze [24].
The viscosity of the beers is an important factor that needs to be considered when studying flavor perception. Hollowood *et al.* [25] stated that flavor perception decreases as viscosity of a solution increases, due to the physical effect on the movement of flavor molecules. Both parameters presented rather homogenous values, with no significant differences between beer groups (p>0.05).
In order to estimate the color characteristics of beers samples, the EBC and CIELAB color values were determined. Analyzing the results presented in Table 3 one can see that the EBC values of beer samples were correlated with the fruit flavor (p<0.05). Lower EBC values ranging from 4.99 to 9.05 were obtained in case of the lemon based beer samples, except for B8 sample which, according to the information provided by the producer, included orange juice and concentrated orange peel extract in addition to the lemon. Significantly higher EBC values were found for grapefruit and raspberry flavored beers (Table 3). As shown by Smedley [26], the EBC method fails to explicitly show the differences in terms of color between beer samples. Different beer samples having similar EBC color values exhibited different visible spectra. In agreement with the EBC color, the L* a* b* color analysis indicated significant differences between beer groups defined based on the fruit flavor (Table 3). L* gives indications about the lightness of the samples, whereas the chromatic components a* and b* are measures of the amounts of green-to-red and blue-to-yellow in the color. The lightness values were higher for lemon beers (L* ranging from 34.9 to 44.1), while raspberry beers were

significantly darker (L* of 2.7 and 10.0 for B11 and B12, respectively). An inverse correlation was found between redness values and lightness of the fruit flavored beer samples. As expected, the highest redness values were measured for raspberry beers and the lowest a* values for the lemon beer type. Finally, the highest yellowness values were found for grapefruit beers (Table 3).

Table 3. *Color characteristics of investigated fruit beer samples*

Sample	EBC color	CIELAB color		
		L*	a*	b*
B1	9.05	40.4±0.02	1.6±0.06	28.5±0.2
B2	4.99	40.8±0.03	1.6±0.09	27.5±0.05
B3	5.55	41.1±0.3	2.5±0.07	31.4±0.23
B4	8.18	39.4±0.04	1.5±0.1	36.8±0.25
B5	6.83	44.1±0.34	-0.5±0.02	23.7±0.29
B6	6.75	41.5±0.09	1.1±0.07	21.6±0.11
B7	5.55	36.9±0.04	5.0±0.23	30.2±0.14
B8	17.36	34.9±0.16	6.0±0.04	35.2±0.29
B9	16.98	30.6±0.03	13.8±0.05	40.5±1.5
B10	17.00	31.5±0.02	17.1±0.09	40.0±0.34
B11	41.18	2.7±0.4	11.9±0.57	4.4±0.6
B12	21.16	10.0±0.06	20.8±0.21	15.7±0.29
B13	5.55	28.3±0.03	19.3±0.13	30.3±0.05

Total phenolic content and antioxidant capacity of fruit beers

Oxidative reactions occurring in different stages of brewing are mainly responsible for flavor instability, being therefore important for determining the shelf-life of packaged beer. The shortcoming caused by oxygen presence may be counteracted by the high endogenous antioxidant activity of beer, due to the presence of phenolic compounds, Maillard reaction products and sulfites [14, 27]. Several naturally occurring antioxidant compounds in beer, which play a key role in delaying or preventing the oxidation processes, arise from malt, cereals and hops [4, 27]. Given the importance of phenolic compounds in defining stability and sensory quality of beer [2], several studies were dedicated to establishing their profile [14, 27]. Beer contains the following phenolic compounds with antioxidant properties: phenolic acids such as benzoic and cinnamic acids derivatives, coumarins, cathechins, proanthocyanidins, tannins and amino phenolic compounds [14, 27]. The total phenolic contents of the investigated beer samples are presented in Figure 1. Phenols presence in studied fruit beers was expressed as ferulic acid, as it represents one of the most important phenolic constituents in beer together with gallic acid [14], originates from barley [12]. The total phenolic content of the beer samples varied within large limits, from 22.16 to 220.43 mg ferulic acid/L. Analyzing the results presented in Figure 1, one can see that raspberry flavored beers presented the highest total phenolic content, while the cranberry flavored beer sample had the lowest quantity, values being significantly different between beer groups ($p<0.05$). Except for B11 sample, all investigated fruit flavored beers exhibited considerably lower total phenolic contents with respect to the lager commercial beers characterized by Zhao *et al.* [14], who reported values in the range of 152.01-339.12 mg gallic acid equivalents·L^{-1}. These differences are most probably due to the dilution of the phenolic compounds of beers, through the addition of different fruit juices or syrups with poor or no phenolic load.

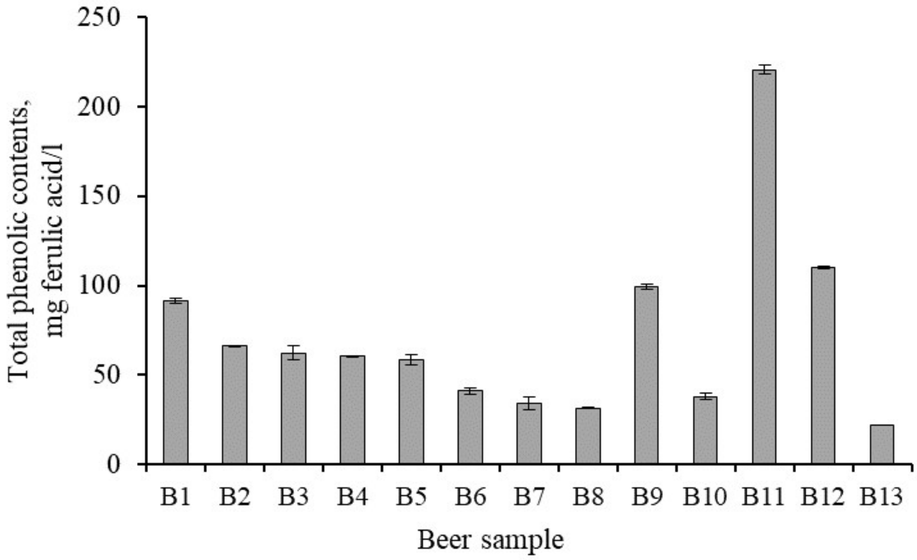

Figure 1. *Total phenolic contents [mg ferulic acid·L^{-1}] of studied fruit flavored commercial beers*

The relationship between phenolic content and antioxidant activity of beer was previously studied [14]. The antioxidant activity of the fruit flavored beer samples was determined by assessing the DPPH-RSA and TEAC (Figure 2 and Figure 3) which are based on the electron transfer of a reduction reaction.

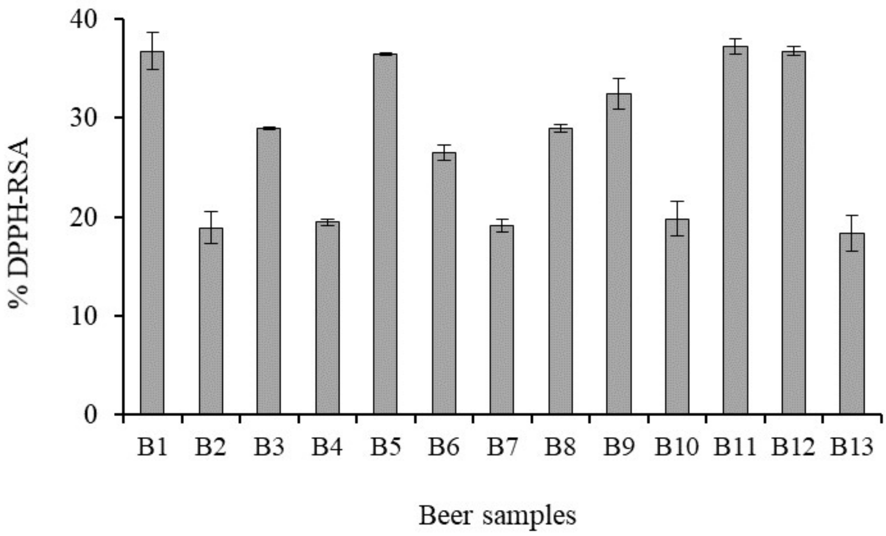

Figure 2. *Antioxidant capacity of fruit flavored beer samples assessed through DPPH-RSA method*

The antioxidant activity varied significantly within the samples and methods used for quantification. Our observation is in agreement with Tafulo *et al.* [4], who compared six different methods for assaying the antioxidant capacity of beers (DPPH, TEAC, total radical trapping antioxidant parameter (TRAP), ferric-ion reducing antioxidant parameter (FRAP), oxygen radical absorbance capacity (ORAC) and cupric reducing antioxidant capacity (CUPRAC)) using three types of standards (ascorbic acid, gallic acid and trolox), and reported significant differences among the results obtained with different methods. So, different antioxidant capacity evaluation methods based on various reaction mechanisms might give different evaluation results [28, 29]. It is therefore difficult to compare the results with the literature data. Moreover, Tafulo *et al.* [4] indicated that flavorings, sweeteners, juice, antioxidants and other additive addition resulted in slightly higher antioxidant activity of beers.

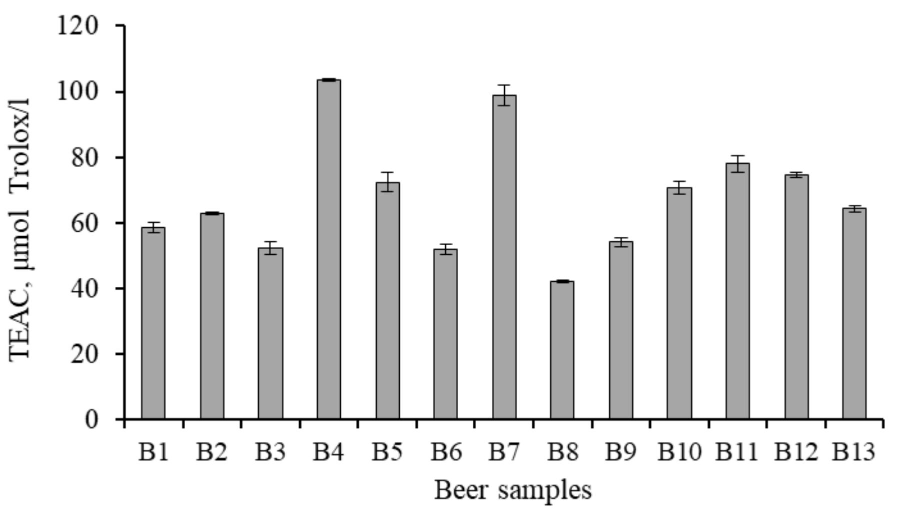

Figure 3. *Antioxidant capacity of fruit flavored beer samples assessed through TEAC method*

Sensory analysis of fruit beers

Sensory analysis revealed young Romanian consumers' perception of fruit flavored beers. The beer attributes considered in the study are general appearance, color, odor, flavor, CO_2 persistency and foam appearance and persistency, and the results are shown in Table 4.

Obtained mean values for the analyzed attributes were similar for all beer samples, with no significant differences between beer groups ($p>0.05$). No significant differences were observed for the overall score ($p>0.05$). Nevertheless, when referring to the lemon beers alone, significant differences in terms of the obtained overall score were observed ($p<0.05$). Thus Ciuc Radler gathered the lowest score with only 14.72 points, while Beck`s lemon obtained the highest acceptance score with 19.47 points.

Table 4. *Sensory evaluation of studied fruit beers*

Sample	General appearance	Color	Smell	Flavor	CO_2 persistency	Foam appearance and persistency	Overall score
B1	2.55	4.00	0.95	6.47	2.77	1.45	17.03
B2	2.02	3.10	0.85	5.25	2.25	1.25	14.72
B3	3.00	4.00	1.00	6.82	2.85	1.80	18.48
B4	2.10	3.00	0.97	5.95	2.55	1.50	19.47
B5	2.93	3.90	0.77	6.30	2.77	1.80	18.20
B6	2.93	3.20	0.97	6.47	2.32	1.70	17.43
B7	2.25	3.50	0.90	6.30	2.62	1.85	18.48
B8	2.47	3.60	0.85	5.42	2.77	1.90	16.83
B9	2.25	3.20	0.77	4.37	2.32	1.80	14.73
B10	2.77	3.80	0.80	4.72	2.55	1.60	16.08
B11	3.00	4.00	0.95	5.77	2.85	1.90	16.25
B12	2.25	3.30	0.90	5.95	2.62	1.80	16.58
B13	2.10	3.50	0.90	5.60	2.62	1.85	17.6

CONCLUSIONS

Thirteen fruit flavored beer samples available on Romanian market, from different brands and origins, were considered in the study. Important quality characteristics were assessed and the results indicated that the physical-chemical properties of the flavored beers comply with those specific to the drink category which they belong. The beer samples exhibited appreciable total phenolic contents and good free radical scavenging activity. Based on the obtained results, and also considering the general low alcohol content, we can conclude that the fruit flavored beers could be considered valuable products, whose quality does not lose ground when compared to regular beer.

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