

DIFFERENCES IN PHYSICOCHEMICAL, MINERAL AND NUTRACEUTICAL PROPERTIES BETWEEN REGULAR, LIGHT AND ZERO BEERS

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Abstract

The differences in physicochemical, mineral and nutraceutical properties between regular, light and zero beers were evaluated. The pH, titratable acidity and colour values of all beers were in the range quality criteria of beer industry. On the other hand, zero beer showed the highest content of Ca, Mg, K and Na, light beer obtained the highest content in Cu, Zn and Fe, while Mn was detected only in regular beer. The highest content of total phenols was found in regular beer, followed by zero and light beers, while in total flavonoids, zero beer obtained the highest content, followed by regular and light beers. Considering the in ABTS, DPPH, FRAP and ORAC antioxidant capacity the results were: regular beer > zero beer > light beer.

Rezumat

Au fost evaluate proprietățile fizico-chimice precum și conținutul în minerale și nutriceutice a berilor normale, ușoare și fără alcool. Berile analizate s-au încadrat în normele de calitate din industria berii privind valorile de pH, aciditate și de culoare. Pe de altă parte, berea fără alcool a prezentat cel mai mare conținut de Ca, Mg, K și Na, berea ușoară a prezentat cel mai mare conținut de Cu, Zn și Fe, în timp ce Mn a fost detectat doar în berea normală. Cel mai mare conținut de fenoli totali a fost găsit în berea normală, urmată de berea fără alcool și de cea ușoară. Conținutul total de flavonoide a fost maxim pentru berea fără alcool, urmat de cea normală și ușoară. Din punct de vedere al capacității antioxidante, evaluată prin metodele ABTS, DPPH, FRAP și ORAC, rezultatele au fost următoarele: bere normală > bere fără alcool > bere ușoară.

Keywords: physicochemical parameters, mineral composition, nutraceutical properties, regular light and zero beers

Introduction

Beer, a complex beverage made from barley (malt), hop, water and yeast is one of the most consumed alcoholic beverages. Is rich in nutrients as well as non-nutrient compounds including carbohydrates, amino acids, minerals, vitamins and phenolics [7]. Beer is available in a wide range of styles and flavours, and when is consumed in sensible amounts it can contribute to a healthy diet [10]. Quality analysis of beer in the brewery laboratory includes numerous determinations such as alcohol content, pH, titratable acidity and colour, among others [19]. Also, phenolic compounds contribute directly to some quality characteristics of beer, such as the colour, flavour, and astringency, and they also protect raw materials from oxidative degradation throughout the brewing process [5].

Substantial numbers of alcohol-related cancers as breast, colorectum, head and neck, oesophagus and liver cancers are associated with consumption of more than 24 g *per* day for men (more than two drinks *per* day) and more than 12 g *per* day for women (more than one drink *per* day) [3]. On the other hand, epidemiological studies suggested that regular and moderate consumption of beer confers cardiovascular protection. Part of this protective effect of beer is due to their alcoholic content (ethanol) and another part to their non-alcoholic components as phenolics [6, 10]. In addition, due to health, diet and safety reasons an increasing demand for low-alcohol and alcohol-free beers has been observed in the last years [23]. Because of the diverse options of different alcohol content in beers for consumers in the market, it is necessary to evaluate their differences in physico-

chemical, nutritional and nutraceutical attributes. For this reason, the aim of this work was to evaluate and compare the physicochemical (alcohol content, pH, titratable acidity, colour) mineral (K, Na, Ca, Mg, Fe, Zn, Cu, Mn) and nutraceutical (total phenols, total flavonoids, ABTS, DPPH, FRAP, ORAC) properties between regular, light and zero beers.

Materials and Methods

Beer samples

Three samples of 355 mL of every type of beer (regular, light and zero) from different lot number were purchased from a local supermarket in the second week of January 2017 and stored at 5°C. The three types of beer were from the same brand, the alcohol content reported in label was 4.5% for regular beer, 3.9% for light beer and 0.0% for zero beer and all samples were produced during 2016. According to labels, regular and light beers were produced using the basic raw materials malt, hop, water and yeast, while zero beer indicated the use of water, barley malt, corn, hop, natural beer flavour, natural malt flavour and potassium citrate.

Physicochemical parameters

Samples were degassed by stirring 30 mL at 100 rpm for 20 min at room temperature and then were filtered using Whatman1 filter paper. The pH was read and after that, samples were titrated with 0.1 N NaOH to a pH 8.2 using an Mettler-Toledo automatic titration equipment T50 titrator (Greifensee, Switzerland) according to Association of Official Analytical Chemist methods [2]. Colour was determined according to Popescu *et al* [19] by reading absorbance of the sample at 430 nm (Ab_{430}) in a Jenway 6705 UV/Vis spectrophotometer (Stone, Staffordshire, UK) using 1 cm cuvettes and the colour of the samples were calculated in EBC (European Brewery Convention) units (colour = $Ab_{430} \times 24$) and ASBC (American Society of Brewing Chemists) units (colour = $Ab_{430} \times 12.7$).

Mineral composition

Mineral analysis was performed based on Association of Official Analytical Chemist methods [2] using an Agilent atomic absorption 240FS spectrometer (Santa Clara, CA, United States). Briefly, 5 mL of 3 M HCl were added to 50 mL of sample and digested-evaporated at boiling temperature until 20 mL of sample were obtained. Afterward, samples were filtered and used for mineral analysis. Potassium and sodium were detected by emission at wavelengths of 589.6 nm and 769.9 nm, respectively, while calcium, magnesium, iron, zinc, copper and manganese were determined by absorption at wavelengths of 422.7 nm, 285.2 nm, 248.3 nm, 213.9 nm, 324.7 nm and 279.5 nm, respectively. Results were expressed as milligrams *per* litre of sample (mg/L) based on calibration curves prepared with 0 mg/L to 100 mg/L for sodium and

potassium; 0 mg/L to 10 mg/L for calcium, iron, magnesium and manganese; 0 mg/L to 5 mg/L for zinc and copper, standards of each mineral.

Nutraceutical properties

Phenolics evaluations were performed according to Niño-Medina *et al* [15]. Total phenols were carried out based in the Folin-Ciocalteu reaction, gallic acid was used as the standard (0 mg/L to 200 mg/L) and results were expressed as milligrams of gallic acid equivalents *per* litre of sample (mg GAE/L). Total flavonoids were evaluated based on the aluminium chloride ($AlCl_3$) reaction, catechin was used as the standard (0 mg/L to 200 mg/L) and results were expressed as milligrams of catechin equivalents *per* litre of sample (mg CatE/L). Total non-flavonoids were obtained by subtraction between total phenols and total flavonoids.

Antioxidant capacity by DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) radicals were measured based on the reduction of the absorbance in the presence of sample according to Niño-Medina *et al* [15]. FRAP antioxidant capacity was measured based on the reaction of ferrous tripyridyltriazine complex in the presence of sample according to López-Conteras *et al* [12] and ORAC antioxidant capacity was measured based on the scavenging of peroxy radicals generated by AAPH (2,2-azo-bis-2-methylpropionamide dihydrochloride) by sample, preventing degradation of fluorescein according to Urias-Orona *et al* [26]. Trolox was used as the standard for the four antioxidant capacity assays (0 μ mol/L to 200 μ mol/L) and results were expressed as micromoles of Trolox equivalents *per* litre of sample (μ mol TE/L). All phenolic and antioxidant evaluations were performed using Jenway 6705 UV/Vis spectrophotometer (Stone, Staffordshire, United Kingdom).

Statistical analysis

All the results were expressed as mean values of three samples \pm standard deviation. Statistical differences among samples were evaluated by analysis of variance (ANOVA) followed by Tukey's test using Minitab 14.0. A level of probability of $p < 0.05$ was set as statistical significance.

Results and Discussion

Physicochemical parameters

The pH and titratable acidity are important quality attributes in alcoholic beverages. They are responsible for the taste, colour and also serve as preservative to extend the shelf life affecting the redox potential and microbial growth [25]. For most of the commercial beers pH values are from 4 to 5. Organic and carbonic acids are responsible for this acidity and they come from metabolic by-products of yeast cells or from malt and hop [4]. Also, the colour is a critical parameter

of beer quality and the main contributors to the beer colour are melanoidins and oxidized polyphenols. They came from raw materials and their chemical

reactions are mainly produced during malting and wort production [9].

Table I

Physicochemical parameters of regular, light and zero beers

Beer	Physicochemical Parameters					
	Alcohol (%) [*]	pH	Total Acidity (%)	Colour (EBC units)	Colour (ASBC units)	Colour View
Regular	4.50	4.05 ± 0.009 ^c	0.177 ± 0.002 ^a	6.29 ± 0.053 ^b	3.19 ± 0.027 ^b	
Light	3.90	4.17 ± 0.004 ^b	0.140 ± 0.001 ^c	5.78 ± 0.035 ^c	2.93 ± 0.018 ^c	
Zero	0.00	4.25 ± 0.001 ^a	0.153 ± 0.003 ^b	7.20 ± 0.071 ^a	3.66 ± 0.036 ^a	

^{*}Alcohol content reported in the label. Values with different letters within same column are significantly different ($p < 0.05$, $n = 3$)

Regarding to the pH and titratable acidity, there were significant difference between the three types of beer. The values of pH were from 4.05 (regular beer) to 4.25 (zero beer), while the titratable acidity ranged from 0.140% (light beer) to 0.177% (regular beer). In addition, the colour values ranged from 5.78 to 7.20 and from 2.93 to 3.66 in EBC and ASBC units, respectively, being light and zero beers the lowest and highest, respectively (Table I).

Our data are in the range of those reported by Pai *et al* [16] who found values from 3.83 to 4.49 in pH and from 0.0957% to 0.2252% in titratable acidity of 15 pale lager beers commercialized in India with an alcohol content from 4.45% to 8.91%. Popescu *et al* [19], produced a light lager beer at laboratory scale and obtained a product with 3.69% of alcohol, a pH of 4.23, a titratable acidity of 0.295% and colour in EBC units of 13.2 (6.7 ASBC units). The physicochemical parameters obtained by these authors are different from our results since their pH, titratable acidity and colour values were higher than the three types analysed in the present study.

On the other hand, Liguori *et al* [11] obtained an alcohol free beer (< 0.5% alcohol) from a regular lager beer (4.95% alcohol) by osmotic distillation and evaluated some physicochemical parameters in

both products. The pH of the original lager beer was 4.13 and the colour in EBC units was 7.6 (3.9 ASBC units) but these values changed to 4.18 in pH and 8.4 in EBC colour units (4.3 ASBC units) after production of alcohol free beer. The pH of the original and alcohol free beers reported by these authors are similar to the data found in our study, but the colour obtained by them is different compared to our results.

Mineral composition

The natural components used for brewing (water, malt, hop and yeast) are the main sources of minerals in beer. According to relatively high contents of some minerals in beer, its moderate consumption can be considered a valuable source of their recommended daily dietary intakes [18].

Regarding to the mineral elements, there were significant differences ($p < 0.05$) in the content of all the minerals quantified in beer samples. Zero beer showed the highest content of calcium (105.47 mg/L), magnesium (93.16 mg/L), potassium (488.16 mg/L), sodium (137.62 mg/L), while light beer contained the highest amount of copper (0.065 mg/L), zinc (0.038 mg/L) and iron (0.065 mg/L). On the other hand, manganese was found only in the regular beer sample (0.048 mg/L) (Table II).

Table II

Mineral content of regular, light and zero beers

Beer	Minor Elements (mg/L)				Major Elements (mg/L)			
	Cu	Mn	Zn	Fe	Ca	Mg	K	Na
Regular	0.044 ± 0.003 ^b	0.048 ± 0.008 ^a	0.035 ± 0.005 ^a	0.076 ± 0.003 ^c	99.49 ± 0.13 ^b	68.38 ± 0.04 ^b	252.09 ± 3.13 ^b	94.09 ± 0.34 ^b
Light	0.065 ± 0.002 ^a	ND	0.038 ± 0.002 ^a	0.112 ± 0.002 ^a	70.66 ± 0.29 ^c	48.35 ± 0.11 ^c	187.76 ± 0.74 ^c	104.79 ± 4.88 ^b
Zero	0.041 ± 0.003 ^b	ND	0.038 ± 0.010 ^a	0.093 ± 0.004 ^b	105.47 ± 0.15 ^a	93.16 ± 0.48 ^a	488.16 ± 9.85 ^a	137.62 ± 0.44 ^a

Values with different letters within same column are significantly different ($p < 0.05$, $n = 3$). ND = Not detected.

Rodrigo *et al* [20], analysed seven Mexican commercial lager beers finding average levels of calcium (50.4 mg/L) and sodium (53.1 mg/L) lower than our results. On the contrary, copper (0.677 mg/L), manganese (0.10 mg/L), iron (0.332 mg/L) and zinc (0.189 mg/L) were higher than found in our samples. In addition, the content of potassium (239 mg/L) and magnesium (57.3 mg/L) was similar to our regular and light beers, respectively.

Alcázar *et al* [1], analysed the content of minerals in commercial lager (10 brands) and low alcohol (18

brands) beers. They observed that the average content of calcium, magnesium and potassium were 13.63%, 33.80% and 21.69% higher in lager beers than in low alcohol beers, respectively. In the present study it was observed the same behaviour in the same elements between regular and light beers being the first one 28.98%, 29.30% and 25.52% higher than the latter one in calcium, magnesium and potassium, respectively. On the contrary to their results, we found that light beer obtained higher values than regular beer in copper, iron and sodium.

Nutraceutical properties

Phenolic compounds are interesting metabolites due to their abundance in the human diet, their antioxidant properties and their potential role in the prevention of several diseases associated with oxidative stress [13]. Several thousands of phenolic compounds have been identified in plants and processed foods. Although it is almost impossible to know the nature of all the phenolics present in a single sample, it is desirable to know the main classes of phenolic compounds present in it [21].

In relation to the phenolics quantified in beer samples, there were significant differences ($p < 0.05$) in the content of total phenolics, total flavonoids and total

nonflavonoids between the three types of beer. In total phenols, regular beer obtained the highest content with 140.70 mg GAE/L, followed by zero beer with 125.30 mg GAE/L and light beer obtained the lowest concentration with 72.32 mg GAE/L. The same behaviour was observed in total non-flavonoids in which regular beer, zero beer and light beer obtained 101.70 mg GAE/L, 65.80 mg GAE/L and 46.82 mg GAE/L, respectively. In total non-flavonoids, zero beer obtained the highest content registering 59.50 mg GAE/L, followed by regular beer with 39.00 mg GAE/L and the lowest content was found in light beer with 25.50 mg GAE/L (Table III).

Table III

Nutraceutical evaluation of regular, light and zero beers

Beer	Phenolics (mg/L)			Antioxidant Capacity ($\mu\text{mol TE/L}$)			
	Total phenols	Total flavonoids	Total non-flavonoids	ABTS	DPPH	FRAP	ORAC
Regular	140.70 \pm 1.91 ^a	39.00 \pm 2.83 ^b	101.70 \pm 0.92 ^a	643.57 \pm 19.19 ^a	684.00 \pm 4.71 ^a	622.28 \pm 7.86 ^a	1252.16 \pm 3.14 ^a
Light	72.32 \pm 1.53 ^c	25.50 \pm 0.71 ^c	46.82 \pm 0.82 ^c	286.43 \pm 17.17 ^c	381.50 \pm 8.25 ^c	474.78 \pm 1.96 ^c	1201.51 \pm 4.77 ^b
Zero	125.30 \pm 1.53 ^b	59.50 \pm 2.12 ^a	65.80 \pm 3.65 ^b	559.29 \pm 21.21 ^b	657.33 \pm 7.07 ^b	520.89 \pm 3.54 ^b	1216.36 \pm 3.37 ^b

Values with different letters within same column are significantly different ($p < 0.05$, $n = 3$)

In terms of percentage, regular beer was 48.59% and 10.94% higher than light and zero beers in total phenols, and 53.96% and 35.29% higher than light beer in total non-flavonoids, respectively. Zero beer was 34.45% and 57.14% higher than regular and light beer in total flavonoids, respectively. The non-flavonoids compounds were the main phenolic group in all samples, accounting for 72.28%, 64.74% and 52.51% of the total phenolic, in regular, light and zero beers, respectively.

Several studies about phenolic compounds have been carried out in regular beers, but data of these evaluations in low and alcohol free beers are limited. Mitić *et al* [14], evaluated 15 regular lagers and 3 alcohol-free commercially beers and they reported that the mean value of regular beers was 19.01% and 10.72% higher than alcohol free beers in total phenolics and total flavonoids, respectively. Piazzon *et al* [17], showed a very similar finding in total phenolics reporting that regular beer had 19.02% more phenolics than dealcoholized beers analysing five different brands of each type. The results obtained by these authors are different to ours, since we found a small difference between regular and zero beer in total phenols, but a large difference between these two beers in total flavonoids.

It is of great interest from different points of view (consumers, food science researchers, nutritional experts) to know the antioxidant capacity of the foods that we consume [8]. The objective to determine the efficacy of antioxidants in protection against oxidative damage is to avoid the loss of commercial and nutritional value and thus a rapid method for determining antioxidant capacity of foods is desirable [22].

In relation to the antioxidant capacity, there were significant differences ($p < 0.05$) in all assays between the three types of beer. The values of antioxidant capacity ranged from 286 $\mu\text{mol TE/L}$ to 643 $\mu\text{mol TE/L}$, 381 $\mu\text{mol TE/L}$ to 684 $\mu\text{mol TE/L}$, 474 $\mu\text{mol TE/L}$ to 622 $\mu\text{mol TE/L}$ and 1201 $\mu\text{mol TE/L}$ to 1252 $\mu\text{mol TE/L}$ in ABTS, DPPH, FRAP and ORAC respectively and the behaviour in all the antioxidant capacity assays was: regular beer > zero beer > light beer (Table III).

Regular beer was higher than zero beer by 13.10%, 3.90%, 16.30% and 2.86% in ABTS, DPPH, FRAP and ORAC assays, respectively. On the other hand, regular beer had a better antioxidant capacity with 55.50%, 44.23%, 23.71% and 4.05% than light beer in the same analyses, respectively.

Tafulo *et al* [24], evaluated the antioxidant capacity using the same assays as in our work, using 10 regular (5% alcohol), 3 light (4% alcohol) and 4 zero (0% alcohol) commercial beers. These authors had the same observations in the FRAP assay in which the average values of regular beers were 5.6% and 14.1% than light and zero beers, respectively. A different behaviour was observed by them in ABTS and ORAC assays in which, zero beers showed the highest antioxidant capacity followed by regular and light beers. According to these authors, zero beer was 1.9% and 2.8% higher than light and zero beers in ABTS assay and 4.3% and 15.2% higher than light and zero beers in ORAC. Finally they observed that light beers had the highest antioxidant capacity in DPPH assay being 14.8% and 30.2% higher than zero and regular beers, respectively.

Conclusions

Due to the diverse options of different alcohol content beers on the market for consumers, it is necessary to evaluate the differences in quality, nutritional and nutraceutical aspects. Although all samples were in the range of the quality criteria in the physicochemical parameters there were statistical differences between them. On the other hand, zero beer showed higher contents of some important nutritional mineral elements (Ca, Mg, K, Na) than regular and light beers. In addition, regular beer presented the highest levels in nutraceutical properties (phenolics and antioxidant capacity). The results obtained in the present work give information to consumers for choosing a beer based on the physicochemical, nutritional and nutraceutical properties.

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