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Evaluation of Phytochemical Composition of Portuguese Beers for Rationale Marketable Selection†

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† Presented at the 1st International Electronic Conference on Food Science and Functional Foods, 10–25 November 2020; Available online: https://foods_2020.sciforum.net/

Submitted: date; Accepted: date; Published: date

Abstract: Beer has been highly appreciated due to its phenolic composition and antioxidant capacity conjugated with its low alcohol content. Although the existence of some studies regarding the phenolic composition and antioxidant capacities of beers, there is no studies related to the determination of these parameters in the most consumed commercial beers in Portugal.

The phenolic composition and antioxidant capacity of twenty-three Portuguese commercial beers of different styles and types from, was studied for the first time.

The total phenolic content, *ortho*-diphenols, and flavonoids ranged between 0.15 ± 0.01 and 0.82 ± 0.07 g Gallic Acid (GA) L^{-1}; 0.07 ± 0.02 and 1.80 ± 0.09 g GA L^{-1}, and 0.02 ± 0.00 and 0.15 ± 0.02 g Catechin (CAT) L^{-1}, respectively. An accurate quantitative phenolic analysis was also performed, and the compound identified in higher amount was gallic acid, followed by the syringic acid. Concerning flavonoids, gallocatechin was the most abundant compound identified (from 21.44 ± 2.87 and 144.00 ± 10.93 μg mL^{-1}). A significant correlation between *ortho*-diphenols and the antiradical capacity (ABTS and DPPH) was found, being the latter negatively correlated. Flavonoids content was also positively correlated with total phenols and antiradical capacity determined by the ABTS assay.

These results evidence that phenolic composition is affected by several factors inherent to beers, namely ingredients, fermentation type, and brewing process.

Keywords: Commercial beers; Phenolic composition; High-Performance Liquid Chromatography–Diode Array Detector; Antioxidant capacity; Correlation

1. Introduction

Beer is the most widely consumed alcoholic beverage in the world and the third most popular drink after water and tea [1]. This is a complex alcoholic beverage made from barley (malt), hop, water, and brewer's yeast, rich in nutrients as well as non-nutrient components, such as phenolic acids and flavonoids, mainly derived from the added malt (70–80%) and hops (20–30%) [2,3].

Although the existence of some studies regarding the phenolic composition and antioxidant capacities of beers, there is very few studies related to the determination of these parameters in the most consumed commercial beers in Portugal. In this sense, the aims of this work were to study and to characterize some of the most common Portuguese beers available on the market, by the assessment of their content in phenolics and antioxidant capacity. Additionally, a principal component analysis (PCA), a pearson correlation test, and a dendogram were conducted to highlight the phenolic contents that best separate beer samples according to their styles.

2. Material and Methods

2.1. Plant Material and Treatments

The present work was carried out on three bottles of twenty-three Portuguese commercial beers purchased in supermarkets (Vila Real, Portugal), including dark beers, pale beers, non-alcoholic beers, and with flavor. The brand names were omitted and represented by number codes, as summarized in Table 1, in which some characteristics were described as reported on the bottles. From all the beers, just three were classified as ale beer according to the beer label. The remaining samples were classified as lager beers.

Before the analysis, beers were firstly degassed by mechanical agitation during 24 h. Then, the samples were stored at 4 °C and analyzed within 48 h. The phytochemical composition and antioxidant capacity of beers have been performed. For the determination of the phenolic content, namely total phenols, flavonoids, and *ortho*-diphenols, spectrophotometric assays were assessed, according to the methodologies previously reported [4]. The free radical scavenging activity was determined by DPPH and ABTS spectrophotometric methods adapted to a microscale, according to the procedure described by Leal et al. [5]. The identification and quantification of phenolics was also performed by Reverse Phase–High-Performance Liquid Chromatography–Diode Array Detector (RP-HPLC-DAD) to obtain a more detailed information about the phytochemical composition of these Portuguese beers. Chromatograms were recorded in the range 200–600 nm range and analyzed at 280 and 330 nm.

3. Results and Discussion

3.1. Total Phenols, Ortho-diphenols and Flavonoid Contents of the Portuguese Beers

The results of the phenolic content of Portuguese beers regarding total phenols, *ortho*-diphenols, and flavonoids are presented in Table 1.

As it can be observed, the sample 2-Black revealed to present the highest concentration in total phenols, with 0.824 ± 0.074 g GA L^{-1}, being significantly different from all the other beers under study, with the exception of the beer 1, both ale beers, thus subjected to a high fermentation process; in opposite, the beer with the lowest concentration was 23-Panache, with 0.153 ± 0.013 g GA L^{-1}, significantly distinguishing itself from other beers.

Similar concentrations to those described above were obtained by Zhao et al. [6], namely between 0.152 and 0.340 g GA L^{-1} for 34 commercial lager-type beers using also the Folin-Ciocalteau method. However, other authors found significantly lower values of total phenols in laboratory, local Canadian and foreign commercial beers, namely between 0.04 and 0.14 g GA L^{-1} [7]. Oladokun et al. [8] also analysed commercial lager beers in terms of total phenols, obtaining concentrations between 0.07 and 0.26 g GA L^{-1}, much lower than those found in the present study.

Regarding the content of *ortho*-diphenols (Table 1), the 7-White sample proved to be the beer with the highest concentration with 1.801 ± 0.087 g GA L^{-1}, being significantly different from all the other beers studied ($p < 0.05$); in opposite, the beer with the lowest concentration of *ortho*-diphenols was 8-White beer with 0.074 ± 0.024 g GA L^{-1}.

Analyzing the flavonoids, the results revealed that 2-Black beer presented again (just like for total phenols) the highest concentration, with a content of 0.151 ± 0.017 g CAT L^{-1}; the beer with the lowest concentration was the 15-Lemon, with 0.020 ± 0.005 g CAT L^{-1}.

In their study, Rahman et al. [7] obtained a flavonoid content in the range of 0.008 and 0.05 g CAT L^{-1}, much lower than those found in this study. Nardini and Garaguso [9] also obtained higher values of phenolic compounds content (total phenols and flavonoids) in ale beers than in lager, which is in agreement with our study, namely concerning the sample 2-Dark, and other studies [10,11].

The differences found in total phenols and flavonoid content between values of the present study and the literature could be due to beer storage conditions, beer type, raw and origin of ingredients, brewing techniques, as well as the fermentation type and time used by the industry [12].

3.2. Antioxidant Capacity of the Portuguese Beers

The antioxidant capacity of the twenty-three beer samples was evaluated and the results are presented in Table 1. Regarding the evaluation of the antiradical capacity through the ABTS method, the results obtained revealed that 2-Black and 4-Black beers were the beers with the highest capacity (0.102 ± 0.002 mmol trolox L^{-1} 0.107 ± 0.002 mmol trolox L^{-1}), significantly different of all the other samples; in opposite, the beers that showed the lowest antiradical capacity were the Lemon beers number 14, 15, and 16 (around 0.008 ± 0.001 mmol trolox L^{-1}), being not also significantly different from beers 11 (White) and 23 (Panache).

According to Zhao et al. [6] the values obtained for ABTS were in the range of 0.55 mmol trolox L^{-1} and 1.95 mmol trolox L^{-1}, clearly above those obtained in the present study (0.008 and 0.107 mmol trolox L^{-1}).Concerning the DPPH methodology, the results obtained (Table 1) revealed the 10-White sample as the beer with the greatest antiradical capacity, with 0.038 ± 0.00 mmol trolox L^{-1}, being not significantly different from other samples. In opposite, 2-Black beer showed the lowest values, with 0.019 ± 0.002 mmol trolox L^{-1}, being not significantly different from beers 1 (Abbey), 3 (Black), 4 (Black), 5 (White), 6 (White), and 7 (White).

Comparing the results of the present study with those of Zhao et al. [6] we found that these DPPH values were higher, ranging from 0.24 to 0.70 mmol trolox L^{-1} in lager-type beers. Rahman et al. [7] also obtained higher DPPH values (between 0.25 and 0.71 mmol trolox L^{-1}) to those of the present study (0.019–0.038 mmol trolox L^{-1}).

Tafulo et al. [13] also determined the antioxidant capacity of 27 commercial beers, 18 of them Portuguese ones. This activity was determined by six different methods and three different standards. Despite the high antioxidant power found in these samples, ORAC method presented the highest values of antioxidant capacity. Furthermore, among the several factors that can influence the antioxidant capacity of beers, it was found that the colour and the method employed were the factors that significantly most affected this activity.

In the present study, the significant differences found in some samples can also be explained by the fact that they come from different manufacturers and, despite the similar base components used in beer production and process, there may be differences in terms of the quantities established by each manufacturer and the origin of each component.

The presence of phenolic compounds in beer can thus provide an antioxidant action, making it able to assist in some physiological disorders of the body. Nonetheless, beer should be moderately consumed.

Table 1. Phenolic content and antioxidant capacity of the Portuguese beers.

		Phenolic Content			**Antioxidant Capacity**	
Beer samples		**Total phenols (g GA L^{-1})**	***Ortho-diphenols* (g GA L^{-1})**	**Flavonoids (g CAT L^{-1})**	ABTS (mmol trolox L^{-1})	DPPH (mmol trolox L^{-1})
1	Abbey	0.706±0.027$^{gh\ X}$	0.401±0.015^{ef}	0.085±0.014^{abcdef}	0.049±0.001^{d}	0.021±0.001^{a}
2	Black	0.824±0.074^{h}	1.552±0.142^{h}	0.151±0.017^{f}	0.102±0.007^{e}	0.019±0.002^{a}
3	Black	0.506±0.065^{ef}	0.704±0.007^{g}	0.098±0.016^{abcdef}	0.016±0.001^{b}	0.020±0.001^{a}
4	Black	0.515±0.034^{ef}	0.718±0.015^{ef}	0.109±0.009^{bcdef}	0.107±0.003^{e}	0.021±0.001^{a}
5	White	0.362±0.027^{ef}	1.634±0.044^{h}	0.041±0.003^{abcd}	0.023±0.001^{c}	0.023±0.001^{a}
6	White	0.269±0.051^{ab}	1.561±0.011^{h}	0.033±0.002^{ab}	0.021±0.001^{bc}	0.023±0.001^{a}
7	White	0.304±0.048^{abcd}	1.801±0.087^{i}	0.043±0.005^{abcde}	0.023±0.001^{c}	0.022±0.001^{a}
8	White	0.278±0.029^{abc}	0.074±0.024^{a}	0.076±0.026^{abcdef}	0.019±0.000^{bc}	0.037±0.001^{de}
9	White	0.354±0.036^{bcde}	0.170±0.011^{abc}	0.042±0.028^{abcd}	0.020±0.000^{bc}	0.032±0.002^{bc}
10	White	0.276±0.034^{abc}	0.185±0.028^{abc}	0.039±0.006^{abc}	0.017±0.001^{b}	0.038±0.001^{e}
11	White	0.396±0.012^{bcde}	0.122±0.009^{ab}	0.041±0.017^{abcd}	0.009±0.002^{a}	0.034±0.003^{cde}
12	White Non-alcoholic	0.485±0.078^{def}	0.082±0.063^{a}	0.045±0.010^{abcde}	0.020±0.002^{bc}	0.034±0.001^{cde}
13	Black Non-alcoholic	0.461±0.012^{cdef}	0.419±0.046^{f}	0.125±0.022^{ef}	0.018±0.001^{bc}	0.036±0.001^{cde}

14	Lemon Non-alcoholic	0.267±0.030^{ab}	0.188±0.008^{abc}	0.042±0.025^{abcd}	0.009±0.001^{a}	0.032±0.003^{bc}
15	Lemon	0.304±0.022^{abcd}	0.099±0.007^{ab}	0.020±0.005^{a}	0.008±0.001^{a}	0.037±0.000^{e}
16	Lemon	0.240±0.022^{ab}	0.163±0.010^{abc}	0.032±0.016^{ab}	0.008±0.001^{a}	0.035±0.001^{cde}
17	90 years (edition)	0.500±0.172^{ef}	0.154±0.020^{abc}	0.060±0.012^{abcde}	0.016±0.002^{b}	0.033±0.002^{cd}
18	Bohemia wheat	0.397±0.099^{bcde}	0.199±0.024^{abc}	0.116±0.042^{cdef}	0.021±0.001^{bc}	0.034±0.003^{cde}
19	Bohemia pure malt	0.607±0.067^{fg}	0.281±0.012^{cde}	0.067±0.005^{abcde}	0.018±0.001^{bc}	0.035±0.001^{cde}
20	Bohemia original	0.500±0.048^{bcde}	0.357±0.050^{def}	0.123±0.093^{def}	0.018±0.001^{bc}	0.034±0.000^{cde}
21	Bohemia IPA	0.487±0.051^{def}	0.228±0.012^{bcd}	0.078±0.028^{abcdef}	0.018±0.001^{bc}	0.035±0.001^{cde}
22	Red fruits	0.312±0.054^{abcd}	0.451±0.026^{f}	0.089±0.020^{abcdef}	0.020±0.001^{bc}	0.034±0.000^{cde}
23	Panache	0.153±0.013^{a}	0.191±0.012^{abc}	0.023±0.012^{a}	0.010±0.001^{a}	0.028±0.003^{b}
	P-value	*** ^{Y}	***	***	***	***

^{X} Values are presented with mean ± SD (n = 3). Different letters indicate significantly different results (ANOVA. P> 0.05). according to the Tukey test. ^{Y} Significance: not significant. N.S. (P> 0.05); * significant with P <0.05; ** significant with P <0.01; *** significant with P <0.001.

3.3. Phenolic Profile of the Portuguese Beers

The identification and quantification of phenolics was also performed by Reverse Phase–High-Performance Liquid Chromatography–Diode Array Detector (RP-HPLC-DAD) to obtain a more detailed information about the phytochemical composition of these Portuguese beers.

It was possible to observe that the most abundant phenolic acids presents in almost all beer samples was gallic acid, followed by the syringic acid. The highest concentration of gallic acid found was in 1-Abbey beer, with 95.80 ± 3.71 μg mL^{-1}, while the highest concentration of syringic acid was for the 12-White non-alcoholic beer, with a concentration of 12.40 ± 1.62 μg mL^{-1}.

Contrarily to the aforementioned compounds, the other phenolic acids were detected in low concentrations in beer samples analyzed. In fact, protocatechuic and ferulic acids were only detected in two samples, namely in 2-Dark and 5-White beers, and 2-Dark and 15-Lemon beers, respectively, being 2-Dark sample the beer with the highest concentration for both compounds (28.43 ± 0.26 and 1.49 ± 0.06 μg mL^{-1}, respectively). This is in agreement with some studies of the literature which quantified less phenolic compounds in lager beers than in ale ones, namely with lower concentrations of *p*-coumaric, syringic, and caffeic acids [9].

Other compounds were only found in one beer sample, namely hydroxybenzoic, caffeic, and vanillic acids in 2-Dark (12.33 ± 0.248 μg mL^{-1}), 22-Red fruits (29.24 ± 3.64 μg mL^{-1}), and 18-Bohemia wheat (1.332 ± 0.086 μg mL^{-1}), respectively. Nardini et al.[9] also found caffeic acid, among other compounds, in fruit beers which are significantly improved in phenolic compounds comparatively to the conventional beers.

Zhao et al. [4] also identified some of these compounds in beer samples. Concerning gallic acid, the concentrations found in the present work were ten times higher than those obtained by these authors (10.39 ± 0.09 μg mL^{-1} and 1.81 ± 0.11 μg mL^{-1}). Concerning protocatechuic acid, in the present study, higher values than those obtained by the authors Zhao et al. [6] were found, which obtained concentrations between 0.02 ± 0.02 μg mL^{-1} and 1.30 ± 0.05 μg mL^{-1}. In contrary, the concentration in ferulic acid in the present study was less than those found by the same authors, which obtained the following values 0.51 ± 0.03 μg mL^{-1} and 2.81 ± 0.04 μg mL^{-1} in their beer samples. Finally, caffeic acid, which was just identified in one beer, was also found at higher concentrations comparatively to the concentrations of the study of Zhao et al. [6] (between 0.12 ± 0.03 μg mL^{-1} and 1.22 ± 0.05 μg mL^{-1}).

Concerning flavonoids, gallocatechin was the most abundant compound found in all the samples (from 21.44 ± 2.87 and 144.00 ± 10.93 μg mL^{-1}), except for 2-Black beer which was not detected. Epicatechin and catechin were also found in high concentrations in almost all beers, with concentrations ranging from 3.28±0.18 and 215.10 ± 22.58 μg mL^{-1} and from 1.41 ± 0.01 and 155.30 ± 2.10 μg mL^{-1}, respectively.

The other compounds identified, including kaempferol derivatives, rutin, and quercetin, were present in few samples, namely in Lemon (14 and 16) and Red fruits beers (22). Concerning kaempferol, this compound was found in eleven beer samples, being in higher concentration in dark and ale samples, namely 1-Abbey, 2-Dark, and 3-Dark (0.22 μg mL^{-1}, on average).

In fact, several differences were found between the beer samples, due to several factors, such as the fermentation type, the origin, concentration, and quality of ingredients, and the brewing process. Furthermore, the phenolic compounds present in beer come from hops and, in the great majority, from barley malt, making the drink a source of polyphenols [14]. However, compounds derived from hops are easier to characterize than those from barley, once during the processing of the drink the last one can undergo changes, making them difficult to characterize [15]. Despite this, during malting and brewing process, phenolic compounds are also subject to changes due to the extraction or enzymatic release, to heat-induced chemical reactions or to precipitation with or adsorbed to hot and cold trub, stabilization agents and yeast cells [16].

Within the several stages that make up the beer production process, filtration emerges as one of the main responsible for the drastic reduction in the content of polyphenols present in the final matrix. The boiling stage also causes a series of changes in the must polyphenols composition, which is already quite complex, making it difficult to predict the fate of the polyphenols in this mixture. Such complexity is partly due to the ease of oxidation and polymerization of several phenolic compounds [17].

Thus, in barley grains, derivatives of hydroxybenzoic and hydroxycinnamic acids have been identified, such as *trans*-ferulic acid, found in greater quantity in the grain, followed by *p*-coumaric and vanillic acids. These acids are known to act as primary antioxidants in the reception of free radicals, interrupting the chain reaction and are present in the aleurone layer and in the endosperm of the grain [18].

Generally, phenolic compounds are found in beer linked to other compounds, such as esters and glycosides, but it is also possible to find them in their free form, and some substances are more likely to be found in malt or hops. Derivatives of hydrobenzoic acids and hydroxinamic acids, such as ferulic, *p*-coumaric and caffeic acids (also identified in this work), are extracted more commonly from malt, while flavonols, chalcones and flavanones are essentially found from hops. Equally detectable, both in hops and in malt, are tannins derived from flavonols, catechins and procyanidins [19].

In addition to the several dependent factors and the contribution to the aroma and color of the beers, the phenolic compounds are correlated with the antioxidant capacity, improving the stability and, consequently the shelf-life of beers.

3.4. Characterization of the Portuguese Beers by PCA Analysis

Principal component analysis (PCA) represents one of the most widely used chemometric tools. PCA is an unsupervised technique, which reduces the dimensionality of the original data matrix while maintaining maximum variability and allows visualization of the original arrangement of the samples in an n-dimensional space, through the information maintained, allowing the relationship between variables and observations to be studied, as well as the recognition of the data structure. The explanation of the differences in the samples is given through the factors obtained from the generalized correlation matrix of the data sets and, at the same time, allows to determine which variables contribute most to this differentiation (Figure 1 (A)).

PCA was applied to evaluate data on the main phenolic content determined, namely *ortho*-diphenols, total phenols, flavonoids and antioxidant capacity (ABTS and DPPH) (Figure 1 (B)). The first main component was able to explain 56.87% of the total variance and the second explained 26.32%, totalizing 83.19%.

The simple dispersion graph (Figure 2) suggests the location of the beers in relation to the phenolic content and antioxidant capacity. It was possible to observe the formation of five groups. The group represented by the dark blue color was the one with the highest DPPH values, the group represented in green was the one with the highest values in *ortho*-diphenols, the group represented

by red showed higher values for the flavonoids and total phenols, and lastly, the group represented by the black color, showed the highest values for antioxidant capacity, namely ABTS.

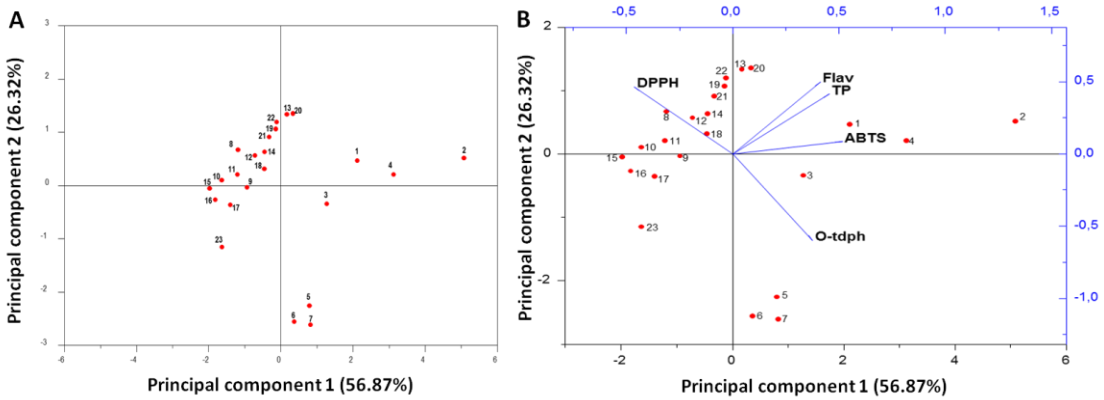

Figure 1. (A) Simple dispersion graph (Principal component 1 x Principal component 2) over the main sources of beer variability; (B) Projection of the values obtained for PC's 1 and 2 for the different samples. The weights of each variable for each of the factors are represented by the position of the abbreviations in the quadrants.

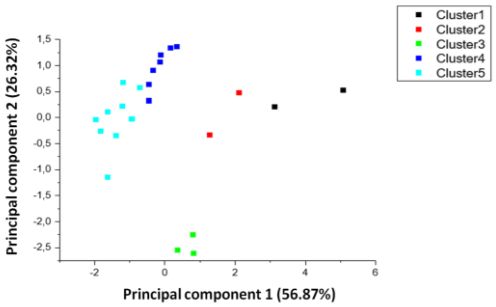

Figure 2. Grouping resulting from the analysis of clusters. Different colors refer to different groups. The distribution of the samples is represented according to the values for CP's 1 and 2.

The similarity of the samples was evaluated using hierarchical analysis of clusters and five groups were suggested, in which they corroborate the results found by the PCA. The means for each response variable were compared statistically. Through the separation of the groups, it was possible to observe that in cluster 1 there are 2-Black and 4-Black beers, which are similar to the antiradical capacity by ABTS assay. In cluster 2, the samples 1-Abbey and 3-Black were contained because they are in the quadrant of antiradical capacity by ABTS and content in *ortho*-diphenols. In cluster 3, the beers 5-White, 6-White and 7-White were grouped, since they have identical content in *ortho*-diphenols, hence they were grouped in the same quadrant. In cluster 4 were the samples 8-White, 12-White without alcohol, 13-Black without alcohol, 17- 90 years (edition), 18-Bohemia wheat, 19-Bohemia pure malt, 20-Original bohemia, 21-Bohemia IPA, and 22-Red fruits due to the common content in flavonoids, total phenols, and antiradical capacity values for DPPH. Finally, in cluster 5 were 9-White, 10-White, 11-White, 14-Lemon without alcohol, 15-Lemon, 16-lemon, and 23-Panache. These beers presented common characteristics in terms of *ortho*-diphenols and the same antiradical capacity for DPPH.

Through pearson's statistical correlation analysis ($p \leq 0.05$) it was possible to observe, through the results obtained, that the analyzes performed obtained a significant correlation with each other.

There is a significant correlation between *ortho*-diphenols and the antiradical capacity of ABTS and DPPH, being the latter negatively correlated.

In the case of total phenols, the correlation was significant for flavonoids, antiradical capacity for ABTS and DPPH, the latter being also negatively correlated. For flavonoids, the correlation was significant for total phenols and for antiradical capacity for ABTS.

Concerning the antiradical capacity, this correlated with *ortho*-diphenols, total phenols and flavonoids, correlating negatively once again with antiradical capacity by DPPH.

4. Conclusions

Beer can be considered a good source of polyphenols, which can come from both malt and hops. Due to its antioxidant capacity and low alcohol content, beer has been extensively studied in its capacity to reduce the risk of coronary heart disease. In this study, an accurate qualitative and quantitative determination of phenolic compounds by chromatographic and spectrophotometric methods has been performed in commercial Portuguese beers. The HPLC-DAD analyses allowed to determine seven phenolic acids and eleven flavonoids in twenty-three commercial beers.

The phenolic profile was characterized by high contents of gallic and syringic acids, kaempferol, gallocatechin and epicatechin, and low contents of vanillic, ferulic, and caffeic acids, quercetin, and rutin. High correlations have been found between some phenolic contents and the antioxidant capacity determined by ABTS and DPPH methods. The several differences found between the samples were undoubtedly due to the ingredients and the brewing and fermentation processes of the different beers.

Author Contributions: Conceptualization, methodology, investigation, writing—original draft preparation, writing—review and editing, I.G. Conceptualization, supervision, funding acquisition, A.B.

Funding: This work was supported by National Funds by FCT - Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020, and FEDER-Interreg España-Portugal programme for financial support through the project 0377_Iberphenol_6_E.

Conflicts of Interest: The authors declare no conflict of interest.

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