# **Evaluation of phytochemical composition of Portuguese beers for rationale marketable selection**



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### Introduction

Beer is the most widely consumed alcoholic beverage in the world and the third most popular drink after water and tea (Machado, 2005). 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%) (Fegredo et al., 2009; Quifer-Rada et al., 2015).

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, a Pearson correlation test, and a Dendogram were conducted to highlight the phenolic contents that best separate beer samples according to their styles.

### Material and Methods

### Samples

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 24h. Then, the samples were stored at 4 °C and analyzed within 48h.

#### Methodology

For the determination of the phenolic content, namely total phenols, flavonoids, and *ortho*diphenols, spectrophotometric assays were assessed, according to the methodologies previously reported (Gouvinhas et al., 2020).

All the assays were performed using 96-well micro plates (Nunc, Roskilde, Denmark) and an Infinite M200 microplate reader (Tecan, Grödig, Austria). For all analyses, three replicates (n=3) of each sample were assessed.

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. (2020).

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Results and Discussion

|              | Phenolic content        |  |  |                               | Antioxidant capacity              |                                |
|--------------|-------------------------|--|--|-------------------------------|-----------------------------------|--------------------------------|
| Beer samples |                         | Total phenols Ortho-diphenols Flavonoids |  |                               | ABTS DPPH                         |                                |
|              |                         | Total phenols<br>(g GA L <sup>-1</sup> ) | Ortho-diphenols<br>(g GA L <sup>-1</sup> ) | (g CAT L <sup>-1</sup> )      | (mmol trolox<br>L <sup>-1</sup> ) | (mmol trolox L <sup>-1</sup> ) |
| 1            | Abbey                   | x0.706±0.027gh                           | 0.401±0.015 <sup>cfZ</sup>                 | 0.085±0.014 <sup>abcdef</sup> | 0.049±0.001 <sup>d</sup>          | 0.021±0.001ª                   |
| 2            | Black                   | 0.824±0.074 <sup>h</sup>                 | 1.552±0.142 <sup>h</sup>                   | 0.151±0.017f                  | 0.102±0.007°                      | 0.019±0.002ª                   |
| 3            | Black                   | 0.506±0.065 <sup>cf</sup>                | 0.704±0.007 <sup>g</sup>                   | 0.098±0.016 <sup>abcdef</sup> | 0.016±0.001 <sup>b</sup>          | 0.020±0.001ª                   |
| 4            | Black                   | 0.515±0.034 <sup>ef</sup>                | 0.718±0.015 <sup>ef</sup>                  | 0.109±0.009 <sup>bcdef</sup>  | 0.107±0.003°                      | 0.021±0.001ª                   |
| 5            | White                   | 0.362±0.027ef                            | 1.634±0.044 <sup>h</sup>                   | 0.041±0.003 <sup>abcd</sup>   | 0.023±0.001°                      | 0.023±0.001*                   |
| 6            | White                   | 0.269±0.051ab                            | 1.561±0.011 <sup>h</sup>                   | 0.033±0.002 <sup>ab</sup>     | 0.021±0.001 <sup>bc</sup>         | 0.023±0.001*                   |
| 7            | White                   | 0.304±0.048 <sup>abcd</sup>              | 1.801±0.087 <sup>i</sup>                   | 0.043±0.005 <sup>abcde</sup>  | 0.023±0.001°                      | 0.022±0.001                    |
| 8            | White                   | 0.278±0.029abc                           | 0.074±0.024 <sup>a</sup>                   | 0.076±0.026 <sup>abcdef</sup> | 0.019±0.000 <sup>bc</sup>         | 0.037±0.001 <sup>de</sup>      |
| 9            | White                   | 0.354±0.036bcde                          | 0.170±0.011abc                             | 0.042±0.028 <sup>abcd</sup>   | 0.020±0.000bc                     | 0.032±0.002bc                  |
| 10           | White                   | 0.276±0.034 <sup>abc</sup>               | 0.185±0.028 <sup>abc</sup>                 | 0.039±0.006 <sup>abc</sup>    | 0.017±0.001 <sup>b</sup>          | 0.038±0.001°                   |
| 11           | White                   | $0.396 \pm 0.012^{bcde}$                 | 0.122±0.009 <sup>ab</sup>                  | $0.041{\pm}0.017^{abcd}$      | $0.009 \pm 0.002^{a}$             | $0.034 \pm 0.003^{cde}$        |
| 12           | White Non-<br>alcoholic | $0.485 \pm 0.078^{def}$                  | $0.082 \pm 0.063^{a}$                      | 0.045±0.010 <sup>abcde</sup>  | $0.020 \pm 0.002^{bc}$            | 0.034±0.001 <sup>cde</sup>     |
| 13           | Black Non-<br>alcoholic | 0.461±0.012 <sup>cdef</sup>              | 0.419±0.046 <sup>f</sup>                   | 0.125±0.022ef                 | 0.018±0.001∞                      | 0.036±0.001 <sup>cde</sup>     |
| 14           | Lemon Non-<br>alcoholic | 0.267±0.030 <sup>ab</sup>                | 0.188±0.008 <sup>abc</sup>                 | 0.042±0.025 <sup>abcd</sup>   | 0.009±0.001ª                      | 0.032±0.003 <sup>bc</sup>      |
| 15           | Lemon                   | 0.304±0.022 <sup>abcd</sup>              | $0.099 \pm 0.007$ ab                       | 0.020±0.005ª                  | 0.008±0.001ª                      | 0.037±0.000°                   |
| 16           | Lemon                   | 0.240±0.022 <sup>ab</sup>                | 0.163±0.010 <sup>abc</sup>                 | $0.032 \pm 0.016^{ab}$        | $0.008 \pm 0.001^{a}$             | $0.035 \pm 0.001^{cde}$        |
| 17           | 90 years<br>(edition)   | $0.500 \pm 0.172^{\rm ef}$               | 0.154±0.020 <sup>abc</sup>                 | 0.060±0.012 <sup>abcde</sup>  | 0.016±0.002 <sup>b</sup>          | 0.033±0.002 <sup>cd</sup>      |
| 18           | Bohemia wheat           | 0.397±0.099 <sup>bede</sup>              | 0.199±0.024 <sup>abc</sup>                 | 0.116±0.042 <sup>cdef</sup>   | 0.021±0.001bc                     | 0.034±0.003 <sup>cde</sup>     |
| 19           | Bohemia pure<br>malt    | $0.607 \pm 0.067$ fg                     | 0.281±0.012 <sup>cde</sup>                 | 0.067±0.005 <sup>abcde</sup>  | 0.018±0.001bc                     | 0.035±0.001 <sup>cde</sup>     |
| 20           | Bohemia<br>original     | 0.500±0.048 <sup>bcde</sup>              | 0.357±0.050def                             | $0.123 \pm 0.093^{def}$       | 0.018±0.001 <sup>bc</sup>         | 0.034±0.000 <sup>cde</sup>     |
| 21           | Bohemia IPA             | 0.487±0.051def                           | 0.228±0.012 <sup>bcd</sup>                 | 0.078±0.028 <sup>abcdef</sup> | 0.018±0.001 <sup>bc</sup>         | 0.035±0.001 <sup>cde</sup>     |
| 22           | Red fruits              | 0.312±0.054abcd                          | 0.451±0.026 <sup>f</sup>                   | 0.089±0.020abcdef             | 0.020±0.001bc                     | 0.034±0.000cde                 |
| 23           | Panache                 | 0.153±0.013ª                             | 0.191±0.012 <sup>abc</sup>                 | 0.023±0.012 <sup>a</sup>      | 0.010±0.001ª                      | 0.028±0.003b                   |
|              |                         |  |  |                               | ***                               | ***                            |

 $^{X}$  Values are presented with mean ± SD (n = 3). Different letters indicate significantly different results (ANOVA. P> 0.05). according to the Tukey test.

 $^{\rm Y}$  Significance: not significant. N.S. (P> 0.05); \* significant with P <0.05; \*\* significant with P <0.01; \*\*\* significant with P <0.001.

## 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.