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ORIGINAL RESEARCH ARTICLE

Analysis of pyranoanthocyanins, polymeric pigments and colour parameters in Port wines

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ABSTRACT

Context & aim: The two major categories of Port wines, Tawny and Ruby, are defined by their ageing conditions, with associated oxidative conditions being much more pronounced in the case of the former than the latter. The main aim of this study was to determine if, independently of the producer, all Port wines can be grouped into these styles based on their chromatic and pigment characteristics.

Methods: A total of 32 Port wine samples comprising eight different styles were provided by five different producers of the Douro region in Portugal for this work. All the samples were filtered prior to analysis for anthocyanin determination using an HPLC-DAD-ESI/MS, and unfiltered samples were used for the determination of colour parameters and CIE Lab coordinates. The samples were measured in triplicate for statistical analysis.

Results: The chromatic analysis of the Port wines showed Ruby style Ports to have a higher colour intensity and lower hue (°) than Tawny style Ports. The PCA analysis of colour parameters and CIE Lab coordinates clearly shows two separate clusters representing Ruby styles and Tawny styles respectively. Overall, Tawny style Ports had traces of or non-detected anthocyanins monoglucosides, a few styles having < 10 mg/L in the form of malvidin-3-*O*-glucoside equivalent; meanwhile Ruby styles showed higher concentrations of anthocyanins as detected by HPLC, sometimes reaching values close to 100 mg/L. The major anthocyanin family found across all wines (even in trace amounts) were pyranoanthocyanins, specifically vitisin A and coumaroylated vitisin A.

Main conclusions: The results prove that in terms of all the parameters studied there is a clear division between the two major styles of Port wines, which can be attributed to the differences between the ageing process of these two types of wines.

KEYWORDS: Port wine, pyranoanthocyanins, colour intensity, hue, ageing conditions

INTRODUCTION

Port wine is a sweet fortified wine vinified from grapes that come from the Douro Demarcated Region (DDR) of Portugal (Hogg, 2013). Based on traditional methods, Port Wine production can be divided into four main steps: short alcoholic fermentation, fortification, making up of lots and blending of the wines, and ageing in old wood (Moreira *et al.*, 2019). Depending on the ageing method applied, Port wines can be grouped into two major styles: Tawny style (or barrel-aged wine) and Ruby style (or bottle-aged wine). Regardless of being classified as bottle-aged, the Ruby style also spend some time in wood, but most of their lives are spent maturing in bottle (Hogg, 2013; Moreira and Guedes de Pinho, 2011; Pereira *et al.*, 2019).

Anthocyanins are water-soluble polyphenolic pigments found in fruits, vegetables and flowers (Oliveira *et al.*, 2019). These pigments, depending on the pH and other complexing agents, are the principal compounds that contribute to the colour of young red wine, being responsible for their red/purple colour (He *et al.*, 2012; Morata *et al.*, 2019; Waterhouse and Zhu, 2020). During red wine ageing, anthocyanins and other phenolic compounds like flavanols play a role in several chemical reactions leading to colour changes; this is no different to what happens during the maturation of Port wines (Ho *et al.*, 2001). Compared to other red wines, Port wines have a more complex wine matrix due to the addition of grape spirit to stop fermentation. Wine spirits are rich in aromatic compounds, such as aldehydes and alcohols; the potential for the formation of new compounds is thus increased as a result of the reactions between these aldehydes and flavonoids (anthocyanins and catechins) (Pissarra *et al.*, 2005).

The colour differences seen in the different types of Port wines are a result of the changes in the phenolic compounds extracted from the grapes during vinification and maturation (Pinho *et al.*, 2012). All young red Ports start with a ruby colour that becomes tawny or orange-brown as they mature (Ho *et al.*, 2001). The evolution of this colour will depend on the length of time for which the wine ages in oak and/or in the bottle, and the size and age of the oak vessel will also have an influence (Prata-Sena *et al.*, 2018). This colour evolution involves polymerisation reactions between anthocyanins, tannins, pyruvic acid and acetaldehydes (Bakker and Clarke, 2011), which give rise to other pigments.

The most common anthocyanin found in Port wine grapes is malvidin in the form of malvidin-3-*O*-glucoside (Mv-3-*O*-glc) and its acetyl and coumaroyl derivatives (Bakker and Timberlake, 1985). The actual composition of these anthocyanins varies notably depending on the variety (Bakker and Timberlake, 1985), year of harvest and the elevation of the vineyard (Hogg, 2013; Mateus and De Freitas, 2001). Pyranoanthocyanins comprise a class of anthocyanin derivatives that plays an important role in the final Port wine colour. Pyruvic acid derivatives of anthocyanins (A-type vitisins) are pyranoanthocyanins that have been detected in high concentrations in Port wines (Fernandes *et al.*, 2017). Port wines have a slightly higher pH value than red table wines, which facilitates the formation of anthocyanin-pyruvic acid adducts (Mateus and De Freitas, 2001). Fortification during alcoholic fermentation increases the availability of pyruvic acid (Romero and Bakker, 1999), a by-product of yeast metabolism during the first stages of fermentation (Morata *et al.*, 2003); this also explains the high amounts of vitisin A found in these wines. These vitisins are relatively stable and sometimes the only anthocyanin present in Port wines (Romero and Bakker, 2001).

Colour differences between the different styles of Port wines are associated with the different ageing conditions (i.e., reductive ageing involving a gradual loss of anthocyanins and oxidative ageing involving a high loss of anthocyanins), but the initial anthocyanin content of the wine also plays an important role (Ferreira and Pérez-Palacios, 2014). Furthermore, differences in anthocyanin content between cultivars, vineyard location, maceration type, fermentation duration and house styles all contribute to the final colour and polyphenol content of the wines

MATERIALS AND METHODS

1. Wine Samples

A total of eight different categories of Port wines (Figure 1) comprising 32 different wines were obtained from five different producers from the Douro region: three bottles of Late Bottled Vintage (LBV), four bottles of Ruby (R), two bottles of Ruby Reserve (RR), three bottles of Tawny (T), five bottles of Tawny Reserve (TR), seven bottles of 10-year-old tawny (10y), six bottles of 20-year-old tawny (20y) and two bottles of 30-year-old tawny (30y). A letter from A to E was assigned to each producer and the samples were labelled according to their port wine category and producer (when different brands of the same producer were analysed 1, 2 or 3 was added to the label).

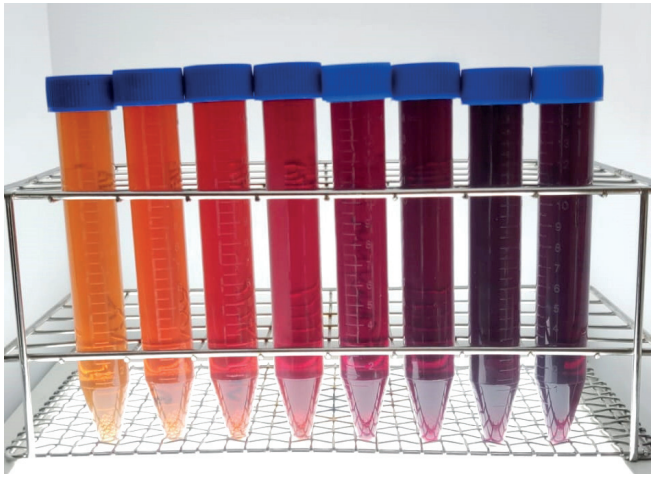

FIGURE 1. Colour grade of the different styles of analysed Port wine.

From Tawny to Ruby, left to right: 30-year-old tawny, 20-year-old tawny, 10-year-old tawny, tawny reserve, tawny, ruby reserve, ruby and late-bottled vintage.

1.1. Wine sample preparing

All the Port wine samples were filtered with a 45 µm methylcellulose membrane before being analysed using the high-performance liquid chromatograph (HPLC) coupled to a mass spectrometer and a gas chromatography with flame ionisation detector (GC-FID). The filtered samples were poured into 2 mL clear glass vials sealed with 9 mm PTFE/silicon septum caps (Phenomenex, Torrance, CA, USA), and labelled based on style of Port and a letter code representing sample origin

Unfiltered samples were used for the determination of the colour parameters using UV-visible spectrophotometer. For ruby style Ports, a dilution 1:1 was performed before the measurement.

2. Anthocyanin determination (HPLC-DAD-ESI/MS)

The identification of all non-acylated monomeric anthocyanins and the derived acylated pyranoanthocyanins, and oligomeric and polymeric pigments in all the wine samples was done using an Agilent Technologies (Palo Alto, CA, USA) series 1100 HPLC chromatograph (high-performance liquid chromatograph) equipped with a diode array detector (DAD) and a quadrupole mass spectrometer with an electrospray interface (MS-ESI). Two solvents were used for pigment separation, Solvent A (water/formic acid, 95:5 v/v) and Solvent B (methanol/formic acid, 95:5 v/v) with the following gradient: 0–2 min, 25 % B; 2–10 min, 25–50 % B linear; 10–11 min, 50 % B; 11–12min, 50–2 % B linear; and 12–17 min, re-equilibration; the working flow was 0.6 mL/min. The column used for the separation of pigments was a reverse-phase Poroshell 120 C18 column (Phenomenex, Torrance, CA, USA) with the following dimensions: 50 mm × 4.6 mm, and particle size 2.7 µm. Detection was performed via scanning in the 400–600 nm range.

Malvidin-3-*O*-glucoside, expressed as milligram per litre (mg/L), was used as an external standard at a wavelength of 525 nm to quantify all the anthocyanins and derived pigments, while identification was carried out via MS positive scanning from 100 to 1500 *m/z* and from time 0 to 17 min. The injection volume was set at 40µL, but for Port samples corresponding to Tawny style ports the injection volume was 100 µL, in order to increase the size of the peaks for better integration.

3. Colour parameters (spectrophotometry)

The colour parameters were measured using two different methods. The first method involved a UV-visible spectrophotometer 8453 from Agilent Technologies (Palo Alto, CA, USA) with a photodiode array detector and a quartz cuvette with a 1 mm path length to avoid signal saturation. Absorbance measurements were done at three different wavelengths, 420, 520 and 620 nm, to compare Colour Intensity (CI) (calculated as the sum of the different absorbances) and tonality (which is the ratio between the absorbance measured at 420 and 520 nm) in all samples; CI and tonality represent the "chromatic profile" of a given wine and change with the ageing (evolution) of the wine. Total polyphenol index was determined at 280 nm using a 1 mm path length cuvette. *p*-coumaric acid, expressed as mg/L with a calibration curve, was used as an external standard at wavelength 280 nm for the quantification of total phenols.

A characterisation of the change in colour between samples using CIE *Lab* coordinates was performed. A Smart Analysis (DNA Phone s.r.l, Parma, Italy) spectrophotometer with a 1 mm quartz cuvette was used for this purpose. The CIE *Lab* is a uniform three-dimensional space, defined by the colorimetric coordinates L*, a* and b*. The values obtained from these coordinates were hue angle (Hº) (i.e., the colour of the object: red, blue, yellow, etc., which varies across the wavelengths of the visible spectrum), and Chroma (C*), also known as saturation and which is a measure of colour intensity or purity relative to neutral grey. These values were also used to analyse the different Port wine samples qualitatively and quantitatively respectively.

4. Statistical analysis

All the data were analysed using the statistical software R (version 4.0.3.) with the user surface RStudio (version 1.4.1103.). The differences between the wine styles in terms of all the studied variables were tested using one factor (Wine style) analysis of variance (ANOVA) and mean significant difference comparison (Tukey HSD) tests. Significance level was set at 5 %.

Using version 19.3.03 of PC Statgraphics Centurion (Graphics Software Systems, Rockville, MD, USA) a principal components analysis (PCA) and a cluster analysis (CA) were performed considering major chromatic and pigment parameters.

RESULTS

1. Colour parameter results

A summary of the main colour parameters and the total polyphenol index (TPI) is given in Table 1.

Figure 2 shows a representation of the colour scheme according to CIE L*, a*, b* coordinates. All the analysed samples are located in positive a* and b* quadrantes and are predominantly red or yellow in colour respectively. Lower b* values are associated with wine samples that are less yellow in colour and tend towards blue. Meanwhile, higher a* and b* values are indicative of brownish wines.

A clear division can be seen between the two styles of port wines. All the ruby samples fall below the dashed line, whilst all the tawny style samples are above it.

A PCA was done on the CIE Lab colour parameters (Figure 3). The distribution is explained by the first two components (98,5 %). Component 1 is positively contributed by b*, hue (°) and L* and component 2 is positively contributed by a*.

Two main clusters can be identified as a result of the PCA (different coloured dots), but other groups can also be seen, as well as one outlier sample (TB). The group highlighted in green comprises the oldest wines, which show the highest values for hue (°) and L*. The other highlighted groups are

TABLE 1. Summary of colour intensity (CI), hue and TPI. Different letters indicate significant statistical differences between samples belonging to the same Port Style. Average, STD (n = 3 and p < 0.05).

Port Wine	CI	Hue	TPI
30yA	2.8 ± 0.05b	2.2 ± 0.01a	35.0 ± 0.54a
30yE	3.1 ± 0.04a	1.9 ± 0.00b	32.8 ± 0.15b
20yA	3.5 ± 0.03d	1.7 ± 0.00c	34.1 ± 0.98c
20yB	3.8 ± 0.00b	2.2 ± 0.00a	38.5 ± 1.10a
20yC1	3.6 ± 0.01cd	1.8 ± 0.00b	37.2 ± 0.39b
20yC2	5.2 ± 0.02a	1.6 ± 0.00e	39.3 ± 0.53a
20yD	3.7 ± 0.01c	1.6 ± 0.00d	30.3 ± 0.32d
20yE	2.8 ± 0.21e	1.6 ± 0.02d	26.0 ± 0.04e
10yA	3.5 ± 0.01f	1.2 ± 0.00f	22.5 ± 0.03d
10yB	5.8 ± 0.00b	1.2 ± 0.00e	39.6 ± 0.18a
10yC1	6.0 ± 0.01a	1.1 ± 0.00g	36.1 ± 1.13b
10yC2	5.4 ± 0.03d	1.4 ± 0.00b	36.6 ± 0.15b
10yC3	4.8 ± 0.01e	1.3 ± 0.00d	32.5 ± 0.39c
10yD	5.5 ± 0.00c	1.3 ± 0.00c	33.3 ± 0.34c
10yE	2.4 ± 0.02g	1.4 ± 0.01a	20.5 ± 0.02e
TRA	6.6 ± 0.02b	1.1 ± 0.00c	35.9 ± 0.45a
TRB	5.8 ± 0.01c	1.0 ± 0.00d	34.7 ± 0.83b
TRC1	7.0 ± 0.01a	1.0 ± 0.00e	35.7 ± 0.05a
TRC3	3.0 ± 0.00d	1.1 ± 0.00b	20.8 ± 0.03c
TRE	2.6 ± 0.05e	1.4 ± 0.00a	17.3 ± 0.10d
TA	5.3 ± 0.08c	1.0 ± 0.00a	26.6 ± 0.22c
TB	8.9 ± 0.01a	0.7 ± 0.00c	36.7 ± 0.72a
TC1	6.0 ± 0.02b	0.9 ± 0.00b	33.6 ± 0.4b
RRA	26.8 ± 0.36a	0.8 ± 0.01a	66.5 ± 0.48a
RRD	12.5 ± 0.04b	0.7 ± 0.00b	45.7 ± 0.08b
RA	14.1 ± 0.79c	0.6 ± 0.04c	54.2 ± 0.12d
RB	14.9 ± 0.03b	0.6 ± 0.00bc	56.4 ± 0.49c
RC1	11.7 ± 0.01d	0.7 ± 0.00a	57.7 ± 0.63b
RC2	17.9 ± 0.10a	0.7 ± 0.00ab	67.8 ± 0.65a
LBVC1	16.0 ± 0.04b	0.7 ± 0.00c	68.7 ± 2.82a
LBVC3	14.5 ± 0.02c	0.8 ± 0.00b	56.8 ± 0.35b
LBVD	17.4 ± 0.01a	0.8 ± 0.00a	58.6 ± 0.20b

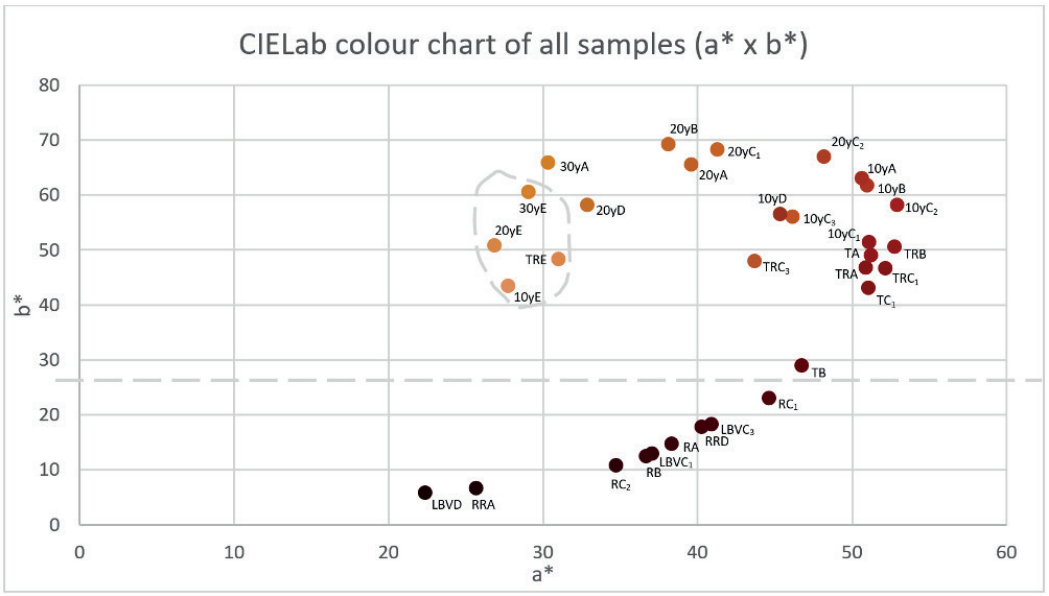

FIGURE 2. Colour chart of all Port wine samples, with the colour representation according to CIELab coordinates.

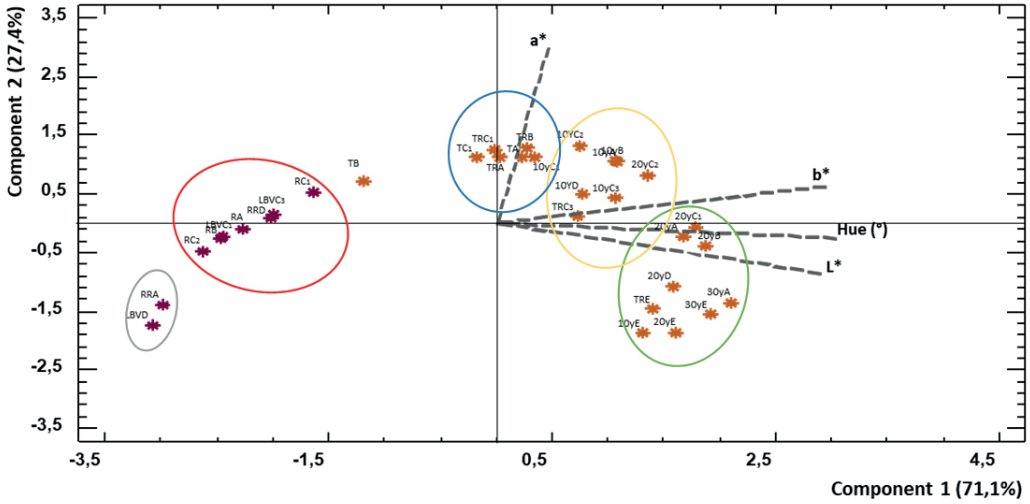

FIGURE 3. Projection of the 32 wines in the first two components of the PCA regarding CIE Lab colour parameters.

grouped almost 100 % according to wine age and wine style, and a few wine samples are outliers (which can be attributed to the different house styles). Two Ruby style samples are highlighted in grey, for which a* has a high negative influence, separating them from the rest of the samples. Chromatically, the Ruby style Ports are very close to each other, showing less variation in the PCA.

2. Anthocyanin results

The anthocyanin concentration results can be seen in Table 2 and Figure 4. Independently of the producer, total concentration of anthocyanins monoglucosides detected by HPLC was always higher for Ruby style Ports than for Tawny style Ports. The highest concentration of anthocyanins was found to be just under 100mg/L of malvidin-3-*O*-glucoside equivalent, for a Ruby style Port from producer C. Anthocyanin concentrations in Tawny style Ports were generally in trace amounts or not detected, apart from Tawny (T) and Tawny Reserve (TR) for producers A, B and C_1.

Total anthocyanins concentration was calculated as the sum of the families of identified anthocyanins. Ruby (R) wines showed the highest concentrations of anthocyanins, ranging from 93.21 mg/L to 29.97 mg/L of Mv-3-*O*-glc equivalent, followed by LBV, which ranged from 36.37 mg/L to 3.30 mg/L, and then by RR, ranging from 34.26 mg/L to 6.28 mg/L. The family of anthocyanins mostly present differed depending on producer and wine type. Regarding the Ruby ports from producer C (R_1 and R_2) the biggest percentage in weight was attributed to the monoglucoside family, in particular to Mv-3-*O*-glc, however, the most abundant pigments in the Ruby ports from all the other producers and in the LBV's from producer C were pyranoanthocyanins, in particular, vitisin A. For the Tawny ports the total anthocyanins range was generally only present in trace

TABLE 2. Summary of anthocyanin content (mg/L). Different letters indicate significant statistical differences between samples belonging to the same Port Style. Average, STD (n = 3 and $p < 0.05$).

Port Wine	Monoglucosides	Vit Type	Acylated glucosides	VinylPhenolic glucosides	Total Anthocyanins
30yA	n.d.	n.d.	n.d.	n.d.	n.d.
30yE	n.d.	n.d.	n.d.	n.d.	n.d.
20yA	n.d.	n.d.	n.d.	n.d.	n.d.
20yB	n.d.	n.d.	n.d.	n.d.	n.d.
20yC1	n.d.	n.d.	n.d.	n.d.	n.d.
20yC2	n.d.	n.d.	n.d.	n.d.	n.d.
20yD	n.d.	n.d.	n.d.	n.d.	n.d.
20yE	n.d.	n.d.	n.d.	n.d.	n.d.
10yA	n.d.	n.d.	n.d.	n.d.	n.d.
10yB	n.d.	n.d.	n.d.	n.d.	n.d.
10yC1	n.d.	n.d.	n.d.	n.d.	n.d.
10yC2	n.d.	n.d.	n.d.	n.d.	n.d.
10yC3	n.d.	n.d.	n.d.	n.d.	n.d.
10yD	n.d.	n.d.	n.d.	n.d.	n.d.
10yE	n.d.	n.d.	n.d.	n.d.	n.d.
TRA	n.d.	1.9 ± 0.06	n.d.	n.d.	1.9 ± 0.06
TRB	n.d.	1.7 ± 0.11	n.d.	n.d.	1.7 ± 0.11
TRC1	n.d.	4.4 ± 0.15	n.d.	0.3 ± 0.02	4.7 ± 0.17
TRC3	n.d.	n.d.	n.d.	n.d.	n.d.
TRE	n.d.	n.d.	n.d.	n.d.	n.d.
TA	0.8 ± 0.09b	1.8 ± 0.02b	n.d.	n.d.	2.6 ± 0.07b
TB	1.0 ± 0.17a	6.8 ± 0.45a	n.d.	n.d.	7.8 ± 0.50a
TC1	0.5 ± 0.20c	0.4 ± 0.12c	n.d.	n.d.	0.9 ± 0.09c
RRA	14.6 ± 1.23	15.1 ± 0.56a	4.6 ± 0.09	n.d.	34.3 ± 1.68a
RRD	n.d.	4.8 ± 0.19b	n.d.	1.5 ± 0.38	6.3 ± 0.36b
RA	14.0 ± 0.09c	18.7 ± 0.15b	4.6 ± 0.14c	n.d.	37.4 ± 0.23c
RB	9.0 ± 0.86d	18.3 ± 1.65b	2.6 ± 0.33d	n.d.	30.0 ± 1.06d
RC1	50.6 ± 0.42a	17.2 ± 0.59c	25.4 ± 0.58a	n.d.	93.2 ± 1.57a
RC2	35.4 ± 0.74b	21.2 ± 1.22a	16.4 ± 1.36b	1.1 ± 0.03	74.1 ± 2.53b
LBVC1	12.7 ± 0.38a	17.1 ± 0.59a	5.1 ± 0.65	1.5 ± 0.45	36.4 ± 1.34a
LBVC3	0.4 ± 0.05b	14.4 ± 0.28b	n.d.	1.6 ± 0.08	16.4 ± 0.23b
LBVD	n.d.	3.3 ± 0.16c	n.d.	n.d.	3.3 ± 0.16c

*n.d.–anthocyanins not detected or below limit of detection.

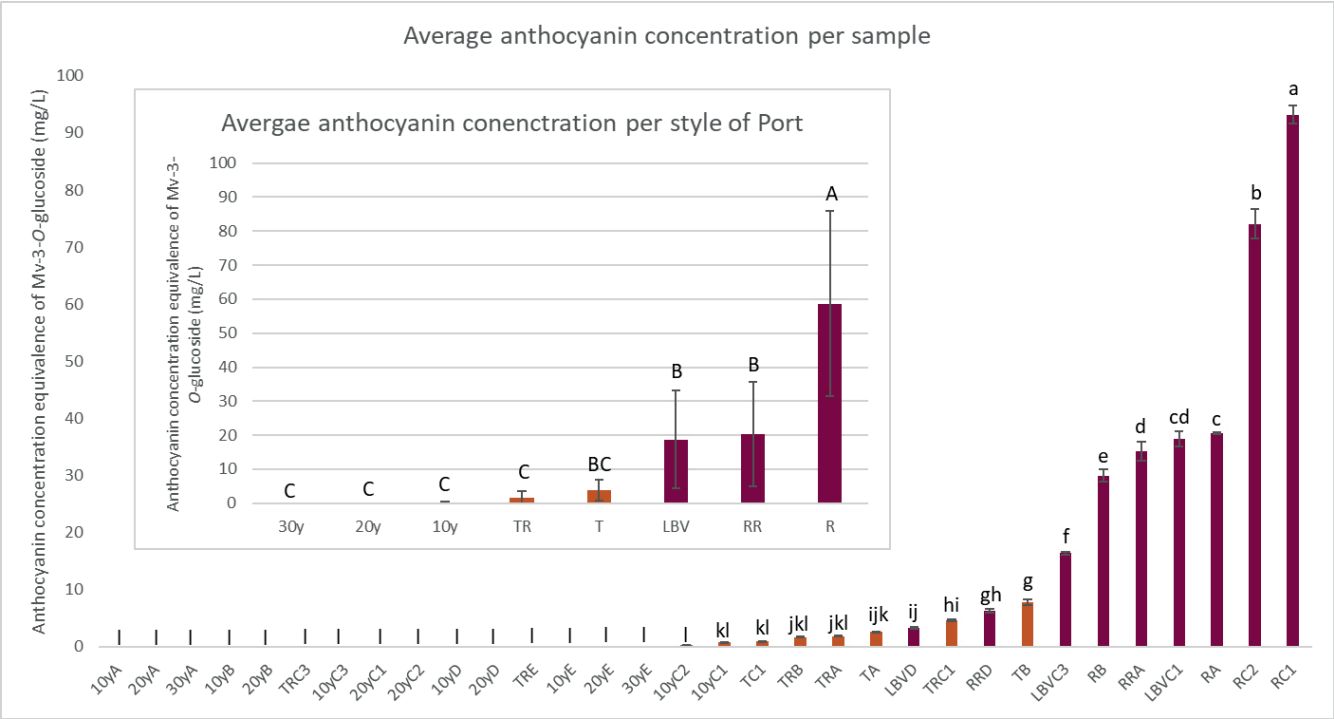

FIGURE 4. Average anthocyanin concentration per sample (1) and per style (2). Difference in letters indicates significant statistical difference (one way ANOVA at a 95 % confidence level). Means ± STD (n = 3).

amounts or not detected, with the exception of Tawny (T) and Tawny Reserve (TR) for producers A, B and C_1, ranging from 7.76 mg/L to 2.63 mg/L in T and from 4.68 mg/L to 1.86 mg/L in TR. The main group of pigments was pyranoanthocyanins, containing notably high amounts of vitisin A. The Tawny wines containing only traces of anthocyanins showed a peak corresponding to coumaroylated vitisin A (eluting at around min 10), and in some cases to visitin A; these are the only peaks that can be observed in the chromatogram.

Figure 5 represents the elution of different wines from each producer at the same scale.

The most identifiable peaks eluted at around min 5, 7, 10 and 12, and they correspond to malvidin-3-*O*-gluoside, vitisin A, coumaroyl vitisin A and malvidin-3-(6”-p-coumaroylglucoside) respectively.

In order to better understand the peaks of these wines, the different wines from producer A were represented in chromatograms (Figure 6 a) and b)). In Figure 6 a) the chromatograms of Ruby, Ruby Reserve and Tawny have been overlaid at the same scale, whilst in Figure 6 b) Tawny, Tawny Reserve, 10-year-old Tawny, 20-year-old Tawny and 30-year-old Tawny are shown. The main identified peaks are labelled from A to G: A) Malvidin-3-*O*-glucoside, B) Vitisin A type Peonidin, C) Vitisin A, D) Acetyl Vitisin A, E) Coumaroyl Vitisin A, F) malvidin-3-(6”-acetylglucoside) and G) Malvidin-3-(6”-p-coumaroylglucoside). The remaining peaks were not identified due to their low intensity and the interferences of the base line compounds that limited the achievement of a correct mass spectrum.

DISCUSSION

Port wines from different categories are known to differ in lightness, colour intensity (CI) and tonality (Table 1). Ruby style Ports are more opaque and more intensely coloured than tawny style Ports, because they have a naturally higher pigment concentration due to the different oxidative ageing conditions. As can be seen in Figure 2, Tawny style Ports, which undergo a more oxidative process during ageing, are more yellow in colour, and have higher tonality and hue (º) values. In fact, colour hue (º) ranges from deep red in the Ruby wines to light gold in the Tawny wines, and these differences in colour are highly influenced by the ageing conditions (Figure 2). The long exposure to oxidative ageing, as well as annual racking, of the Tawny style Ports compared to the Ruby style Ports are the main reason for the colour evolution during ageing, oxygen being the primary factor affecting this evolution (Ho *et al.*, 2001). The deep red colour of the Ruby style ports means they have higher values for colour intensity compared to Tawny style wines (results not shown). The higher expression of colour in these wines can be linked to the concentration of pyranoanthocyanin-type anthocyanins found in them. These types of anthocyanins are known for having a higher molar extinction coefficient than anthocyanin monoglucosides, contributing to a large extent to the overall colour expression of the wine (de Freitas and Mateus, 2006; de Freitas and Mateus, 2011).

Independently of the producer, the concentration of anthocyanins was always higher in Ruby style Ports. These findings are in agreement with findings by previous authors of other studies on Port wines (Pinho *et al.*, 2012; Romero and Bakker, 2001).

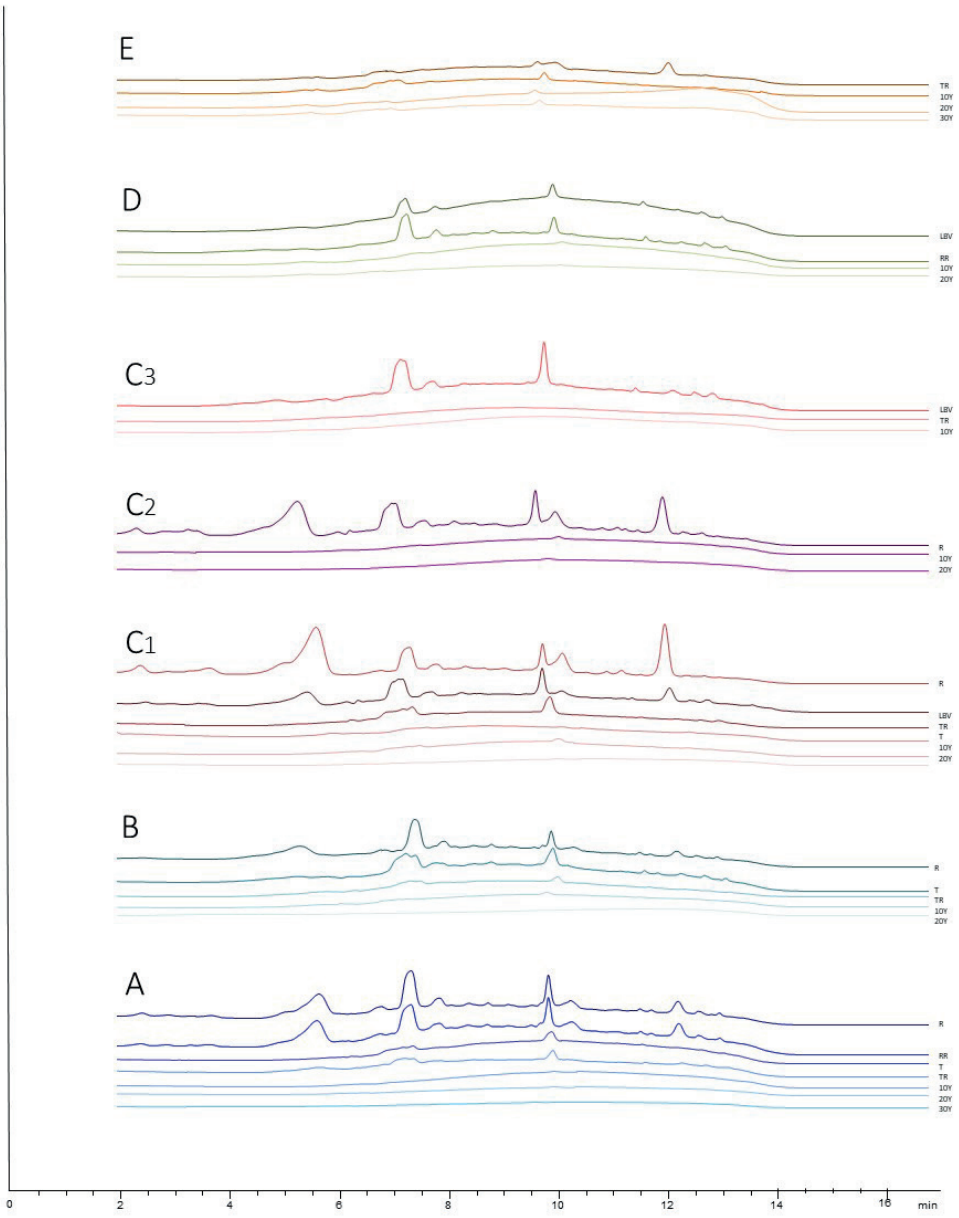

FIGURE 5. Representative chromatograms of all wines from all producers at the same scale.

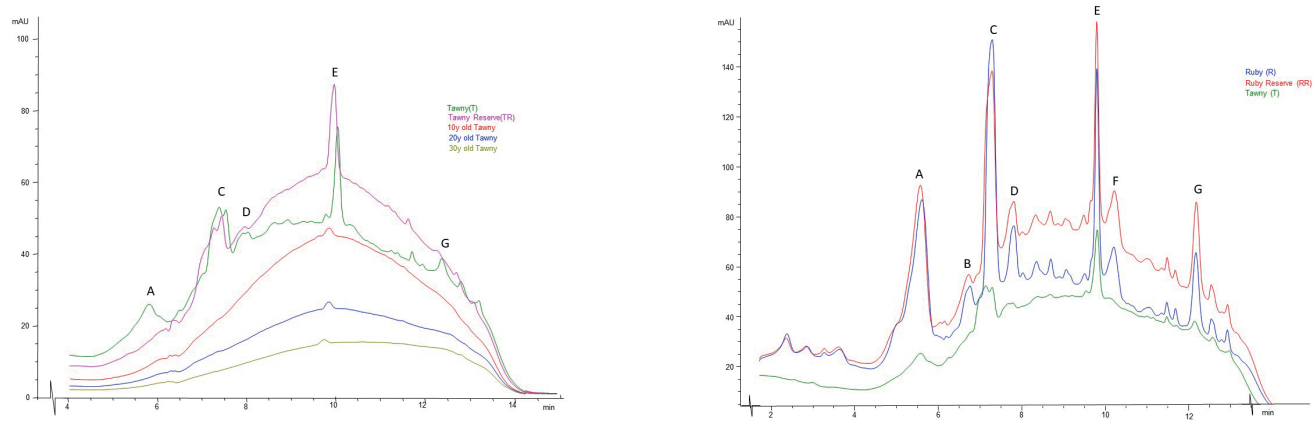

FIGURE 6. Chromatographic profiles of anthocyanins in Port wines at 525 nm from producer A. 1) Ruby style ports and Tawny (T); 2) Tawny style Ports. A) Malvidin-3-O-glucoside, B) Vitisin A type Peonidin, C) Vitisin A, D) Acetyl Vitisin A, E) Coumaroyl Vitisin A, F) Malvidin-3-(6″-acetylglucoside) and G) Malvidin-3-(6″-p-coumaroylglucoside).

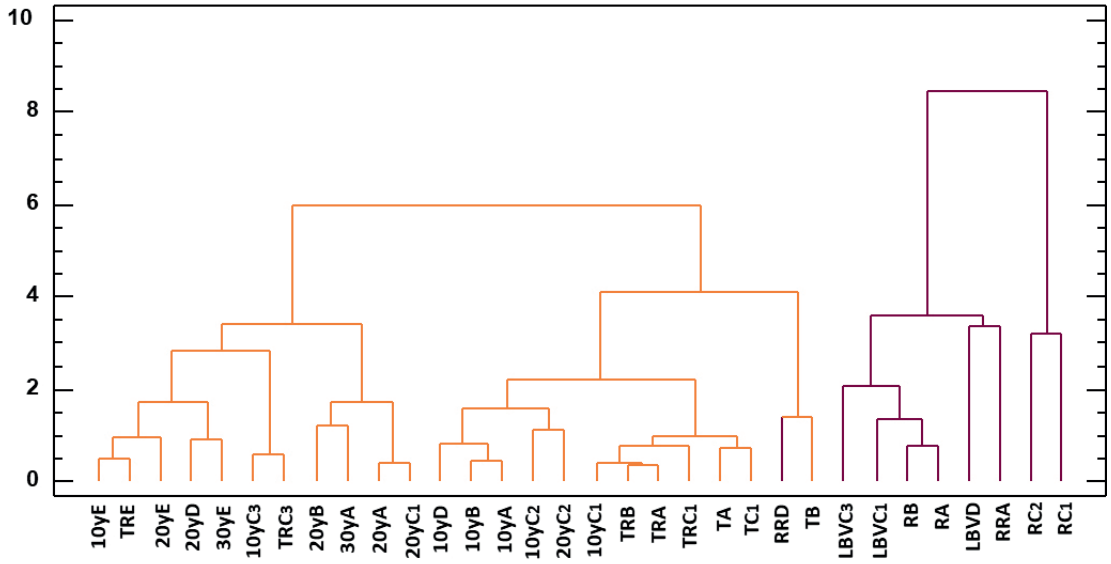

FIGURE 7. Cluster analysis using the furthest neighbour method and Euclidean distances.

As a general rule, these Port wines are matured in either stainless steel vessels, or very big oak vessels for a minimum period of 2 years for Ruby ports, 3 years for Ruby Reserve and 4 years for LBV. This latter category, in contrast to Vintage Ports, does not carry on ageing in the bottle under reductive ageing conditions. This results in a very slow loss of anthocyanins compared to Tawny style Ports, which are aged in small (up to 600L) oak casks, thus resulting in a faster loss of anthocyanins. The evaporation of the wines during ageing also makes the wine more viscous, making analysis more difficult and less efficient due to potential clogging, and back-pressure build-up.

Even though in some full-bodied red wines anthocyanin concentrations can be higher than 2 g/L, they are typically around 500 mg/L (He *et al.*, 2012). In the present study, the highest concentration that was found was just under 100 mg/L in a Ruby Port. The concentration of anthocyanins is much lower in Port wines than in red table wines; thus, the identification and quantification of the peaks in Port wines is much more complicated, not only due to the size of the peak (which is directly correlated with the concentration) but also to the complex matrix of the Port wine (high sugar content and high alcohol). The elution time provided information about the peaks that were difficult to recognise due to their low concentrations, and, in some cases, were close to the limit of quantification and detection; for example, the peaks eluting at min 10 as can be seen in Figure 6. The chromatograms differ due to the changes in anthocyanins composition, and also largely due to the differences in concentration of these pigments in each type of Port wine.

A cluster analysis (Figure 7) on the major studied chromatic and pigment parameters was performed to assess the feasibility of using these results to differentiate between the different Port categories.

Two clusters were formed in this analysis: one of the Ruby style Ports (in purple) and the other of the Tawny style Ports (in orange). Distinct groups can be identified within each cluster, depending on the distances established in the furthest neighbour method. In the Ruby cluster, pigment concentration parameters play a more significant role in group differentiation, whilst in the Tawny cluster the chromatic parameters contribute more to group formation. Nevertheless, it is possible to characterise the wines in terms of total pigments concentrations: the higher concentrations are associated with the cluster to the right, whilst the bigger cluster to the left comprises all the Tawny style Ports whose anthocyanin concentrations have considerably decreased in comparison to Ruby style Ports. A more detailed analysis reveals the neighbouring Ports to be more similar to each other in anthocyanin composition, not only in terms of concentration but in the chemical nature of the pigments as well. Thus, Tawny style Ports that underwent different ageing are also grouped together in 6 sub-clusters at level 2, suggesting similar anthocyanins concentration and chemical composition.

CONCLUSIONS

Depending on type of Port wine, the concentrations of non-acylated and acylated anthocyanins were found to vary, with Ruby style Ports having the highest levels and Tawny style Ports the lowest. The chromatic characteristics observed in each style are caused by the nature of these molecules and other pigments associated with ageing, and not just their concentration. Some ranges show a fit in terms of overall concentration and nature of anthocyanins for each of the Port wine styles, notwithstanding the differences in concentrations recorded for the same Port style from different producers. In this regard, tools that would authenticate the style of such distinctive Portuguese wines include the characterisation of anthocyanins with HPLC-DAD/MS, and chromatic values with UV-Vis and CIELab coordinates.

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