PHENOLIC COMPOSITION AND TOTAL ANTIOXIDANT CAPACITY ANALYSIS OF RED WINE VINEGARS COMMERCIALIZED IN PORTUGUESE MARKET

ANÁLISE DA COMPOSIÇÃO FENÓLICA E DA CAPACIDADE ANTIOXIDANTE TOTAL DE VINAGRES DE VINHO TINTO COMERCIALIZADOS NO MERCADO PORTUGUÊS

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SUMMARY

In the last years, there has been an increase in consumption of wine vinegars in Portugal. Thus, the aim of this work was to evaluate the phenolic composition and total antioxidant capacity from several commercial red wine vinegars commercialized in Portuguese market. Several parameters were evaluated: general phenolic composition, chromatic characteristics, individual anthocyanins and phenolic acids by HPLC, and total antioxidant capacity by two methodologies (DPPH and ABTS). For the different parameters analyzed, the red wine vinegars samples studied differed significantly. Vinegars with higher phenolic content tend to have lower lightness, but higher values of the red component color. High diversity of anthocyanins was detected, with some of the vinegars being distinguished by having significantly higher values of anthocyanins compared to the others, as was detected for the generality of the other phenolic parameters. The total antioxidant capacity was positively correlated with the different phenolic parameters. Finally, higher total antioxidant capacity was detected for the phenolic fraction containing anthocyanins and polymeric proanthocyanidins. The results obtained confirm that red wine vinegars are good sources of phenolic compounds and antioxidants. However, there is a great diversity of values for the various red wine vinegars commercialized in the Portuguese market.

RESUMO

Nos últimos anos tem ocorrido um aumento do consumo de vinagres de vinho em Portugal. Assim, o objetivo deste trabalho foi avaliar a composição fenólica e a capacidade antioxidante total de diversos vinagres de vinho tinto comercializados no mercado português. Vários parâmetros foram avaliados: composição fenólica geral, características cromáticas, antocianinas individuais e ácidos fenólicos por HPLC, e a capacidade antioxidante total por duas metodologias (DPPH e ABTS). Para os diferentes parâmetros analisados, as amostras de vinagres de vinho tinto estudadas diferiram significativamente. Vinagres com elevado conteúdo fenólico tenderam a ter baixos valores de luminosidade, mas elevados valores da componente vermelha da cor. Elevada diversidade de antocianinas foi detetada, com alguns dos vinagres a poderem ser significativamente distinguidos por apresentarem valores elevados de antocianinas comparativamente com os restantes, tal como foi detetado para a generalidade dos outros parâmetros fenólicos. A capacidade antioxidante total foi correlacionada positivamente com os diferentes parâmetros fenólicos. Finalmente, elevada capacidade antioxidante total foi detetada para a fração fenólica contendo antocianinas e proantocianidinas poliméricas. Os resultados obtidos confirmam que os vinagres de vinho tinto são boas fontes de compostos fenólicos e de antioxidantes. No entanto, existe uma grande diversidade de valores para os vários vinagres de vinho tinto comercializados no mercado português.

Key words: phenolic compounds, antioxidant capacity, red wine vinegars.
Palavras-chave: compostos fenólicos, capacidade antioxidante, vinagres de vinho tinto.

INTRODUCTION

Vinegars are the result of a two-step fermentation process over almost any fermentable carbohydrate source (fruits, honey, cereals, etc.). First, during alcoholic fermentation, yeasts transform sugars into ethanol, which is then converted into acetic acid during the second fermentation by acetic bacteria. Vinegars have been produced by humankind since the early days of agriculture until today, throughout the

102

different continents and different cultures. This product has been employed as food ingredient and preservative, as flavor enhance, and also as ordinary remedy against illness (Mazza and Murooka, 2009). Thus, several authors reported several health properties of vinegars, namely antimicrobial activity (Luo *et al.*, 2004; Medina *et al.*, 2007; Ozturk *et al.*, 2015), positive action on blood glucose regulation (Ebihara and Nakajima, 1998; Johnston and Buller, 2005), blood pressure control, digestion aid, appetite stimulation (Xu *et al.*, 2007) and promotion of calcium absorption (Hadfield *et al.*, 1989; Xu *et al.*, 2007).

In the products derived from fruits and cereals, like vinegars, phenolic compounds are present (Andlauer *et al.*, 2000; Verzelloni *et al.*, 2007; Cerezo *et al.*, 2008). Vinegars made from red wine could usually have higher content of phenolics. According to several authors (Alonso *et al.*, 2004; Verzelloni *et al.*, 2007; Budak and Guzel-Seydin, 2010), red wine vinegars contain higher concentration of benzoic acid, caftaric acid, coutaric acid, chlorogenic acid, caffeic acid and ferulic acid. Furthermore, the red wine composition used, production technology and aging process has an important effect on functional properties of wine vinegars, namely in phenolic composition (Morales *et al.*, 2001; Budak and Guzel-Seydin, 2010; Cerezo *et al.*, 2010). According to Mas *et al.* (2014), the acetic acid bacteria species determine the quality of vinegars, although the final quality is a combined result of production method procedures, wood contact, and aging. In addition, according to several authors one of the key factors that will determine wine vinegars quality is the raw material used, particularly the quality of wine used (Tesfaye *et al.,* 2002; Ho *et al.,* 2017). All of these factors determine the vinegar chemical composition and sensory properties.

Consistent with several studies, the strong antioxidant effect of vinegars is due to their bioactive compounds including: carotenoids, phytosterols and also phenolic compounds, represented by, among other, flavonoids, tannins, anthocyanins or phenolic acids (Masino *et al.*, 2008; Charoenkiatkul *et al.*, 2016; Slobodníková *et al.*, 2016). Recently, Kawa-Rygielska *et al.* (2018) found a highest concentration of biologically-active compounds in the vinegars obtained from different cherry cultivars, particularly from vinegars made with a red-fruit cherry variety.

In Portugal, in 2017 the market for vinegar production represented a value around of 11.4 million euros, having grown 5% compared to the year of 2016. On the other hand, the consumption of vinegar is mainly vinegar made from white wine, which accounts for 67% of the total vinegar consumed, following the cider vinegar (Gonçalves, 2017). Thus, red wine vinegar consumption is still poorly representative.

Nowadays, the presence of diverse red wine vinegars in the market and consumer demand for quality condiments stimulates the characterization and establishment of parameters for quality control. Therefore, considering the phenolic compounds contribution to human health, the study of this compounds group and the potential antioxidant capacity associated will be very relevant for red wine vinegars characterization. In addition, it is important to take into consideration that there is a considerable lack of information about this topic, in particular for the red wine vinegars commercialized in the Portuguese market. Thus, the main purpose of this study was to investigate the phenolic composition of red wine vinegars from different sources commercialized in the Portuguese market and respective total antioxidant capacity.

MATERIAL AND METHODS

Chemicals

Gallic acid and malvidin-3-monoglucoside standards were purchased from Extra-Synthese (Genay, France) while caffeic acid standard was purchased from Sigma-Aldrich (St. Louis, USA). Solvents used for HPLC analysis (methanol, formic acid and acetonitrile) were purchased from Fisher Scientific (Loughborough, United Kingdom). For total antioxidant capacity evaluation, 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2.2-diphenyl-1-pirylhydrazyl (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Finally, Folin-Ciocalteau reagent, ethyl acetate, methanol and hydrochloric acid were purchased from Merck (Darmstadt, Germany).

Red wine vinegars samples

A total of seven different representative commercial Portuguese red wine vinegars were purchased from the market in 2016. A total of twenty one bottles or red wine vinegars were purchased (three bottles per brand) for physicochemical analysis from retail stores in the Portuguese market (Viseu, Portugal). All commercial red wine vinegars were stored in the laboratory at a constant temperature of 20 °C ± 2°C prior to analysis. General characteristics, namely the sample codification used in this work, titratable acidity and other additional information provided by the producing company on the label of each vinegar

bottle are listed in Table I. All red wine vinegars tested were previously filtered (pore diameters 20 μm) before analysis.

Vinegars sample coding	Titratable acidity (g/100 mL)[a]	Additional information from vinegar producer on the labels of the vinegar bottles
RV1	7.0	Aged in American oak wood barrels
RV2	7.0	Aged in American oak wood barrels
RV3	6.5	[b]
RV4	7.0	Aged in oak wood barrels
RV5	6.0	[b]
RV6	6.0	[b]
RV7	7.0	Aged

[a] Values expressed in acetic acid and mentioned by the producers on the labels of the vinegar bottles; [b] Without additional information from the vinegar producer.

General chemical and phenolic composition analysis

The red wine vinegars samples tested in our study were analyzed for pH, titratable acidity, fixed acidity, volatile acidity, dry extract and ashes using the analytical methods recommend by the AOAC (2016).

The total polyphenols content of the red wine vinegars was determined using the Folin-Ciocalteau spectrophotometric method according to the methodology described by Prior *et al.* (2005), while non-flavonoid phenols and flavonoid phenols were determined using the methodology described by Kramling and Singleton (1969). Results were expressed as mg/L of gallic acid-equivalent means of calibration curves with standard gallic acid. Total anthocyanins were determined by the sulfur dioxide bleaching procedure using the method described by Ribéreau-Gayon and Stonestreet (1965). All measurements were performed in triplicate for each red wine vinegar sample.

Chromatic characteristics analysis

Using the CIELab method, red wine vinegars chromatic characteristics (scanned from a range of 380-770 nm) was determined by the calculation of several chromatic parameters: L^* (%) (lightness), a^* (redness), b^* (yellowness), chroma $[C^* = [(a^*)^2 + (b^*)^2]^{1/2}]$ and hue-angle $[h^o = \tan g^{-1}(b^*/a^*)]$, according to OIV (2012) method. All measurements were performed in triplicate for each red wine vinegar sample.

Chromatographic analysis of individual anthocyanins

Individual monomeric anthocyanins from the commercial red wine vinegars were analyzed using HPLC-DAD Dionex Ultimate 3000 Chromatographic System (Sunnyvale, California, USA) equipped with a quaternary pump Model LPG-3400 A, an auto sampler Model ACC-3000, an thermostatted column compartment (adjust to 25 ºC) and a multiple Wavelength Detector MWD-300. The column (250 x 4.6 mm, particle size 5 μm) was a C_{18} Acclaim® 120 (Dionex, Sunnyvale, California, USA) protected by a guard column of the same material. The solvents were: (A) 40% formic acid, (B) pure acetonitrile and (C) bidistilled water. The individual anthocyanins were analyzed by HPLC using the method described by Dallas and Laureano (1994). Thus, initial conditions were 25 % (A), 10 % (B), and 65 % (C), followed by a linear gradient from 10 to 30% (B), and 65 to 45 % (C) for 40 min, with a flow rate of 0.7 mL/min. Each red wine vinegar sample was previously concentrated up to 25 times. The injection volume was 20 μl. The detection was made at 520 nm. A Chromeleon software program version 6.8 (Sunnyvale, California, USA) was used. The quantification of the individual anthocyanins was made by mean of calibration curve obtained with standard solutions of malvidin-3-monoglucoside. The chromatographic peaks of anthocyanins were identified according to reference data previously described by Dallas and Laureano (1994). All analyses were done in triplicate from each red wine vinegar sample.

Chromatographic analysis of individual phenolic acids

For the individual phenolic acids, the chromatographic system, including the column and software program, was the same already described for individual monomeric anthocyanins. However, the elution conditions used were implemented based on the methodology described by Guise *et al.* (2014). Thus, solvent (A) was 5% aqueous formic acid and solvent (B) was pure methanol. The elution program was the following: 5% (B) from zero to 5 min followed by a linear gradient up to 65% (B) until 65 min and from 65 to 67 min down to 5% (B). The flow was 1 mL/min and column temperature was

maintained at 35 °C during the run. Detection was performed at 280 and 325 nm with sample injection volume of 20 µL. Each red wine vinegar sample was previously concentrated up to 25 times. The chromatographic peaks of the individual phenolic acids were identified according to reference data previously analyzed also by Guise *et al.* (2014). The quantification of each individual phenolic acid was made by mean of calibration curves obtained with standard solutions of caffeic acid. All analyses were done in triplicate from each red wine vinegar sample.

Total antioxidant capacity

The total antioxidant capacity from the commercial red wine vinegars studied was determined by the use of two different methods: ABTS and DPPH. ABTS method is based on decolouration that occurs when the radical cation ABTS$^+$ is reduced to ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) (Re *et al.*, 1999). The radical was generated by reaction of a 7 mM solution of ABTS in water with 2.45 mM potassium persulphate (1:1). The assay was made up with 980 µl of ABTS$^+$ solutions and 20 µL of the sample (at a dilution of 1:50 in water). The reaction takes place in darkness at room temperature. Absorbance measurements at 734 nm were made after 15 min of reaction time.

The procedure used to determine antioxidant capacity using DPPH method is described by Brand-Williams *et al.* (1995). Briefly, 0.1 mL of different sample concentrations was added to 3.9 mL of 2.2-diphenyl-1-pirylhydrazyl (DPPH) methanolic solution (25 mg/L). The DPPH solution was prepared daily and protected from the light. Absorbance at 515 nm was measured after 30 min of reaction at 20 ºC. The reaction was carried out under shaking in closed eppendorf tubes at 20 ºC. Methanol was used as a blank reference. Total antioxidant capacity results were expressed as Trolox equivalents (TEAC mM), using the calibration curve previously made. All measurements were done in triplicate from each red wine vinegar sample.

The total antioxidant capacity from the commercial red wine vinegars studied was also determined in three different phenolic fractions, by the use of the methodology previously described by Sun *et al.* (2006). Thus, each sample was passed through the preconditioned neutral DSC-18a column. For precondition of DSC-18a column, 60 mL of methanol was used to activate the column and then the column was washed with 120 ml of distilled water, followed by preconditioning with 60 mL of commercial pH 7.0 phosphate buffer before utilization. Fractionation started with 50 mL of diluted red wine vinegar sample to elute fraction I (phenolic acids). After, the column was washed with 100 mL of distilled water and dried under vacuum. Elution with 100 mL of ethyl acetate allowed to isolate fraction II (monomers and oligomers of proanthocyanidins), which was recovered with methanol/water (20:80 v/v). Finally, fraction III (anthocyanins and polymeric proanthocyanidins) fixed on the column was eluted with 100 mL of methanol acidified with 0.1% hydrochloric acid.

Statistical analysis

Results from triplicate experiment are expressed as mean value ± standard deviation. In order to determine whether there was a statistically significant difference between the results obtained for the different analytical parameters studied from the commercial red wine vinegars samples analyzed, an analysis of variance and comparison of treatment means (ANOVA, one-way) were carried out. Differences between means were tested using Tukey test ($p < 0.05$). In addition, a principal component analysis (PCA) was also used to analyze the data and study the relations between the commercial red wine vinegars studied and their composition.

All analyses were performed using SPSS Software version 25.0 (SPSS Inc. Headquarters, Chicago, Illinois, U.S.A.).

RESULTS AND DISCUSSION

General chemical and phenolic composition

The pH, total, fixed and volatile acidity, dry extract and ashes content of all commercial red wine vinegars samples are summarized in Table II. The pH ranged from 2.93 to 3.12 and the average value was 3.04. For titratable acidity the values quantified varied from 6.42 to 7.68 g of acetic acid/100 mL and the average value was 7.11 g of acetic acid/100 mL, while volatile acidity ranged from 6.31 to 7.49 g of acetic acid/100 mL and the average value was 6.88 g of acetic acid/100 mL. Acetic acid is the major acid which contributes for volatile acidity of vinegars as a consequence of ethanol conversion into acetic acid during the acetous fermentation by acetic bacteria.

TABLE II

General chemical composition of the commercial red wine vinegars studied

Composição química geral dos vinagres de vinho tinto comerciais estudados

Red wine vinegars	pH	Titratable acidity (g/100 mL)[a]	Fixed acidity (g/100 mL)[a]	Volatile acidity (g/100 mL)[a]	Dry extract (%)	Ashes content (g/L)
RV1	3.12±0.01	7.56±0.03	0.11±0.01	7.45±0.03	1.68±0.02	2.73±0.08
RV2	3.02±0.02	7.68±0.05	0.19±0.01	7.49±0.04	2.11±0.03	2.66±0.05
RV3	2.93±0.01	6.51±0.02	0.17±0.02	6.34±0.02	1.68±0.01	2.40±0.04
RV4	2.97±0.01	7.53±0.06	0.19±0.01	7.31±0.04	1.68±0.03	2.72±0.02
RV5	3.10±0.02	6.42±0.003	0.11±0.03	6.31±0.03	1.35±0.01	2.37±0.01
RV6	3.05±0.01	6.51±0.02	0.14±0.01	6.37±0.03	2.01±0.02	2.38±0.05
RV7	3.10±0.01	7.59±0.01	0.16±0.02	7.43±0.05	2.11±0.03	2.98±0.03
AV	3.04±0.07	7.11±0.55	0.15±0.03	6.88±0.54	1.82±0.28	2.59±0.22
CV	2.19	7.75	20.60	7.82	15.25	8.68
R	2.93-3.12	6.42-7.68	0.11-0.19	6.31-7.49	1.35-2.11	2.37-2.98

[a] Values expressed in acetic acid; AV, average values; CV (%), coefficient of variation; *R*, range; Values are given as the mean ± SD of the three experiments.

The total dry extract of vinegar represents the mineral and organic material of a vinegar, while ashes represents the mineral residue of the sample. Thus, concerning to these two parameters and as expected, a very low values were obtained. The total dry extract quantified in the different commercial red wine vinagers ranged from 1.35 to 2.11 % and the average value was 1.82 %. In addition, for ashes the values ranged from 2.37 to 2.98 g/L with an average value of 2.59 g/L.

In general, all of these general chemical parameters are according to the results previously obtained by other authors in different red wine vinegars (Rizzon and Miele, 1998; Pinsirodom *et al*., 2008; Budak *et al*., 2010) and also according to portuguese legislation for vinagers comercialized in Portugal (Decreto-Lei nº 174/2007).

Total phenolic compounds, flavonoid and non flavonoid phenols and also total anthocyanins are presented in Table III. In general, the results show that the levels of all general phenolic parameters in the seven commercial red wine vinegars differed significantly, particularly for non flavonoid phenols and total anthocyanins. Thus, total polyphenols values ranged from 720.7 to 1052.7 mg/L of gallic acid equivalents (average value of 893.8 mg/L of gallic acid equivalents), while flavonoid phenols ranged from 535.8 to 766.1 mg/L of gallic acid equivalents (average value of 670.8 mg/L of gallic acid equivalents). Non flavonoid phenols values ranged from 70.7 to 305.3 mg/L of gallic acid equivalents (average value of 223.0 mg/L of gallic acid equivalents). The coefficient of variation was lower for total phenols (11.0 %) and flavonoid phenols (10.3 %) than the coefficient of variation calculated for non flavonoid phenols (38.8 %) and total anthocyanins (27.54 %). For total anthocyanins the values quantified varied from 14.29 to 31.08 mg/L of malvindin-3-monoglucoside equivalents and the average value was 21.11 mg/L of malvindin-3-monoglucoside equivalents.

For three of the seven commercial red wine vinegars studied, they are mentioned by producers as having had an aging period in contact with oak wood (Table I), in particular in contact with American oak wood (RV1 and RV2 samples), while for one of them, only aging with oak wood is mentioned (RV4 sample). In addition, another label stated that it was simply subjected to an aging process (RV7 sample), without mentioning the aging form.

TABLE III

General phenolic composition and chromatic characteristics by CieLab method of the commercial red wine vinegars studied

Composição fenólica geral e características cromáticas pelo método CieLab dos vinagres de vinho tinto comerciais estudados

Red wine vinegars	General phenolic composition				CieLab coordinates				
	Total phenolic compounds (mg/L)[a]	Flavonoid phenols (mg/L)[a]	Non flavonoid phenols (mg/L)[a]	Total anthocyanins (mg/L)[b]	$L*$	$a*$	$b*$	$c*$	h^o
RV1	886.9[a]±13.5	667.3[a]±5.0	219.6[a]±1.2	20.82[a]±3.11	49.25[a]±1.23	41.28[a]±0.65	49.69[a]±0.45	42.87[a]±2.32	82.72[a]±1.21
RV2	720.7[b]±5.1	650.0[a]±1.8	70.7[b]±0.5	14.29[b]±0.78	53.57[a]±0.98	38.07[b]±1.03	33.72[b]±1.21	39.67[a]±3.41	78.87[a]±0.98
RV3	840.1[a]±17.6	535.8[b]±1.2	304.3[c]±0.9	16.36[b]±0.71	55.87[a]±2.31	36.38[b]±2.31	45.34[a]±0.98	38.00[a]±0.65	98.61[b]±1.78
RV4	980.8[c]±9.0	658.7[a]±1.8	322.1[c]±1.4	18.98[a]±1.67	51.73[a]±1.03	39.43[a]±1.50	57.77[c]±1.47	41.03[a]±0.99	97.93[b]±2.09
RV5	1052.7[d]±16.9	747.4[c]±2.7	305.3[c]±2.4	28.38[c]±1.36	36.54[b]±0.81	51.39[c]±0.98	27.96[b]±1.01	52.93[b]±2.31	39.88[c]±1.11
RV6	921.8[a]±0.7	766.1[c]±1.2	155.7[d]±1.2	31.08[c]±1.59	41.06[c]±1.21	47.65[c]±2.12	9.37[d]±0.98	49.20[b]±1.21	33.20[d]±0.58
RV7	853.6[a]±22.1	670.3[a]±3.9	183.3[d]±3.3	17.84[a]±1.33	47.89[d]±1.98	42.31[a]±1.10	43.46[a]±1.43	43.89[a]±0.76	73.50[a]±3.45
AV	893.8±98.6	670.8±69.4	223.0±86.6	21.11±5.81	47.99±6.41	42.36±4.98	38.19±14.88	43.94±4.95	72.10±24.15
CV	11.0	10.3	38.8	27.54	13.36	11.75	38.90	11.26	33.49
R	720.7-1052.7	535.8-766.1	70.7-305.3	14.29-31.08	36.54-55.87	36.38-51.39	9.37-57.77	38.00-52.93	33.20-98.61

[a] Values expressed in mg/L of gallic acid equivalents; [b] Values expressed in mg/L of malvidin-3-monoglucoside equivalents; AV, average values; CV (%), coefficient of variation; R, range; $L*$ (%) (lightness); $a*$ (chromatic coordinate from green to red); $b*$ (chromatic coordinate from blue to yellow); $C*$ (Chroma); h^o (hue-angle); Values are given as the mean ± SD of the three experiments. Different letters in a column indicate statistically significant differences between the red wine vinegars tested according to the Tukey test ($p < 0.05$).

On the other hand, for three of the commercial red wine vinegars (RV3, RV5 and RV6 samples), the producers did not mention if any kind of aging process occurred. In this sense, it is clear that when the values of the phenolic composition from the vinegars studied, in particular total phenolic content, is related with the information provided by the vinegars producers, it is not possible to verify a clear relation, in particular considering the mention of the aging process. Several works related the red wine vinegars production with the phenolic composition. Thus, the maceration time used during red wine production (Yokotsuka *et al*., 2000; Jordão *et al*., 2012), the vinegar aging time and particularly the use of different wood species (Tesfaye *et al*., 2004; Durán *et al*., 2011; Cerezo *et al*., 2014), have an important impact on the phenolic composition of vinegars. Thus, according to these authors, a long maceration process during the first fermentation and the use of aging process in contact with wood, increase the phenolic content of red wine vinegars. However, it is important to note that acetic fermentation is associated with higher decrease in polyphenols than alcoholic fermentation, in particular anthocyanins (Andlauer *et al*., 2000; Ubeda *et al*., 2013; Ordoudi *et al*., 2014). In addition, the substrate selection for vinegars production is an important parameter to take

into account the final phenolic content of fruit vinegars, including for the vinegars produced from red wines (Kelebek *et al*., 2017).

Regarding the red wine vinegars color parameters, the results obtained for the chromatic characteristics by the CIELab method are shown also in Table III. For lightness values ($L*$), significantly lower values were detected for RV5 and RV6 red wine vinegars samples, 36.54 and 41.06 expressed by the CIELab coordinates, respectively. The remaining red wine vinegars had in general similar $L*$ values that ranged from 47.89 to 55.87 expressed by the CIELab coordinates. It is important to note that the red wine vinegars that showed the lowest $L*$ values corresponded to the vinegars sample that exhibited the higher phenolic content, in particular total phenols and total anthocyanins. This higher phenolic content contributed to a less brightness and as such a more pronounced color.

Concerning $a*$ (redness) values, they varied from 36.38 to 51.39 expressed by the CIELab coordinates (average value of 42.36). Again RV5 and RV6 red wine vinegars samples showed the significantly higher $a*$ values, respectively 51.39 and 47.65 expressed by the CIELab coordinates. The higher red color component showed by these two red wine

vinegars is a consequence also of the higher total anthocyanin contents obtained for these vinegars (Table III). The determinant role of anthocyanins for the red color component of the generality of fermented beverages, such as red wines (Cristino *et al.*, 2013; Tavares *et al.*, 2017) and fruit vinegars (Ubeda *et al.*, 2013), is well known.

For *b** values (yellowness), the values quantified showed a higher variability with a coefficient of variation of 38.9%. Thus, *b** values varied from 9.37 to 57.7 expressed by the CIELab coordinates and the average value was 38.19 expressed by the CIELab coordinates. In general, it is well know that the contact with wood, induce an extraction of several phenolic wood components which may imply an increase in brownish tones and consequently a potential increase of *b** values may occur (Tavares *et al.*, 2017). In our study, on the basis of the information provided by the producers (Table I), this was the case, because vinegars conserved in contact with wood (RV1 and RV4 samples) showed the highest *b** values (except for RV2 sample). RV5 and RV6 vinegars showed the significantly lower *b** values, probably as a consequent of a high red color values obtained. In addition, for these vinegars samples, no information was provided by the producers about the potential aging process used. Furthermore, other factors could determine the increase of brownish tones in red wine vinegars such as oxidation conditions that occurs during the acidification process, the use of antioxidants during the vinegars production and also the phenolic content of the red wines used.

Finally, for *c** values (chroma) the values ranged from 38.0 to 52.93 expressed by the CIELab coordinates and the average value was 43.94. As expected, due to the phenolic content and the CIELab coordinates values, RV5 and RV6 vinegars samples showed the significantly highest *c** values.

Individual anthocyanins and phenolic acids

Individual monomeric anthocyanins quantified in all commercial red wine vinegars studied are shown in Table IV. Although only low levels of some individual anthocyanins were present, and some of them were not detected in several red wine vinegars samples, nine different individual monomeric anthocyanins were detected: delphinidin-3-monoglucoside, petunidin-3-monoglucoside, peonidin-3-monoglucoside, malvidin-3-monoglucoside, delphinidin-3-acetylglucoside, cyanidin-3-acetylglucoside, peonidin-3-

acetylglucoside, peonidin-3-coumaroyl glucoside and malvidin-3-coumaroyl glucoside. Delphinidin 3-acetylglucoside and malvidin-3-monoglucoside were the individual anthocyanins with the highest values in all red wine vinegars analyzed (varying from 81.5 to 730.6 µg/L, averaging 272.1 µg/L and varying from 110.2 to 233.9 µg/L, averaging 133.9 µg/L, respectively) followed by petunidin-3-monoglucoside (varying from 19.6 to 134.9 µg/L, averaging 94.0 µg/L) and peonidin-3-monoglucoside (varying from 29.4 to 237.7 µg/L, averaging 93.0 µg/L). Cyanidin 3-acetylglucoside (varying from 5.6 to 47.6 µg/L, averaging 23.1 µg/L) and peonidin 3-coumaroylglucoside (varying from 16.3 to 71.6 µg/L, averaging 30.2 µg/L) were the individual anthocyanins quantified in the lowest concentrations. In addition, for several red wine vinegars samples it was not possible to detect some monomeric anthocyanins, such as delphinidin 3-monoglucoside (RV1, RV2 and RV3 vinegars samples) and petunidin 3-monoglucoside (RV1, RV2 and RV5 vinegars samples). The pattern of the anthocyanin chemical groups showed that the simple glucoside group was the main group (total average value of 383 µg/L) followed by acetyl glucosides (total average value of 347 µg/L) and the coumaroyl glucoside group (total average value of 113.5 µg/L).

Although a high coefficient of variation obtained (ranged from 28.5 to 89.7 %), RV5 and RV6 vinegars samples showed in general, significantly higher values for the different individual anthocyanins (for example 470.1 and 730.6 µg/L for delphinidin 3-acetylglucoside, respectively). The use of red wines with high anthocyanin content and also the potential absence of an aging process (Table I), may justify the high individual anthocyanin levels quantified in RV5 and RV6 vinegars samples. In addition, the high variation of the individual anthocyanin values quantified could be also attributed to the different red wine anthocyanin composition used for vinegar production, to the pH value of the vinegar and also the vinegar production technology used. According to several authors (Natera *et al.*, 2003; Ubeda *et al.*, 2013; Kawa-Rygielska *et al.*, 2018) vinegar polyphenolic composition depends most of all on the type of raw material, as well the production technology, namely, the fermentation and aging conditions.

The data in Table V shows the individual phenolic acids quantified in the commercial red wine vinegars tested. As shown in this Table, six different phenolic

TABLE IV

Individual monomeric anthocyanins of the commercial red wine vinegars studied

Antocianinas monoméricas individuais dos vinagres de vinho tinto comerciais estudados

Red wine vinegars	Delphinidin 3-monoglucoside	Petunidin 3-monoglucoside	Peonidin 3-monoglucoside	Malvidin 3-monoglucoside	Delphinidin 3-acetylglucoside	Cyanidin 3-acetylglucoside	Peonidin 3-acetylglucoside	Peonidin 3-coumaroylglucoside	Malvidin 3-coumaroylglucoside
RV1	n.d.	n.d.	n.d.	110.2^a±0.2	81.5^a±1.2	5.6^a±0.4	19.4^a±0.3	17.8^a±0.5	84.9^a±4.6
RV2	n.d.	n.d.	29.4^a±0.1	114.0^a±5.1	121.9^b±2.0	7.1^a±2.8	18.7^a±4.4	16.3^a±2.9	61.8^b±0.9
RV3	n.d.	110.5^a±4.8	41.2^b±4.0	119.0^a±9.9	n.d.	n.d.	n.d.	n.d.	34.0^c±0.1
RV4	55.6^a±0.3	111.2^a±4.7	63.9^b±2.1	144.6^b±12.7	122.7^b±0.4	n.d.	19.1^a±0.3	n.d.	n.d.
RV5	32.2^b±2.1	n.d.	93.3^c±14.0	135.6^b±21.6	470.1^c±37.8	43.0^b±2.0	107.7^b±7.1	27.3^a±12.3	89.4^a±33.6
RV6	98.6^c±2.0	134.9^a±11.8	237.7^d±22.0	233.9^c±45.5	730.6^d±15.9	47.6^b±4.3	125.0^b±8.1	71.6^b±8.5	107.9^d±8.0
RV7	n.d.	19.6^b±0.5	n.d.	122.5^a±5.0	106.1^d±0.9	12.3^a±4.9	23.3^a±2.0	17.6^a±2.6	121.8^d±2.8
AV	62.1±27.5	94.0±44.0	93.0±75.5	133.9±39.9	272.1±244.3	23.1±18.3	52.2±45.6	30.2±21.1	83.3±28.9
CV (%)	44.2	46.8	81.7	28.5	89.7	79.1	87.4	70.0	34.7
R	55.6-98.6	19.6-134.9	29.4-237.7	110.2-233.9	81.5-730.6	5.6-47.6	18.7-125.0	16.3-71.6	34.0-121.8

Individual anthocyanins expressed in malvidin-3-monoglucoside equivalents (µg/L); n.d., not detected; AV, average values; CV (%), coefficient of variation; *R*, range; Values are given as the mean ± SD of the three experiments. Different letters in a column indicate statistically significant differences between the red wine vinegars tested according to the Tukey test ($p < 0.05$).

TABLE V

Individual phenolic acids of the commercial red wine vinegars studied

Ácidos fenólicos individuais dos vinagres de vinho tinto comerciais estudados

Red wine vinegars	Protocatechuic acid	Chlorogenic acid	Caffeic acid	Syringic acid	*p*-Coumaric acid	2-Hydroxycinnamic acid
RV1	n.d.	1.47^a±0.05	1.79^a±0.04	5.14^a±0.05	2.77^a±0.05	n.d.
RV2	n.d.	0.89^b±0.03	n.d.	n.d.	0.32^b±0.0.2	0.12^a±0.02
RV3	1.39^a±0.05	n.d.	1.87^a±0.03	5.33^a±0.10	0.45^b±0.03	n.d.
RV4	1.57^a±0.04	n.d.	2.56^b±0.09	7.88^b±0.09	0.75^c±0.08	n.d.
RV5	0.78^b±0.02	1.52^a±0.04	5.00^c±0.11	3.96^c±0.07	4.44^d±0.11	0.66^b±0.05
RV6	1.46^a±0.07	1.13^c±0.05	4.65^d±0.07	3.61^c±0.03	2.14^a±0.07	n.d.
RV7	1.81^c±0.05	1.47^a±0.06	2.94^b±0.05	5.07^a±0.06	3.35^e±0.02	0.36^c±0.04
AV	1.40±0.34	1.30±0.25	3.43±1.26	5.17±1.37	2.03±1.47	0.38±0.22
CV (%)	24.39	18.99	40.24	26.54	72.46	58.13
R	0.78-1.81	0.89-1.52	1.79-5.00	3.61-7.88	0.32-4.44	0.12-0.66

Individual phenolic acids expressed in caffeic acid equivalents (mg/L); n.d., not detected; AV, average values; CV %, coefficient of variation; *R*, range; values are given as the mean ± SD of the two experiments; different letters in a column indicate statistically significant differences according to the Tukey test ($p < 0.05$).

acids were quantified: protocatechuic, chlorogenic, caffeic, syringic, *p*-coumaric, and 2-hydroxycinnamic acids. In general, syringic and caffeic acids were the individual phenolic acids detected in the highest concentrations (varying from 3.61 to 7.88 mg/L, averaging 5.17 mg/L and varying from 1.79 to 5.0 mg/L, averaging 3.43 mg/L, respectively), while 2-hydroxycinnamic acid was the individual phenolic acid quantified in the lowest concentrations (varying from 0.12 to 0.66 mg/L, averaging 0.38 mg/L). In addition, 2-hydroxycinnamic acid was only quantified in three red wine vinegars (RV2, RV5 and RV6

samples); *p*-coumaric acid was the only phenolic acid quantified in all red wine vinegars tested (varying from 0.32 to 4.44 mg/L, averaging 2.03 mg/L). Furthermore, RV5 and RV7 red wine vinegar samples showed the higher values for the total phenolic acids quantified (16.36 and 15.0 mg/L, respectively), while RV2 sample showed the lowest value (1.33 mg/L).Phenolic acids quantified from the commercial red wine vinegars samples studied were in general in accordance with previous data published by Natera *et al*. (2003) and Cerezo *et al*. (2008), but lower than values obtained by Kelebek *et al*. (2017). On the other hand, according to the results obtained in the present work, the vinegar aging process in contact with wood seems to have not influenced in the content of phenolic acids quantified. Concerning the levels of individual phenolic acids quantified in the vinegars samples analyzed, syringic and caffeic acids were the most abundant, which is in accordance with previous data reported by other authors for grape vinegars (Kelebek *et al*., 2017) and apple vinegars (Nakamura *et al*., 2010). Budak and Guzel-Seydim (2010) also reported higher content of chlorogenic and syringic acids for wine vinegars, while other authors (Bakir *et al*., 2017) reported higher content of *p*-coumaric and caffeic acids for different fruit vinegars.

Total antioxidant capacity

Total antioxidant capacities from the commercial red wine vinegars studied were measured using two different methods: ABTS and DPPH. It is well know that there are several methods to measure the antioxidant capacity of substances. In addition, one single method cannot demonstrate the antioxidant capacity of substances comprehensively. First, organisms have more than one antioxidant system and second, different free radicals have different antioxidant clearance mechanisms. The two methods currently employed (ABTS and DPPH) to measure total antioxidant capacity are mainly *in vitro* determinations and thus cannot simulate the physiological environment.

The data in Table VI show the total antioxidant capacity results quantified in the commercial red wine vinegars tested. As shown in this Table, the results obtained varied from 2.59 to 3.93 mM TEAC, averaging 3.47 mM TEAC by ABTS method, while the values obtained by DPPH method varied from 1.44 to 1.90 mM TEAC, averaging 1.66 mM TEAC. These values are similar to those reported by Kelebek *et al*. (2017) for grape vinegars but lower than the values obtained by the same authors for apple vinegars. In addition, similar data were also recently

obtained by Kawa-Rygielska *et al*. (2018) in cherry vinegars by the application of ABTS method.

TABLE VI

Total antioxidant capacity obtained by ABTS and DPPH methodologies of the commercial red wine vinegars studied

Capacidade antioxidante total obtida pelos métodos ABTS e DPPH dos vinagres de vinho tinto comerciais estudados

Red wine vinegars	ABTS (mM) [a]	DPPH (mM) [a]
RV1	3.33[a]±0.23	1.67[a]±0.01
RV2	2.59[b]±0.22	1.45[b]±0.03
RV3	3.16[a]±0.23	1.44[b]±0.05
RV4	3.50[a]±0.09	1.61[a]±0.06
RV5	3.89[d]±0.10	1.73[a]±0.05
RV6	3.90[d]±0.31	1.63[a]±0.01
RV7	3.93[d]±0.10	1.90[d]±0.05
AV	3.47±0.46	1.66±0.18
CV (%)	13.17	10.82
R	2.59-3.93	1.44-1.90

[a] Values expressed in Trolox equivalents (TEAC); AV, average values; CV %, coefficient of variation; *R*, range; Values are given as the mean ± SD of the three experiments. Different letters in a column indicate statistically significant differences according to the Tukey test ($p < 0.05$).

RV7 vinegar sample presented the significantly highest values for total antioxidant capacity (3.93 and 1.90 mM TEAC, respectively for ABTS and DPPH method), while RV2 and RV3 samples showed the lowest values (2.59 and 3.16 mM TEAC, respectively for ABTS method, and 3.16 and 1.44 mM TEAC, respectively for DPPH method).

The values obtained with ABTS assay were higher than those obtained with DPPH assay in each red wine vinegar sample analyzed. The difference in the antioxidant capacity obtained with ABTS and DPPH assays could be due to the different reaction mechanism involved. For Villaño *et al*. (2006), this variance is due to the different reagents of the polyphenols with each method applied. According to other authors (Wang *et al*., 2004), ABTS[+] and DPPH radicals have a different stereochemical structure and a different method of genesis and thus they lend, after the reaction with the antioxidants, a qualitatively different response to the inactivation of their radical. Thus, it is clear that no single assay can provide all the information needed to evaluate antioxidant capacity, and multiple assays are therefore required to build up an antioxidant profile of different food

products. In addition, it was also evident that total antioxidant capacity values of the red wine vinegars analyzed, showed slight quantitative differences among the values obtained from each antioxidant method applied as well as differences in the range of variation. Thus, a lower coefficient of variation was shown for both methodologies (13.17 and 10.82% for ABTS and DPPH methods, respectively), which indicated that these methods were sensitive to the lower intrinsic variability of total antioxidant capacity values obtained for the red wine vinegars studied.

To verify the contribution of each phenolic fraction on the overall antioxidant capacity of commercial red wine vinegars, it was tested in this study the total antioxidant capacity from three different phenolic fractions isolated: fraction I (containing phenolic acids), fraction II (containing monomeric and oligomeric proanthocyanidins) and fraction III (containing polymeric proanthocyanidins and anthocyanins). Thus, Figure 1 show the total antioxidant capacity results for each phenolic fraction isolated from the commercial red wine vinegars samples studied. As reported in Figure 1, for all vinegars samples, fraction III showed the significantly highest values of total antioxidant capacity (varying from 0.930 to 1.259 mM TEAC for

DPPH method and from 1.290 to 2.72 mM TEAC for ABTS method), followed by fraction I (varying from 0.250 to 0.490 mM TEAC for DPPH method and from 0.491 to 0.962 mM TEAC for ABTS method), and finally fraction II which showed the lowest total antioxidant capacity values (varying from 0.026 to 0.110 mM TEAC for DPPH method and from 0.100 to 0.250 mM TEAC for ABTS method). With these results, it was clear that the phenolic component containing polymeric proanthocyanidins and anthocyanins (fraction III) showed the highest contribution for total antioxidant capacity of commercial red wine vinegars studied, while monomeric and oligomeric proanthocyanidins (fraction II) showed the lowest contribution for total antioxidant capacity. Tagliazucchi *et al.* (2008) reported for traditional balsamic vinegars that polymeric tannins were the phenolic compounds group which contributed significantly for high antioxidant capacity of these vinegars analyzed. In addition, Rivero-Pérez *et al.* (2008) reported that anthocyanin fraction is mainly responsible for the total antioxidant capacity in red wines.
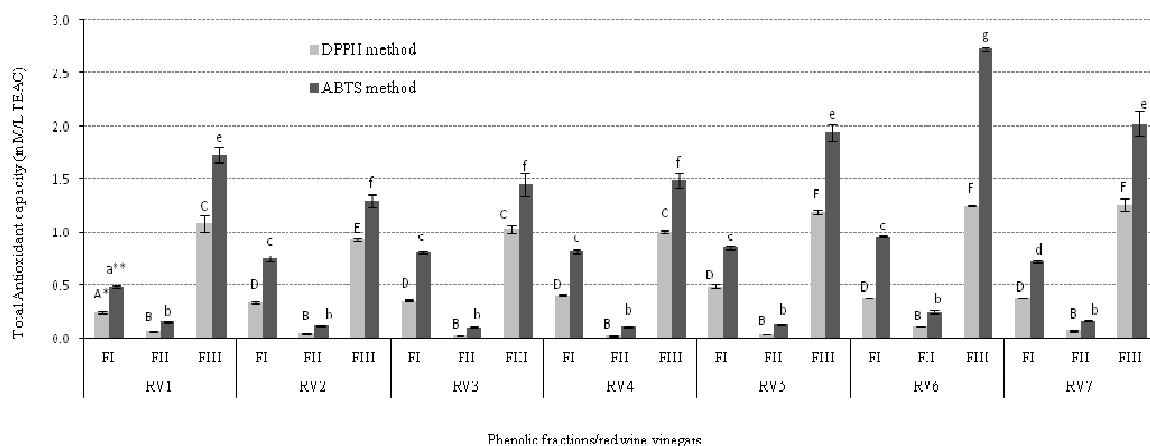

Figure 1. Total antioxidant capacity for each phenolic fraction isolated from the commercial red wine vinegars studied.

Phenolic fractions: FI, phenolic acids; FII, monomeric and oligomeric proanthocyanidins; FIII, polymeric proanthocyanidins and anthocyanins. Values are given as the mean ± SD of the three experiments. Different letters for each phenolic fraction and same antioxidant method (* capital letters for DPPH method and ** lowercase letters for ABTS method) indicate statistically significant differences according to the Tukey test (*p* < 0.05).

Capacidade antioxidante total de cada fração fenólica isolada nos vinagres de vinho tinto comerciais estudados.

Frações fenólicas: FI, ácidos fenólicos; FII, proantocianidinas monoméricas e oligoméricas; FIII, proantocianidinas poliméricas e antocianinas. Os valores são apresentados como médias ± desvio padrão de três repetições. Letras diferentes para cada fração fenólica e mesmo método da atividade antioxidante (letras grandes para o método DPPH e ** letras pequenas para o método ABTS) indicam diferenças estatisticamente diferentes de acordo com o teste de Tukey (p < 0.05).*

A linear regression analysis was performed to determine the correlation between the different phenolic parameters and their total antioxidant capacity of commercial red wine vinegars studied. As shown in Table VII, the correlation coefficients calculated, indicated good correlations among different phenolic parameters (total phenolic compounds, flavonoid phenols, which includes anthocyanins and proanthocyanidins, and total anthocyanins) and total antioxidant capacity. These results were independent of the antioxidant capacity method used. Thus, the values ranging from 0.67 to 0.71 and from 0.66 to 0.72 for total phenols and flavonoid phenols, respectively. For total anthocyanins, the correlations varied from 0.62 to 0.83. These high correlation values between phenolic composition and total antioxidant capacity are according to previous works for different grape vinegars (Budak and Guzel-Seydin, 2010; Kelebek *et al.*, 2017; Kawa-Rygielska *et al.*, 2018). Regarding the correlation between no flavonoid phenols (which includes phenolic acids) and total antioxidant capacity, low correlation values were found ($R^2 <$ 0.50 for both antioxidant methods).

TABLE VII

Correlations coefficients between the different phenolic parameters and total antioxidant capacity of the commercial red wine vinegars studied

Coeficientes de correlação entre os diferentes parâmetros fenólicos e a capacidade antioxidante total dos vinagres de vinho tinto comerciais estudados

Phenolic parameters	DPPH	ABTS
Total phenolic compounds	0.67	0.71
Flavonoid phenols	0.66	0.72
No flavonoid phenols	< 0.50	< 0.50
Total anthocyanins	0.83	0.62
Total phenols from each phenolic fraction		
Total phenols from FI	0.76	0.56
Total phenols from FII	0.88	0.79
Total phenols from FIII	0.75	0.86

Phenolic fractions: FI, phenolic acids; FII, monomeric and oligomeric proanthocyanidins; FIII, polymeric proanthocyanidins and anthocyanins.

Finally, the correlations among total phenols from the different phenolic fractions previously isolated (FI, FII and FIII) and their total antioxidant capacity are also show in Table VII. In general, the correlation coefficients indicated good correlations among total phenolic content from the several phenolic fractions

and total antioxidant capacity varying the values between 0.56 and 0.88. The lowest correlation value was obtained between total phenolic content of fraction I and the total antioxidant capacity by the use of ABTS method. Probably a less reactivity will occur between the phenolic content of fraction I (which includes namely phenolic acids) and the ABTS reagent. This proves once again that one single antioxidant method cannot demonstrate the antioxidant capacity of substances comprehensively.

Principal components analysis applied on commercial red wine vinegars samples

To better understand the relationship between different commercial red wine vinegars concerning to the main chemical parameters, a principal component analysis (PCA) was performed. The corresponding loading plots that established the relative importance of each variable are shown in Figure 2. Thus, Figure 2A and 2B shows the relationship between the different commercial red wine vinegars and the most relevant independent chemical parameters evaluated (pH, titratable acidity, fixed acidity, volatile acidity, dry extract, ashes content, total polyphenols, flavonoid phenols, non flavonoid phenols, total anthocyanins, total antioxidant capacity by ABTS and DPPH methods).

The PCA (Figure 2A) showed that the first two PCs explained 69.80% of the total variance. The first PC (PC1, 47.52% of the variance), was positively correlated with the variables, total polyphenols (TP), non flavonoid phenols (NFP) total antioxidant (ABTS and DPPH methods) and negatively correlated with the titratable acidity (TAC), fixed acidity (FA), volatile acidity (VA), ashes content (AC) and dry extract (DE). The second PC (PC2, 22.28% of the variance) was positively correlated with pH, flavonoids phenols (FP) and total anthocyanins (TAT).

In Figure 2B it is possible to visualize the spatial distribution of the commercial red wine vinegar samples evaluated concerning to the global parameters considered. Thus, after a cluster analysis, one group is formed by the red wine vinegars aged in wood barrels, according to the information from vinegar producers showed on the labels of the bottle; these vinegars are positioned in the negative side of PC1 (RV1, RV2, RV4 and RV7 samples). These red wine vinegar samples aged were characterized by higher values of titratable acidity, volatile acidity, ashes content and dry extract, while red wine vinegar samples without aging process formed two separate groups (one with RV3 sample and other group with RV5 and RV6 samples). For red wine vinegar samples without any aging process mentioned by the

producers, one group, formed by the RV5 and RV6 samples were characterized by higher values of total antioxidant capacity (ABTS and DPPH methods) and total polyphenols, while other group formed by RV3 sample was characterized by lower pH and flavonoid phenols content.
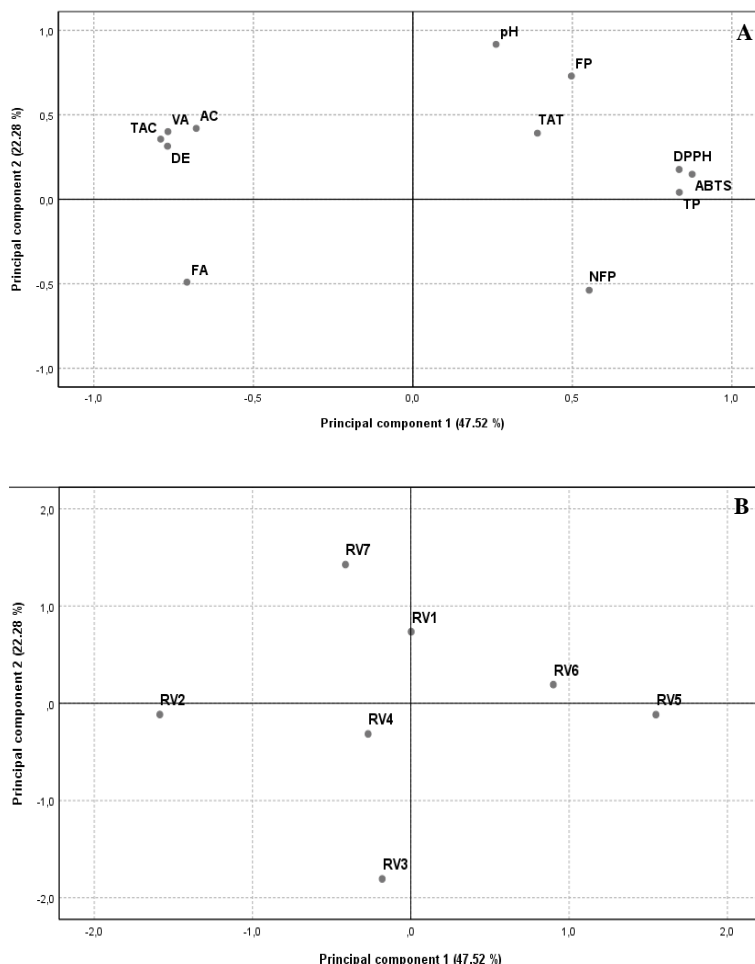

Figure 2. Principal component analysis (PC1 and PC2) for different chemical parameters (A - projection of chemical parameters, B - projection of red wine vinegar samples) in the commercial red wine vinegars studied.
Chemical parameters: TAC - titratable acidity; FA - fixed acidity; VA - volatile acidity; DE - dry extract; AC - ashes content; TP - total polyphenols; FP - flavonoid phenols; NFP - non flavonoid phenols; TAT - total anthocyanins; ABTS - total antioxidant capacity by ABTS method; DPPH - total antioxidant capacity by DPPH method. RV1; RV2; RV3; RV4; RV5; RV6; RV7 - different commercial red wine vinegars samples studied.

Análise em componentes principais (PC1 e PC2) para os diferentes parâmetros químicos (A - projeção dos parâmetros químicos, B - projeção das amostras de vinagre de vinho tinto) nos vinagres de vinho tinto comerciais estudados.

Parâmetros químicos: TAC - acidez titulável; FA - acidez fixa; VA - acidez volátil; DE - extrato seco; AC - conteúdo em cinzas; TP - polifenóis totais; FP - fenóis flavonóides; NFP - fenóis não flavonóides; TAT - antocianinas totais; ABTS - capacidade antioxidante total pelo método ABTS; DPPH - capacidade antioxidante total pelo método DPPH. RV1; RV2; RV3; RV4; RV5; RV6; RV7 - diferentes vinagres de vinho tinto comerciais estudados.

CONCLUSIONS

The commercial red wine vinegars used in this study constitute a quite heterogeneous group, and accordingly with important differences in their phenolic composition and also in total antioxidant capacity. So, in general, the high coefficients of variation for the different phenolic parameters analyzed and antioxidant values were in agreement with the heterogeneity of the samples as cited. However, in a specific point of view, it was proved that red wine vinegars are a good source of phenolic compounds and with a great diversity of individual phenolic composition. In addition, it is important to

consider that this phenolic composition has an important role on total antioxidant capacity of red wine vinegars, in particular anthocyanins and polymeric proanthocyanidins. This is demonstrated by the good linear correlations between the different phenolic parameters and total antioxidant capacity quantified. Thus, it is essential to consider that a study of the antioxidant capacity and the phenolic composition of any food, such as vinegars, should always take into account the structure-activity of antioxidant components, the contribution of specific polyphenolic fractions, raw material, production technology used and the possible aging process. Concerning to the aging process mentioned for some of the red wine vinegars analyzed, it was not possible to verify a clear relation between the aging process mentioned by the producers and phenolic content or total antioxidant capacity values.

Finally, the comparison of all of these results should be conducted with caution since they are obtained from commercial red wine vinegars samples and consequently the results could vary considerably.

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