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Development of a new procedure for the determination of the reactivity of brandies used in wine fortification

Joana Azevedo[1], João Pissarra[2], Filipa Amaro[3], Luís Guido[1], Joana Oliveira[1], Paulo Lopes[4], Paula Guedes de Pinho[3], Nuno Mateus [1] and Victor de Freitas[1,*]

[1] LAQV – REQUIMTE - Laboratório Associado para a Química Verde - Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre, 687, 4169-007 Porto, Portugal
[2] UTAD- Universidade de Trás os Montes e Alto Douro, Quinta de Prados, Vila Real, Departamento de Agronomia, 5001-801 Vila Real, Portugal
[3] UCIBIO@REQUIMTE/Laboratório de Toxicologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[4] Amorim Cork S.A. Rua dos Corticeiros 830, 4536-904 Santa Maria de Lamas, Portugal

**corresponding author*: vfreitas@fc.up.pt

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ABSTRACT

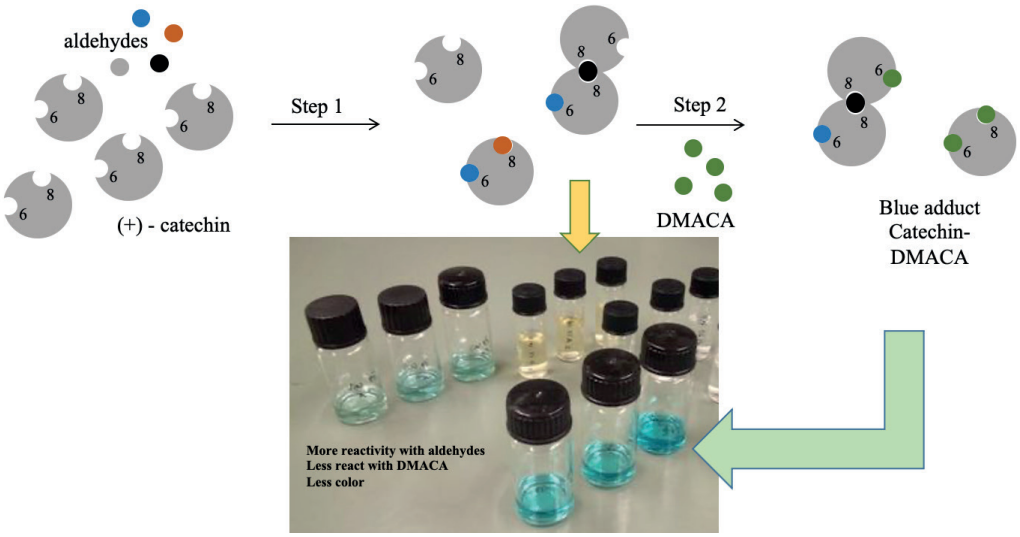
The main purpose of this work was to describe and validate a new methodology for the determination of a brandy reactivity index (BRI) towards flavan-3-ol compounds in wines (catechins and condensed tannins) and to correlate this index with the amount and type of aldehydes present in those brandies. This new method consists of two main reactions: a) the ability of aldehydes to react with C8 and/or C6 positions in the phloroglucinol ring of a catechin present in excess (A ring); b) further reaction of the remaining free catechin with *p*-dimethylaminocinamaldehyde (DMACA) yielding to the formation of a blue compound that can be quantified by Visible spectroscopy at 640 nm. The impact of different experimental conditions such as reagent concentrations and reaction time on BRI were also evaluated. The method was validated through the determination of repeatability (intra-day variability) and reproducibility. The repeatability was considered acceptable with a *CV* of 11.87 %. The analysis of the reproducibility variance S^2_R (11.59), the reproducibility limit R (9.5) and the reproducibility coefficient of variation, CV_R (15.25 %) postulates BRI methodology to be reliable and robust.

Using the developed methodology, the BRI of fourteen different commercial brandies and some pure standards aldehydes were determined. The aldehydes present in brandies, and the content of total aldehydes were determined by GC-MS and then correlated with the BRI. In general, it was observed a direct correlation between the BRI and the concentration of total aldehydes, in particular acetaldehyde.

KEYWORDS

Reactivity Index, Brandy, Wine, GC-MS, aldehydes, *p*-dimethylaminocinamaldehyde (DMACA), (+)-catechin.

GRAPHICAL ABSTRACT



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INTRODUCTION

The world's best-known fortified wines are Ports, Sherry, Madeira, Marsala, Moscatel de Setúbal, Vermouth and Commandaria. All these wines have a high alcohol content (19–21 %) due to the addition of brandies during the winemaking process after being fully or partially fermented, producing a wide range of styles (Amerine, 1980). During Port wine production, brandies are added to stop the fermentation when about half of the sugar has been converted into ethanol, which represents about 20 % of the final volume of these wines. For the Port and Douro Wine Institute (IVDP) which supervises and regulates the wine sector, brandies used in Port Wine production are obtained by distillation at less than 86 % vol. of wine or fermented grape pomace or by redistillation of a distillate of less than 86 % vol.

These brandies have a wide variety of compounds that are distilled from wines, being particularly rich in volatiles (higher alcohols, aldehydes, esters, among others). Therefore, the chemical composition of brandies is an important feature having a strong impact on the final characteristics of Port wines due to the presence of aroma compounds like aldehydes and higher alcohols, and also due to the interaction of some brandies components with other molecules present in the wine must (anthocyanins and tannins) (Canas, 2017; Mateus *et al.,* 2006; Pissarra *et al.,* 2005a; Pissarra *et al.,* 2003). Moreover, the chemical composition of the different commercial brandies varies every year and among producers, which will differently impact the physical-chemical and sensory characteristics of the final Port wine.

The most common brandies usually used in the Port wine industry have a high aldehyde content within a range of 30–250 mg/L with acetaldehyde, propionaldehyde, isovaleraldehyde, isobutyraldehyde and benzaldehyde being the most important and with methylglyoxal and glyoxal being present in trace amounts (Pissarra *et al.,* 2005b; Pissarra *et al.,* 2003). Acetaldehyde is the most abundant aldehyde present in Port wine, which results from the addition of the brandy (Rogerson and De Freitas, 2002), as a fermentation intermediary product and also from oxidation of ethanol (Santos-Buelga *et al.,* 1995; Wildenradt and Singleton, 1974). Except for benzaldehyde which has a pleasantly bitter almond aroma, the generality of aldehydes present (acetaldehyde, propionaldehyde, 2-methylbutyraldehyde, isovaleraldehyde, methylglyoxal, formaldehyde and isobutyraldehyde) have an unpleasant aroma (green leaves and unripe fruit) that can contribute negatively to the Port wine aroma (Pissarra *et al.,* 2005a). However, it has been reported that the condensation of anthocyanins with proanthocyanidins mediated by aldehydes is expected to play an important role in Port wine colour stability and evolution (He *et al.,* 2012). The contribution of acetaldehyde to the formation of ethyl-linked pigments with catechin and procyanidin dimers has been widely reported (Dallas *et al.,* 1996; Francia-Aricha *et al.,* 1997; Timberlake and Bridle, 1976).

Pissarra *et al.,* 2003 studied the impact of the different aldehydes present in brandies used for Port wine fortification on the wine colour and concluded that brandies induced some chromatic changes in wine model solutions. Brandies with high concentrations in aldehydes induced "blueing" and "darkening" colour effects using the CIELAB system. These changes were attributed to the formation of adducts between anthocyanins and flavanols, linked by alkyl groups formed from different aldehydes (propionaldehyde, isovaleraldehyde, benzaldehyde, isobutyraldehyde and others) (Bakker *et al.,* 1993; Pissarra *et al.,* 2003).

These reactions have been described to be acid-catalysed and to involve in the first step the addition reaction of an aldehyde to the (+)-catechin A ring (de Pascual-Teresa *et al.,* 2000; Di Stefano *et al.,* 1989; Ivanova *et al.,* 2011; Ivanova *et al.,* 2010; Ivanova *et al.,* 2012). This nucleophilic attack of the flavanol unit can occur at carbon C8 or C6 of ring A, with carbon C8 being more reactive as it is described as a stronger nucleophile centre (Bendz *et al.,* 1967; Timberlake and Bridle, 1976).

It was also demonstrated that the reactivity depends on the structure of the aldehyde. For instance, the formation of condensed pigments between anthocyanins and flavanols occurs differently and in dissimilar yields depending on the aldehyde present. Acetaldehyde and isovaleraldehyde were found to be the most reactive aldehydes, followed in decreasing order by propionaldehyde, formaldehyde, benzaldehyde, isobutyraldehyde and 2-methylbutyraldehyde (Pissarra *et al.,* 2005a; Pissarra *et al.,* 2003).

There are different methods to determine the quality of brandies before their approval by the IVDP, to be used in Port wine production. This analysis involves the determination of the content of aldehydes by GC, as determined for alcoholic beverages and spirits (NP 3262 1990).

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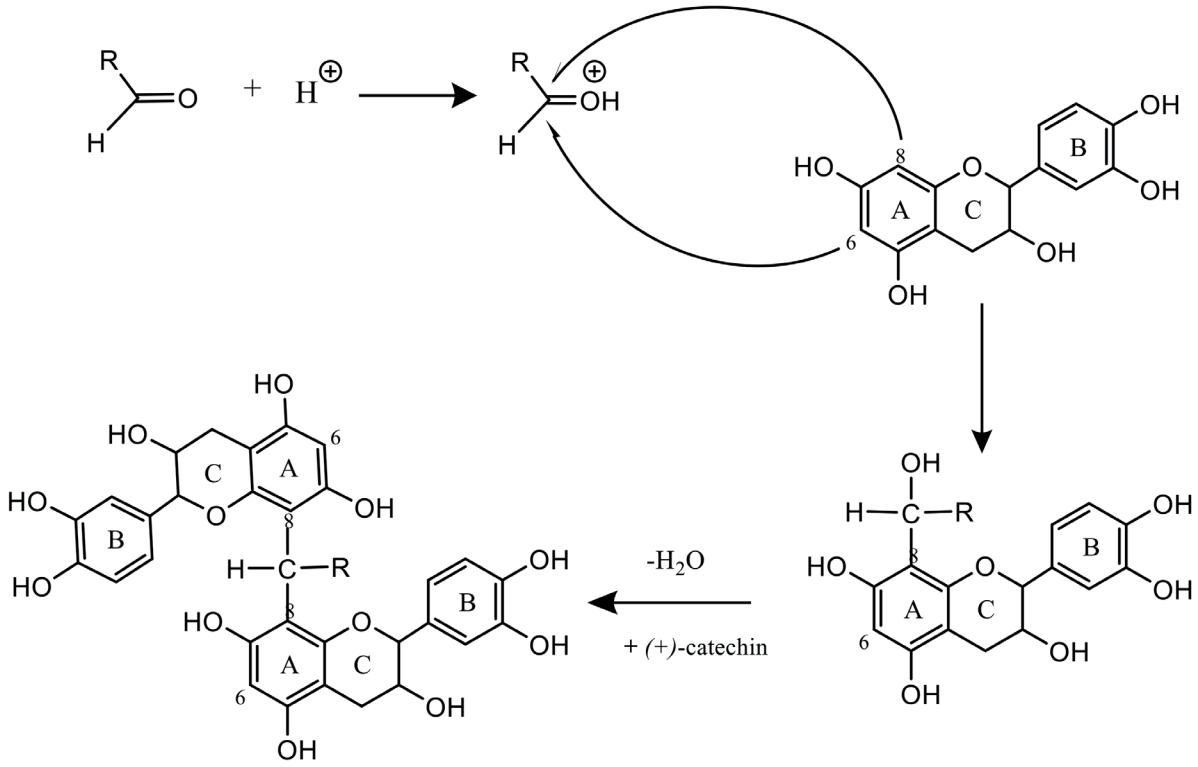


FIGURE 1. Formation of aldehyde-catechin and catechin-aldehyde-catechin adducts.

TABLE 1. Commercial brandies (B1-14) used in Port winemaking and some quality parameters provided by the supplier: Total Higher Alcohols and Acetaldehyde concentration was determined by Gas Chromatography (GC) and total acidity determined by Volumetric titration.

	Total Higher Alcohols (mg/100 cm^3)	Acetaldehyde (mg/L)	Alcohol (%)	Total acidity (mg acetic acid/L)
B1	264	80	77.0	96
B2	270	80	77.1	114
B3	243	90	77.1	54
B4	254	90	77.1	47
B5	236	90	77.4	111
B6	198	90	77.3	23
B7	272	90	77.2	71
B8	228	90	77.3	50
B9	237	90	76.7	70
B10	260	90	77.4	37
B11	273	90	77.2	67
B12	222	90	77.0	95
B13	212	50	77.1	*
B14	255	90	76.9	27

* No information available

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In addition, there are reports in the literature for the determination of some volatile and non-volatile substances by GC-MS to quantify carbonyl compounds in different categories of Port wines (Moreira *et al.,* 2019), but none of these methods are direct to evaluate the putative impact of brandies on Port Wine quality.

Bearing this, this work aimed to develop a new colorimetric method for the determination of a brandy reactivity index (BRI), based on the ability of aldehydes to react with flavan-3-ol compounds and to correlate BRI with the amount and type of aldehydes present in 14 commercial brandies and by this, to determine their possible impact on the characteristics of fortified wines (colour).

BRI methodology can be a very powerful tool to the fortified wines sector, especially Port wine, allowing to properly determine the impact of brandies on the Port wine characteristics.

MATERIALS AND METHODS

1. Reagents

L-(+)-tartaric acid (99 %), ethyl acetate, *p*-dimethylaminocinamaldehyde (DMACA) and methanol were obtained from ChemLab®. Ethanol was purchased from AGA®, sulfuric Acid (95–97 %) from ChemLab® and Hydrochloridric acid, 37 % (v/v) from Fluka®.

O-(2,3,4,5,6-pentafluorobenzyl) hydroxylamine (PFBHA, ≥ 99 %), acetaldehyde (99.5 %), formaldehyde (37 %), propanal (97 %), isobutanal (98 %), 2- methyl-butanal (95 %), 3-methyl-butanal (97 %) and nonanal (95 %) were obtained from Sigma-Aldrich® (St. Louis, Missouri, USA).

2. Brandies samples

The 14 commercial brandies samples were provided by Fladgate Partnership company (Table 1)

3. Brandy Reactivity Index (BRI)

The determination of the Brandy reactivity index (BRI) is based on the ability of the phloroglucinol ring (ring A) of a 3-flavanol unit to act as a nucleophile and to react with carbonyl groups in an acidic medium (Figure 1). This method involves two main steps:

Step 1. To a 10 mL glass flask with cap, 1 mL of a mixture methanol/sulfuric acid (1:1), 1 mL (+)-catechin (0.1 mg/mL prepared in methanol) and 1 mL of brandy were added. The mixture was left to react under stirring at 40 ºC (oven) for 3 h.

Step 2. An aliquot (500 µL) from step 1 reaction was left to react with 2.5 mL of *p*-dimethylaminocinamaldehyde (DMACA), used to react with the free positions of the catechin to form a blue adduct easily identified at 640 nm, at a concentration of 1 mg/mL (prepared in methanol) for 20 minutes in the dark. The absorbance of the solution was determined at 640 nm using a 2 mm glass cell.

A control sample (C) was prepared similarly by the substitution of 1 mL of brandy by an ethanol aqueous solution, 77 % (v/v). Each sample was analysed six times.

The BRI is obtained using the following expression:

$$\% \text{ BRI} = (\text{Abs}_{C} - \text{Abs}_{S}) / \text{Abs}_{C} \times 100$$

3.1 Brandy Reactivity Index (BRI) method development

The limits and effectiveness of this method depend on the experimental condition of the reaction in step 1, which involves the reaction of all aldehydes present in the brandy with (+)-catechin. Bearing this, it was studied the effect of various factors such as

a) Reagent concentrations: for step 1, different concentrations of (+)-catechin of 100, 200 and 400 mg / L in methanol were evaluated. For step 2, two different concentrations of DMACA were studied:1.0 and 2.0 g/L.

b) Reaction time: to determine the time need for the aldehydes in the brandies to react completely with (+)-catechin in step 1, two different times of reaction were tested, 2 h and 3 h. In step 2, the final absorbance was determined 15, 20 and 30 minutes after the addition of DMACA.

4. Validation of method (Statistical Treatment)

The robustness of the BRI method was evaluated by measuring how sensitive it is to small variations that may occur in test conditions. The greater the robustness of a method, the greater will be the confidence of its precision. Precision is a general term that aims to evaluate the dispersion of results between repeated assays of the same sample, under defined conditions. To minimise the matrix effects, it is crucial to evaluate the precision of realistic samples.

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Robustness can be evaluated by assessing repeatability and reproducibility. Between these two extreme accuracy measures, there is an intermediate situation, called intermediate precision or intra-laboratory variability. The validation of the method herein presented was determined by measuring repeatability (intra-day variability) and reproducibility.

To determine the repeatability, a series of 11 measurements on the same sample was carried out in triplicate, under repeatability conditions (same laboratory, same analyst, same equipment, same reagents, same day). This procedure was repeated on a series of samples, at various concentration levels, covering the entire application domain of the method (ISO 5725-2).

To determine the reproducibility, the precision of the method was carried out under different test conditions, using the same method on the same sample, varying the conditions of measurements such as the time of the test assay, and the assay reagents. These assays were performed in six days and repeated 3 times for each sample.

The repeatability limit (r) was calculated for a 95 % confidence level according to (ISO 5725-6):

$$r = t \cdot \sqrt{2} \cdot S_{ri} = 1.96 \cdot \sqrt{2} \cdot S_{ri}$$

$$r = 2.8 \cdot \sqrt{S_{ri}^2}$$

The repeatability coefficient of variation (CV_r), expressed in percentage, was calculated by:

$$CV_r = \frac{S_{ri}}{\bar{x}} \cdot 100$$

The reproducibility limit (R) and the reproducibility coefficient of variation (CV_R) were calculated for a 95 % confidence level in the same way.

5. GC-FID and GC-MS analysis of compounds present in brandies

The GC-FID was only used for acetaldehyde quantification. A volume of 10 µl of the internal standard solution of methyl acetate (1.23 mM; Merck®) was added to 1ml of wine spirit. A volume of 2 µl of this solution was directly injected in a Varian Star 3400 CX gas chromatograph with a flame ionization detector (FID) and described in (Pissarra *et al.,* 2005a). A linear calibration curve was established using standard model solutions of acetaldehyde.

For all the others the sample preparation and extraction were performed as previously described in the literature (Moreira *et al.,* 2019; Moreira *et al.,* 2013; Pinto *et al.,* 2018) with some modifications. Briefly, 250 µL of each brandy was transferred to a 20 mL glass vial with 11.3 µL of the derivatizing agent PFBHA (O-(2,3,4,5, 6-pentafluorobenzyl) hydroxylamine, 40 g/L), to enhance the detection of aldehydes and ketones. After an incubation time of 10 min at 38 ºC, the compounds were extracted by HS-SPME with a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre using a Combi-PAL automatic autosampler (Varian, Palo Alto, CA). The extraction time was 30 min at 38 ºC, under continuous stirring (250 rpm).

Volatile carbonyl compounds detection was accomplished using a 436-GC model (Bruker Daltonics, Fremount, CA) coupled to an EVOQ triple quadrupole (TQ) mass detector from Bruker and a Bruker MS workstation software (version 8.2). A fused silica capillary column (30 m × 0.25 mm internal diameter × 0.25 µm; Restek Corporation, U.S., Bellefonte, Pennsylvania) was used and helium C-60 was the carrier gas (flow rate 1 mL/min). The oven temperature was fixed at 40 °C for 1 min, increasing to 250 ºC (rate 5ºC/min), held for 5 min, followed by increasing to 300 ºC (rate 5 ºC/min). The mass detector was operated in electron impact (70 eV) at 270 ºC and the temperature of the transfer line and manifold were 260º and 40º, respectively. Data acquisition was performed in full scan mode considering a 35–600 *m/z* mass range.

The volatile carbonyl compounds were identified by comparison of the MS fragmentation with the mass spectra present in the National Institute of Standards and Technology (NIST 14) database, comparing the experimental Kovats retention index (RI) with literature and, when possible, by comparison of the retention time and mass spectra with commercially available standard compounds.

6. BRI of pure aldehydes

As different aldehydes exhibit dissimilar reactivity towards catechin, in this work the determination of the reactivity index of each of the main aldehydes present in brandies was performed. For this purpose, model solutions of brandy (77 % ethanol in water (v/v)) were prepared for each aldehyde (10^{-5} M), Acetaldehyde, Formaldehyde, Propanal, Methylglyoxal, 2-Methylpropanal, 2-Methylbutanal, 3-Methylbutanal, Benzaldehyde were evaluated and the respective BRI was determined as described in 3. point of this section.

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7. Statistical Analysis

Values are expressed as the arithmetic means ± standard deviation. In BRI determination the statistical significance between brandies were evaluated by One-way ANOVA with Sidak´s multiple comparisons test using the GraphPad Prism 7. Differences were considered significant when $p < 0.0001$. For correlations were applied Pearson (normal, parametric) and Spearman (nonparametric) tests.

RESULTS AND DISCUSSION

The evolution of catechin in wines was described to start with the reaction with acetaldehyde, giving rise to more polymerised structures (Somers, 1971). This ability of aldehydes to react with catechin has been used by several authors to study the degree of polymerization and the molecular weight of condensed tannins (flavanols) by the reaction with specific aldehydes, yielding coloured tannin-aldehyde adducts that are easily quantified by spectrophotometry (Ribéreau-Gayon and Stonestreet, 1966; Vivas and Glories, 1996; Vivas *et al.,* 1994; Wildenradt and Singleton, 1974). It is known that the reactivity of condensed tannins towards aldehydes decreases with the increase of the degree of polymerization due to the diminution of the number of nucleophilic sites (C6 or C8) available to react, and also due to the steric hindrance observed in more complex structures (Es-Safi *et al.,* 1999; Es-Safi *et al.,* 2000a; Es-Safi *et al.,* 2000b).

1. Development of the Brandy Reactivity Index (BRI)

BRI is based on the reaction of aldehydes present in brandies with (+)-catechin (in excess) in its nucleophile's sites of ring A (C6 or C8 carbon) (step 1) (Figure 2).

In step 2, any (+)-catechin that did not react with aldehydes present in brandies (step 1) reacts with *p*-dimethylaminocinamaldehyde (DMACA) to form a blue adduct that can be quantified at 640 nm (Thies and Fischer, 1971). Hence, the final blue colour is more intense when lower concentrations of aldehydes are present in samples, which will react to a small extent with (+)-catechin (first step) and allow that a higher amount of free (+)-catechin C6-/C8-nucleophilic sites will be available to react with DMACA in step 2. By this, a more intense blue colour indicates a minor reactivity index of the brandy. DMACA was selected since it has been reported to have specificity regarding the reaction with structures that present a phloroglucinol ring, similar to ring A of the basic structure of catechin and proanthocyanidins (McMurrough and McDowell, 1978; Vivas *et al.,* 1994). Furthermore, these reactions were described to occur easily at very acidic conditions (acid-catalysed reaction) and using methanol as solvent (Vivas *et al.,* 1994).

To optimise the methodology, various factors such as reagent concentrations and reaction time were studied and described below.

2. (+)- Catechin and DMACA concentration

The response of BRI was studied at different concentrations of (+)-catechin (100, 200 and 400 mg/L in methanol) in the presence of an excess of DMACA to evaluate the maximum range of absorbance at 640 nm (Table 2). For 200 and 400 mg/L of (+)-catechin, absorbance values at 640 nm were higher than 2, which is clearly out of the Beer–Lambert Law. In these cases, the absorbance recorded would not be directly proportional to the concentration of the coloured DMACA-catechin adducts present in the solution. Considering this, a concentration of (+)-catechin of 100 mg/L was chosen.

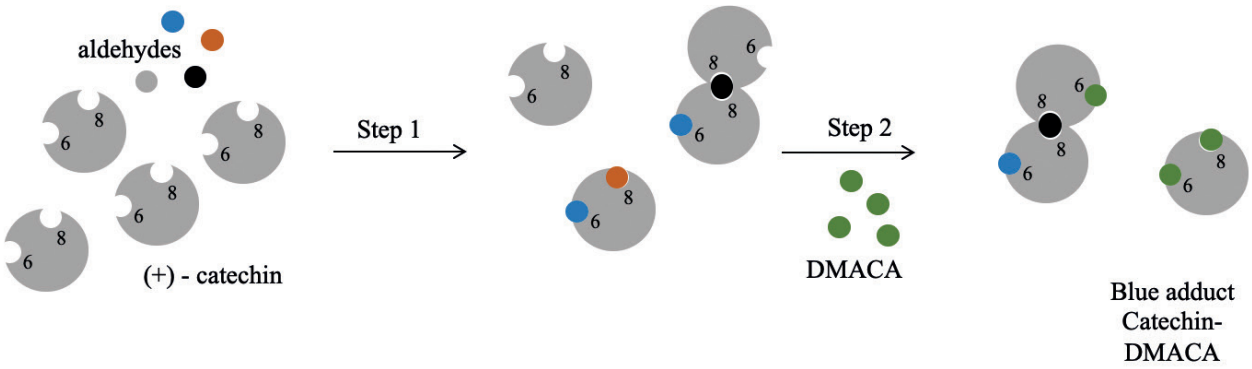


FIGURE 2. Scheme of the different steps involved in determining the BRI

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Regarding the DMACA concentration, two different concentration values were tested, 1.0 and 2.0 g/L and in this case, the absorbance values were very similar, and by this, a concentration of 1.0 g/L was adopted.

TABLE 2. Catechin concentration effect in BRI.

(+)-Catechin (mg/L)	BRI (%)
100	44.3 ± 1.8
200*	78.7 ± 0.05
400*	5.5 ± 0.7

* Abs measurement higher than 2 (possible deviation from the Beer–Lambert law)

2.1. Reaction times (Step 1 and Step 2)

To determine the reaction time required for brandy aldehydes to react with (+)-catechin (step1), different reaction times were tested, 2 h and 3 h. In Table 3 it is possible to verify that the BRI of the solutions does not differ from 2 h to 3 h. However, the results at the end of 3 h show a smaller standard deviation, which indicated that 3 h of reaction was ideal to ensure that aldehydes fully react with (+)-catechin.

To determine the impact of the time of the reaction of the free (+)-catechin with DMACA (step 2), the BRI was determined 15, 20 and 30 min after the addition of DMACA. From the analysis of the results presented in Table 3, it can be observed that the time of reaction seems to significantly impact the BRI. When free (+)-catechin is left to react with DMACA for only 15 minutes it is possible to observe a higher standard deviation contrarily to what is observed for 20 and 30 minutes and a lower value of BRI. For this reason, a 20 minutes' reaction time was chosen as optimal to determine an accurate BRI value.

TABLE 3: Reaction times effect, in BRI (%).

	Reaction Time	BRI (%)
step 1	2 h	31.1 ± 0.4
	3 h	30.19 ± 0.06
step 2	15 min	27.4 ± 0.7
	20 min	36.1 ± 0.2
	30 min	37.5 ± 0.5

3. Method validation

The experimental validation of the method was performed according to the procedure described in the Material and Methods section.

The precision of the method was evaluated as the repeatability variance, S^2_r (8.53), the repeatability limit r (8.17) and the repeatability coefficient of variation, CV_r (11.87 %). The calculations were carried out from the results obtained after eliminating one outlier, according to the standard practice for dealing with outlying observations (ISO, 1994) (ISO 5725-2, ASTM E178).

In general, the results of two determinations of reactivity index carried out under repeatability conditions are accepted if $| Xi - Xi\text{-}1 | \leq 8.17$. A reasonably good judgement on the acceptability of a particular CV value can be made for a given type of experiment. In the field of agricultural sciences CV values between 10 and 20 % can be considered good (Gomez and Gomez, 1984). The repeatability CV herein determined (11.87 %) could thus be considered acceptable.

The reproducibility variance, S^2_R (11.59), the reproducibility limit R (9.5) and the reproducibility coefficient of variation, CV_R (15.25 %) were calculated from all the results obtained (no outliers were eliminated). As expected, the precision of the method slightly decreases under different assay conditions. However, the decrease is very small showing that the method presented herein is reproducible, which means that it generates consistently accurate values every time it is used. Overall, we can conclude that the reported method to measure the reactivity index of aldehydes is reliable and robust.

4. Determination of BRI of samples

The determination of the BRI of the different brandies was determined, using the methodology previously developed. The BRI values may allow differentiating these brandies in terms of their potential to interact with the phenolic compounds present in musts, having consequently an impact on the characteristics of the fortified wines.

Figure 3 shows that sample B1 stood out from all other samples with a value about 3 times higher (75 ± 3) than all the other samples with values ranging from 6 to 30, following B4 and B14 with values around 30, and then the group constituted by B2, B3, B7 and B13 (values around 20). Most of the brandies (B5, B6, B8, B9 and B12) showed the highest BRI values (about 10), whereas B10 and B11 showed the lowest BRI values.

To correlate BRI with the concentration of aldehydes present in the solution, a GC-MS analysis was performed to identify and quantify these aldehydes (Table 4).

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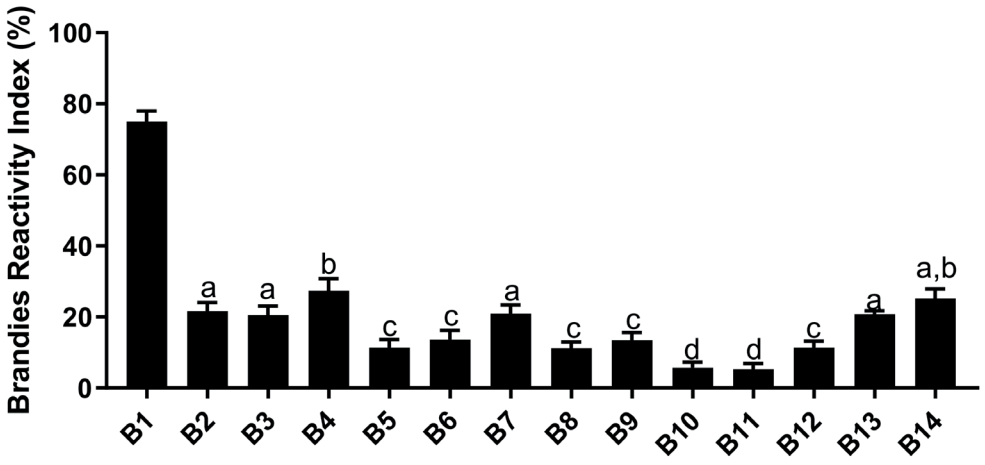


FIGURE 3. Reactivity Index of brandies (BRI), each column represents mean of six measurements ± standard deviation of six measurements.
A column with the same letter does not differ statistically.

TABLE 4. Identification and quantification of aldehydes present in brandies samples by GC-FID and GC-MS.

	Acetaldehyde mg/L	Formaldehyde µg/L	Propanal µg/L	Isobutanal µg/L	2-Methyl-Butanal µg/L	3-Methy-1-Butanal µg/L	Nonanal µg/L
B1	155.4	0.447	68.1	< LOQ	1.31	25.3	< LOQ
B2	58.8	0.304	40.3	< LOQ	0.2	18.2	< LOQ
B3	81,4	0.347	20	5.33	1.02	24.5	< LOQ
B4	96.1	0.309	70.5	< LOQ	0.19	26.4	< LOQ
B5	62.1	0.243	16.8	16.6	0.84	31.8	2.61
B6	51.3	0.257	43.7	0.447	1.36	20.9	< LOQ
B7	50.0	0.257	38.7	< LOQ	1.79	53.4	< LOQ
B8	60.2	0.283	6.1	< LOQ	0.49	9.07	< LOQ
B9	62.9	0.294	33.1	< LOQ	1.94	50.5	2.41
B10	34.1	0.207	35.5	< LOQ	0.85	41.3	< LOQ
B11	31.1	0.614	56.9	< LOQ	0.56	32.7	0.211
B12	66.8	0.316	10.3	12.954	0.48	13.2	33.5
B13	83.5	0.652	12.2	< LOQ	3.28	14.2	1.45
B14	88.6	0.703	67.3	< LOQ	1.49	29.8	< LOQ

From the analysis of the results presented in Table 4 and using a GC-MS method (Moreira *et al.,* 2019) and GC-FID for acetaldehyde quantification**,** it was possible to observe that acetaldehyde was the most abundant aldehyde detected in all brandies with concentrations ranging from 34.1 to 155.4 mg/L followed by propanal, 3-methyl-1-butanal, 2-methyl-butanal and formaldehyde in very small quantities.

Isobutanal was only identified in four samples (B3, B12, B5 and B6) and nonanal was quantified in B13, B11, B9 and B5 in small amounts and a higher concentration in B12 (33.5 µg/L).

Data regarding the quantification of aldehyde compounds present in brandies used in Port winemaking is scarce in the literature. Studies performed by Vanderlinde with brandies used in the production of Cognac, Armagnac and brandies showed similar results with acetaldehyde, formaldehyde 3-methyl-l butanal, propanal, benzaldehyde, isobutyraldehyde and 2-methylbutyraldehyde being the most abundant aldehydes (Vanderlinde, 1995). Similar results were also described by Matias-Guiu and collaborators (Matias-Guiu *et al.,* 2018). A GC-MS method had already been developed for the quantification of carbonyl compounds in

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Port wines (Moreira *et al.,* 2019) and the results showed that all Port wines presented the same aldehydes detected in brandies presented herein. On the other hand, the quantification of these compounds on brandy-containing products such as fortified wines (Port wine) or brandies that were aged in oak barrels can be found in the literature and in the latter, the high amounts of aldehydes found were those extracted from oak barrels such as vanillin, furfural, hydroxymethylfurfural (HMF) and others (Barnaba *et al.,* 2015; Canas, 2017; Canas *et al.,* 2019).

Brandy B1 was the one with the highest concentration of acetaldehyde (155.4 mg/L), followed by B14 and B4. The brandies in the group B2, B3, B7 and B13 did not differ statistically between them, following by the group of brandies B5, B6, B9, B8 and B12.

Comparing the concentration of aldehydes in brandies with the respective BRI, B10 and B11 were the brandies with lower BRI and the lowest contents of acetaldehyde and total aldehydes. It is also possible to observe that brandy B8 had the lowest level of total amounts of aldehydes, however, and due to the higher concentration and reactivity of acetaldehyde, its BRI was not the lowest. The concentration of acetaldehyde in the sample showed to be crucial and to be directly correlated with the reactivity index. For that reason, Pearson (normal, parametric) and Spearman (nonparametric) correlation were applied to the results. It is possible to see in Figure 4 the graph that represents Pearson correction with $r = 0.930$, indicating a strong correlation between BRI and the acetaldehyde content. In a Spearman rank correlation, a coefficient $r_s = 0.716$ was obtained, T statistic = 3555 and p-value = 0.003, indicating a significant positive correlation between the BRI and acetaldehyde, supporting all the results described.

Indeed, several authors have postulated that acetaldehyde, one of the most abundant aldehydes present in brandies, is very reactive and induces tannin polymerization and copolymerization between 3-flavanols and anthocyanins (Jurd and Somers, 1970; Pissarra *et al.,* 2003; Rivas-Gonzalo *et al.,* 1995; Somers, 1971; Timberlake and Bridle, 1976).

Despite the obtained strong correlation between BRI and the acetaldehyde contents, for some brandies that have higher BRI the respective levels of acetaldehyde were lower. For instance, B11 had the lower BRI but there were other brandies (e.g., B2, B8, B13) with a lower level of acetaldehyde. These results suggest that different aldehydes may affect the BRI of brandies differently. To assess the differences in reactivity's, the BRI of pure aldehydes were determined.

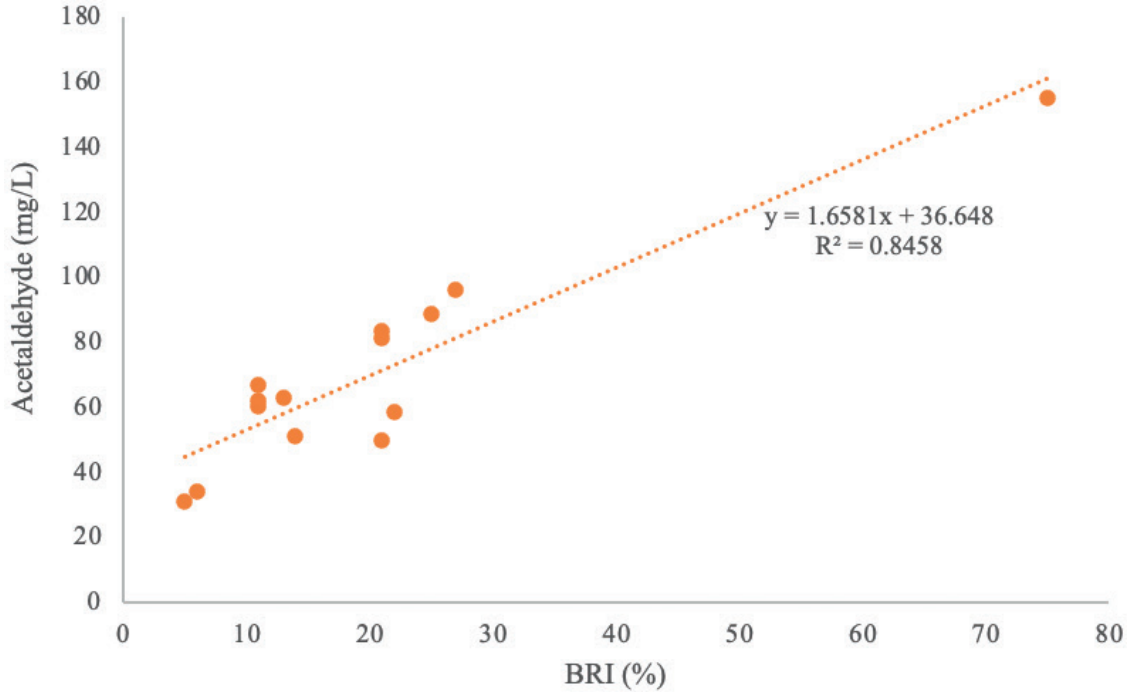


FIGURE 4. Graph correlating BRI and acetaldehyde (analysed by GC-FID) for each brandy. Pearson correlation (r = 0.930)

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5. BRI of pure standard aldehydes

From the analysis of the results presented in Table 5, the aldehydes showed a dissimilar reactivity, with acetaldehyde standing out for having a higher BRI, followed by 3-methylbutanal and propanal. BRI of the remaining aldehydes such as methylglyoxal, formaldehyde, benzaldehyde and 2-methylpropanal decreased in this order, with 2-methylbutanal being the one with the lowest BRI. These differences in reactivity of the aldehydes suggest that, in addition to the concentration, the type of aldehyde present in the spirit has a significant impact on its BRI value. This fact highlights the importance and usefulness of the individual quantification of each of the main aldehydes present in the spirits to clarify the potential effect of these spirits on the final product (Port wine). Furthermore, from the analysis of Table 5, it can be observed that acetaldehyde showed the highest BRI, followed 3-methylbutanal and propanal. Acetaldehyde was the most abundant aldehyde in brandy samples and also the most reactive.

TABLE 5. Determination of BRI the pure standard aldehydes (10-5 M) in hydroalcoholic solution 77 % (v/v).

Aldehydes	BRI (%)
Acetaldehyde	26.6 ± 1.1
Formaldehyde	5.4 ± 0.2
Propanal	11.8 ± 0.5
Methylglyoxal	7.6 ± 0.3
2-Methylpropanal	2.4 ± 0.1
2-Methylbutanal	0.1 ± 0.001
3-Methylbutanal	15.0 ± 0.6
Benzaldehyde	3.5 ± 0.1

Results are expressed in mean ± standard deviation of three analyses $p < 0.05$.

CONCLUSIONS

From this work, it was possible to develop and validate a new method to quantify the reactivity of brandies and to correlate this reactivity with the content in aldehydes. The validation of this method was determined by measuring repeatability (intra-day variability) and reproducibility. The repeatability *CV* herein determined (11.87 %) could be considered acceptable. The reproducibility variance, S^2_R (11.59), the reproducibility limit R (9.5) and the reproducibility coefficient of variation, CV_R (15.25 %) showed that the method is reliable and robust.

In addition, for each brandy, the BRI, the identification and quantification of volatile aldehydes (individually) as well as the content of total aldehydes were determined and subsequently correlated. B1 sample showed a higher reactivity index which was correlated with higher quantities of total aldehydes, especially acetaldehyde. It was possible to demonstrate that acetaldehyde seems to be the aldehyde that most influences the possible interaction that the brandy will have after contact with the wine.

Therefore, the method developed herein indicates that a high BRI represents brandies with higher levels of most reactive aldehydes, which translates into a very important knowledge for the fortified wine industry (such as Port wine) as it allows to determine in advance the impact of the spirits in some wine sensory characteristics (e.g., colour).

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