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Unveiling the Evolution of Madeira Wine Key Metabolites: A Three-Year Follow-Up Study

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Abstract: Madeira wine (MW) encompasses an unusual oxidative ageing process that makes it distinctive. Several metabolites have been related to its quality and safety, such as 5-hydroxymethylfurfural (HMF), furfural, sotolon, and ethyl carbamate (EC). These compounds were quantified over a three-year period to assess their formation rate according to the ageing procedure used: *canteiro* vs. *estufagem*. *Estufagem*, which includes thermal processing of young MWs, promoted greater HMF, furfural, and sotolon accumulation, especially in sweet wines, in which sotolon contributed significantly to aroma (odour active values up to 17.5). Tinta Negra revealed a higher predisposition to form EC while Malvasia and Sercial were less prone to its formation. The formation of furfural, HMF, and EC strongly correlated with the ageing time. Sotolon had a strong correlation with the ageing time in *canteiro* (r = 0.79) and a moderate correlation in *estufagem* (r = 0.65). In both ageing procedures, sotolon, furfural, and HMF formation trends strongly correlated with each other (r = 0.74–0.90). In turn, EC also correlated with all furans (r = 0.51–0.85). Yellow tones (b^*) correlated with these metabolites only when wines undergo *estufagem*. This study provides valuable insights to improve MW quality and safety management procedures.

Keywords: wine oxidation; sotolon; ethyl carbamate; furfural; 5-hydroxymethylfurfural; fortified wines

Citation: Pereira, V.; Leça, J.M.; Freitas, A.I.; Pereira, A.C.; Pontes, M.; Albuquerque, F.; Marques, J.C. Unveiling the Evolution of Madeira Wine Key Metabolites: A Three-Year Follow-Up Study. *Processes* **2022**, *10*, 1019. https://doi.org/10.3390/pr10051019

Academic Editors: Chi-Fai Chau and Jasna Čanadanović-Brunet

Received: 11 April 2022
Accepted: 18 May 2022
Published: 20 May 2022

1. Introduction

Madeira wine (MW) is a fortified alcoholic beverage from Portugal, known worldwide for its unique characteristics. It holds an alcoholic content between 17% and 22% (alcohol by volume, ABV), and it can be made from red and white *Vitis vinifera* L. grapes. The most recognised varieties are white ones, such as Sercial, Verdelho, Boal, and Malvasia, which are usually used to produce dry, medium-dry, medium-sweet, and sweet wines, respectively. However, the bulk production is made from Tinta Negra, a versatile red variety used to produce all wine styles [1]. The ageing process makes these wines distinctive, particularly because it can include intentional thermal processing.

Briefly, after fermentation is stopped by adding neutral grape spirit, according to the sweetness and desired marketing features, the wines undergo one of two ageing processes: *estufagem* or *canteiro*. In the *estufagem* ageing process, wines are industrially heated at about 45 °C for at least 3 months and then kept at room temperature for 90 days. After this period, they are submitted to another maturation period, either in stainless steel tanks or

wooden casks. The *canteiro* process is typically employed for producing high-quality wines. It exclusively utilises wooden casks stored in sun-warmed lofts of the wine cellars, wherein temperatures are higher, for usually more than 5 years [2–5].

Both ageing processes involve the synthesis, conversion, and decomposition of several metabolites, such as sugars, amino acids, volatile organic compounds (VOCs), polyphenols, and others, due to their oxidative nature. It is well known that during the ageing period, the intense and complex flavours of MW are developed [3]. Thus, it is plausible, that these compounds contribute to the longevity and robustness that make MWs singular.

Several studies have described the impact of these ageing processes on wine characteristics, focusing mostly on the physicochemical and sensory properties [2,3,6–9]. In general, MWs are not rich in varietal and fermentative aromas because they are reduced during maturation. Consequently, the freshness and fruitiness, typical of younger wines, becomes less pronounced. The freshness of MW results from its surprising acidity originating from the volcanic soils. As a result of oxidation and elevated temperature, these wines are endowed with tertiary aromas, including caramel, dried fruits, spicy, toasty, musty, mushroom, brown sugar, and woody. Notably, increased concentrations of several metabolites, particularly furans, have been proposed as potential ageing markers of MWs [10].

Furans are heterocyclic compounds composed of a five-membered aromatic ring with one oxygen atom. In thermally processed foodstuffs, these compounds are widespread and mainly formed during non-enzymatic browning reactions, such as caramelisation, Maillard reaction, and acid-catalysed sugar degradation [11–13]. The most well-known furans in fortified wines are sotolon, 5-hydroxymethylfurfural (HMF), and furfural.

Sotolon (3-hydroxy-4,5-dimethyl-2(5H)-furanone, also known as caramel furanone, sugar lactone, or fenugreek lactone) imparts a fenugreek, nutty, caramel, curry, rancid, maple syrup, or burnt sugar odour depending on its concentration and enantiomeric distribution [14,15]. It is considered a key aroma of several fortified wines [16,17], including MWs, with concentrations as high as 2000 μg/L [18], well above its odour threshold of 23 μg/L [19]. There is evidence that its occurrence in MW may be related to sugar degradation mechanisms [13,18]. Despite being reported as an important ageing marker of MWs [10,18], little is known about its formation rate during the ageing processes traditionally used to age these fortified wines.

Concerning HMF and furfural, both are also formed from the degradation of monosaccharides but can also be extracted from wood during ageing [20]. Previously, furfural was reported to impart typical notes of forced-aged MWs [2]. Despite being a quantitatively important MW metabolite [12,21], HMF is not always relevant to the aroma of these fortified wines [3]. These furans have been suspected to be hazardous to humans due to toxic, mutagenic, and carcinogenic effects when present at high doses [22,23]. The Joint FAO/WHO Experts Committee on Food Additives (JECFA) has set an acceptable daily intake of 0.5 mg of furfural per kg of body weight. Regarding HMF, its risk to human health is not fully elucidated, but its toxicological potential seems to be lower, because several animal experiments have shown no adverse effects between 80 and 100 mg/kg body weight per day [23]. Moreover, HMF occurrence in alcoholic beverages does not seem to constitute an additional risk for average drinkers [24]. In contrast, while HMF is approved as a flavouring agent (accredited within EU regulation No 2232/96, FL-No. 13.139), its use as a food nutritional supplement has been gaining attention due to positive effects that have been reported more recently [25,26].

Due to the influence of temperature and maturation time, ethyl carbamate (EC), also known as urethane, is naturally developed in MWs, usually at ppb levels [27,28]. Most EC production is due to reactions between ethanol and carbamoyl compounds, such as urea and citrulline, derived from arginine metabolisation [29]. The International Agency for Research on Cancer classifies EC as a probable carcinogen (group 2A) [30], raising food safety concerns. Some markets have established EC limits in alcoholic beverages, namely in fortified wines of 100 μg/L [31].

This three-year study aimed to monitor the levels of key ageing-related metabolites in wines submitted to the two typical MW ageing processes and assess their formation rate. The selected compounds, sotolon, HMF, furfural, and EC, are related to wine quality and safety, with some also recognised as ageing markers. Moreover, CIELAB parameters were monitored, given that the accumulation of these compounds is usually accompanied by colour changes, consequently influencing the typical colour attributes of MW. Therefore, understanding the production and accumulation of these compounds could improve MW quality control processes and safety.

2. Materials and Methods

2.1. Chemicals

Ethyl acetate and phosphoric acid were purchased from Fisher Scientific (Leicestershire, UK), ammonium dihydrogen phosphate and formic acid from Panreac (Barcelona, Spain), HPLC-grade methanol was purchased from Chem-Lab (Zedelgem, Belgium), and acetonitrile from Honeywell Riedel-de-Haën (Seelze, Germany). Butyl carbamate internal standard was acquired from Sigma-Aldrich (Steinheim, Germany) while the EC, HMF, and furfural standards were obtained from Acros Organics (Geel, Belgium). All chemicals had a purity grade higher than 97%. Type 1 water (ultra-pure) was obtained from a Simplicity® UV ultrapure water system from Millipore (Milford, MA, USA).

2.2. Samples

At least 1000 kg of each grape variety were used to produce 1 initial batch of the following MWs: Sercial, Malvasia, Tinta Negra Dry (TND), and Tinta Negra Sweet (TNS), which were vinified in separated stainless steel tanks, according to common practices used by a local winery. The densities of the initial musts varied between 1061 (Sercial) and 1070 g/L (Tinta Negra). A solution of 10% sulphite was used to obtain a concentration of about 60 mg/L in the musts. Pectinases and diammonium phosphate were also added to all musts. After separating the skins, the fermentative process was controlled below 25 °C, in stainless steel tanks. Commercial yeasts were not added during the fermentation process and grape skins, together with the grape juice, followed 24 h of maceration. The fermentation process was stopped at different stages by adding vinous alcohol (containing 95% (v/v) of ethanol), according to the grape variety and the desired wine style. The alcohol content was adjusted to about 17% (v/v). The fermentation was stopped according to the typical densities of dry (about 1005 g/L) and sweet wines (about 1046 g/L). Then, the wines were clarified and stabilized through bentonite clays and albuminocol gelatins (post-fermentation treatments). The pH of the resulting wines varied from 3.12 to 3.48, and the total acidity ranged from 7.0 to 11.2 g/L expressed as tartaric acid.

As shown in Figure 1, each wine was divided into two new batches. One batch was immediately transferred to wooden casks and aged following the *canteiro* ageing process for a total of 1080 days or 3 years. The other batch was preliminarily submitted to the *estufagem* process (i.e., stainless steel tanks at about 45 °C for 4 months) and then transferred to wooden casks and *canteiro* aged for the remaining period. For each wine sample, 2 independent replicates (2 × 750 mL) were collected at 0, 180, 360, 720, and 1080 days of ageing. Despite this exploratory study having some limitations regarding the number of repetitions and non-repeatability of experiments for the previously described wines, the sample size is enough to draw preliminary comparisons between the ageing processes (4 *estufagem* aged wines vs. and 4 *canteiro* aged wines), comparing wines derived from different wine styles (4 sweet wines vs. 4 dry wines) and grape varieties (4 whites vs. 4 reds). Before all analyses, samples and extracts were passed through 0.22 µm polypropylene (PP) syringe filters (BGB, Rheinfelden, Germany). All samples' replicates were analysed in duplicate (n = 4).

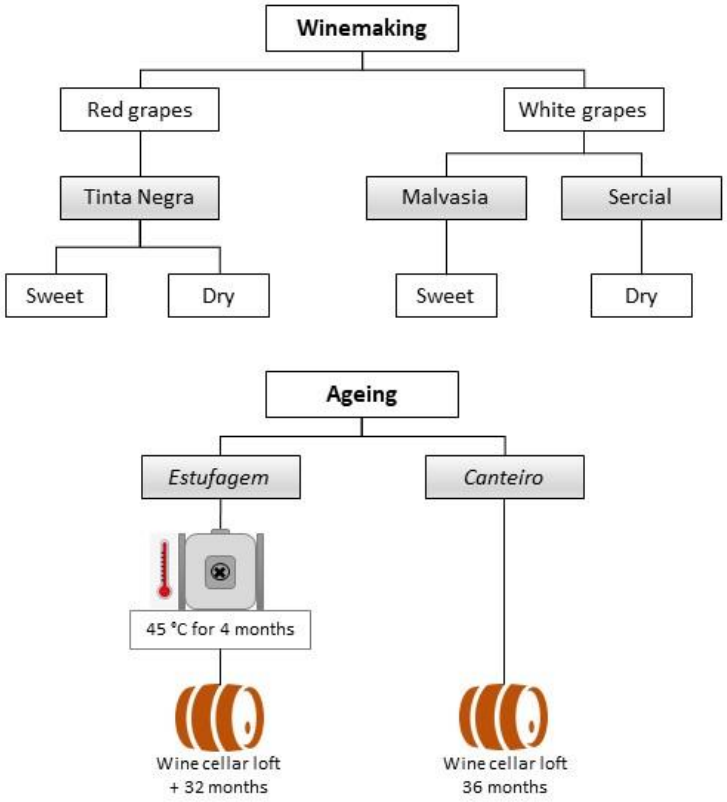

Figure 1. Schematic representation of the experimental trial.

2.3. HMF and Furfural Quantification

Liquid chromatography with photodiode array detection (LC-DAD) was used to determine HMF and furfural concentrations, following an adapted methodology [32]. Briefly, 10 µL of the sample were directly injected into a Nexera X2 UHPLC system outfitted with an SIL-30AC autosampler, LC-30AD binary pumps, a DGU-20 A5 degassing unit, and a CTO-20A column oven, all obtained from Shimadzu (Kyoto, Japan). Separation was performed on a reversed-phase Atlantis T3 C18 column (4.6 mm × 250 mm, 5 µm) from Waters (Milford, MA, USA), maintained at 30 °C. A 10 mM phosphate buffer solution (pH 2.70, corrected with 1 M phosphoric acid) and acetonitrile were used as elution solvents A and B, respectively. The following elution gradient was used: 95% solution A decreased to 88% in 10.5 min, decreased to 30% in 0.5 min, increased to 40% in 2 min, increased to 95% in 1 min, and held at 95% for 6 min, at a flow rate of 1 mL/min. Prior to use, the elution solvents were passed through hydrophilic PP membrane filters with a 0.2 µm pore size from Pall Corporation (Ann Arbor, MI, USA). Spectra were acquired in the 190 to 300 nm range, and furfural and HMF were quantified at 274 and 281 nm, respectively, using an SPD-M20A photodiode array detector from Shimadzu. Compounds were identified by comparing their UV-vis spectra with those of the standards and quantified using an external standard calibration. Data were acquired and processed using the LabSolutions 5.7 software (Shimadzu, Kyoto, Japan). The sample replicates were analysed in duplicate.

2.4. Sotolon and Ethyl Carbamate Quantification

The concentrations of sotolon and EC were determined by miniaturised liquid-liquid extraction procedures followed by liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis, as previously reported [28,33]. The Shimadzu UHPLC system described in Section 2.3 was employed for both analyses. However, for these experiments, that UHPLC system was coupled to an LCMS 8040 triple-quadruple mass spectrometer equipped with an ESI ionisation module set for acquisition in the positive ion mode, using the multiple reaction-monitoring (MRM) mode, between 0.5 and 9.0 min. Settings were

fixed as follows: the desolvation line and the block heater were set to 250 and 400 °C, respectively; the nebulising gas flow was set to 2.5 L/min; and the drying gas flow to 17.5 L/min.

In the case of sotolon, the m/z transition 129.1 → 83.0 was used for identification purposes and the m/z transition 129.1 → 55.1 for quantification. For EC, identification and quantification were achieved by monitoring the m/z transitions 90.10 → 44.50 and m/z 90.10 → 62.05, respectively. Replicates of samples were analysed in duplicate. Data were acquired and processed using the LabSolutions 5.7 software.

2.5. CIELAB Analyses

The wine colour was monitored by determining the CIELAB parameters using UV-vis spectrophotometry, as recommended by the OIV (International Organization of Vine and Wine: OIV-MA-AS2-11: R2006) [34]. The CIELAB parameters were determined by measuring transmittance in the 380 to 780 nm range in 5 nm intervals. Four replicates were analysed for each wine, and each sample's readings were performed in duplicate, using quartz cells with an optical path length of 1 cm and ultra-pure water as the blank. Analyses were carried out on a Shimadzu UV-vis 2600 spectrophotometer (Shimadzu, Kyoto, Japan). Data were acquired using the Shimadzu UVProbe 2.42 software (Shimadzu, Kyoto, Japan). The L^*, a^*, and b^* coordinates were determined using the Color Analysis software version 3.10 (Shimadzu, Kyoto, Japan), with the following settings: *D65* illuminant and 10° incidence angle.

2.6. Data Analysis

The MATLAB® computational platform (Version 9.10.0.1602886, R2021a, The Mathworks, Inc., Natick, MA, USA) was used for the correlation analyses. In particular, the Spearman correlation coefficient was chosen to examine the relationship between the variables under study: the HMF, furfural, sotolon, and EC concentrations; the CIELAB colour parameters; and the storage time (the three years of ageing).

3. Results and Discussion

3.1. HMF and Furfural Quantification

The concentrations of HMF and furfural were determined after 0, 180, 360, 720, and 1080 days of ageing for each wine submitted to each traditional ageing system: *estufagem* and *canteiro*. As depicted in Figure 2a,b, both furans increased with ageing time, especially in the sweet wines, TNS-E and M-E, exposed to thermal processing in the first year of ageing before undergoing oak ageing. The *estufagem* process significantly accelerated their formation rate by up to about 47% in the first year of ageing. Higher amounts were formed during this period while their formation slowed to less than half during the oak ageing period. After 1080 days of ageing, one can observe that the HMF concentration levels varied widely and are strongly dependent on the wine style and ageing process, with levels ranging from 31.2–142.0 mg/L in sweet wines and 6.4–10.1 mg/L in dry wines. Sweet *estufagem* aged wines presented HMF amounts 3.4-fold higher than those aged by *canteiro* while dry ones were only 1.4-fold higher. Furfural levels were quite lower, varying between 6.2 and 12.9 mg/L. There were no major differences in the furfural levels in sweet and dry wines. The odour activity value (OAV) is commonly used to measure the odorant impact of molecules in foods and is calculated by dividing the concentration of the compound by its odour detection threshold. Considering the odour threshold values of 10.0 and 14.1 mg/L, for HMF and furfural in 10–12% (*v*/*v*) ethanolic solutions at 20 °C [5], only HMF was perceptible by the human nose, suggesting that this compound has a significant contribution to the caramel-like aromas of sweet MWs, with OAVs ranging between 3.1 and 14.2.

Considering the acceptable daily intake of furfural previously suggested (0.5 mg/kg bw/d) and that no observed adverse effect levels of around 80 to 100 mg/kg bw/d were suggested

for HMF, moderate consumption of these wines does not seem to pose additional risk to human health.

In general, the results presented herein agree with those previously reported [12].

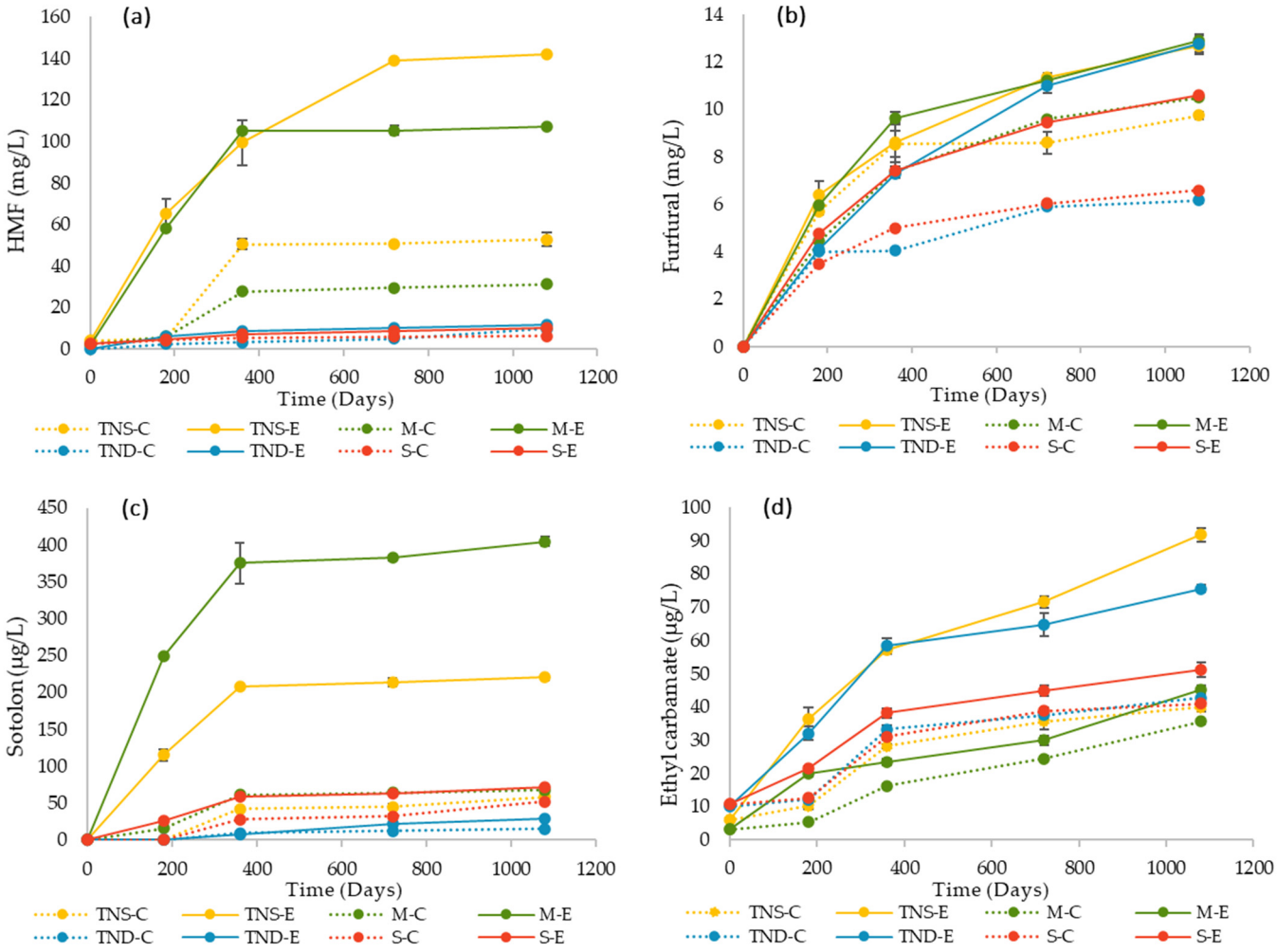

Figure 2. Average concentrations (n = 4) of (**a**) HMF, (**b**) furfural, (**c**) sotolon, and (**d**) EC after 0, 180, 360, 720, and 1080 days of ageing. TNS-C—Tinta Negra Sweet under *canteiro*; TNS-E—Tinta Negra Sweet under *estufagem*; TND-C—Tinta Negra Dry under *canteiro*; TND-E—Tinta Negra Dry under *estufagem*; S-C—Sercial under *canteiro*; S-E—Sercial under *estufagem*; M-C—Malvasia under *canteiro*; M-E—Malvasia under *estufagem*.

3.2. Sotolon Quantification

The levels of sotolon before the ageing period were not quantifiable, but with ageing, they increased, regardless of the ageing procedure employed. It was previously shown that sotolon levels increase in wines exposed to oxidative conditions, such as increased temperature and/or oxygen exposure [35]. The concentrations of sotolon determined at each sampling point are shown in Figure 2c. The levels of this metabolite after 1080 days of ageing ranged from 29.0 ± 2.1 to 404.5 ± 6.4 µg/L. Higher concentrations of sotolon were detected in sweet wines, up to 404.5 ± 6.4 and 220.3 ± 3.5 µg/L in M-E and TNS-E, respectively. In contrast, TND wines had the lowest concentrations after 3 years of ageing, with 15.3 ± 1.5 µg/L in the TND-C and 29.0 ± 2.1 µg/L in the TND-E wines. The disparities found within the levels between wine styles are related to sugar degradation, given that the occurrence of this compound in fortified wines has mainly been related to this formation pathway [13]. It is noticeable that the ageing process employed impacted on the formation of sotolon. In this regard, the wines that underwent *estufagem* ageing contained higher

concentrations of sotolon at the end of the ageing period, reaching levels up to 6.0-fold higher than *canteiro* ones.

Previously, an odour detection threshold of 23 μg/L was reported for sotolon in MWs [19]. Considering this, one can observe that sotolon significantly contributes to the aroma of sweet *estufagem* aged wines, reaching OAVs of up to 17.5. In the corresponding wines aged under the *canteiro* ageing system, its odorant impact was less expressive, and OAVs were lower than 2.9. Moreover, the average OAVs of 3-, 5-, and 10-year-old MWs were found to be 2.8, 6.3, and 9.8, respectively [19]. Thus, the soloton odorant impact in the sweet wines aged by the *estufagem* process surpassed the average value obtained for 10-year-old MWs while those aged by *canteiro* were comparable to 3-year-old MWs.

3.3. Ethyl Carbamate Quantification

Before starting the ageing process (i.e., time = 0 days), EC varied between 3.1 ± 0.2 and 10.7 ± 0.4 μg/L. The EC levels increased with the ageing time, ranging from 35.6 ± 0.9 to 92 ± 2 μg/L after 1080 days of ageing (Figure 2d). The increased temperature that these wines were exposed to during *estufagem* was enough to promote its formation, a result that is consistent with a previous study [29]. The analyses performed at 180 days of ageing of the previously heated wines revealed that the EC concentrations were higher (9–26 μg/L) than the corresponding wines submitted to *canteiro*. Notably, the EC formation rate was higher in the first year of ageing, yielding levels of 40% to 78% of the final concentration.

Tinta Negra wines submitted to *estufagem*, TNS-E, and TND-E had the highest predisposition to form EC, reaching 91.8 ± 2.0 μg/L after 1080 days of ageing. A previous study showed that TN wines presented higher amounts of arginine in comparison to Malvasia, decreasing during *estufagem* [36]. The current findings corroborate those previously reported by Leça et al. [37], who found that residual arginine can contribute to EC accumulation during wine ageing.

The remaining wines varied between 35.6 ± 0.9 and 51.2 ± 2.1 μg/L. White varieties appear to be the less prone to EC formation, even when submitted to *estufagem*. It is important to highlight that none exceeded 100 μg/L, the limit established by most markets [31].

3.4. CIELAB Analyses

The obtained CIELAB coordinates (L^*, a^*, and b^*) are represented in Figure 3. The initial coordinates of a^* were all positive (average value of 13.0), indicating a moderate occurrence of red tones in young MWs. There was a general tendency for the a^* values to decrease during ageing. This decrease may be related to tannin and anthocyanin oxidation reactions, which are also present in white wines but usually to smaller extents [38]. The *estufagem* process accelerated the a^* decrease in all samples and was more pronounced in TNS wines. The b^* values were also always positive, suggesting a strong predominance of yellow tones, even at the initial stage (average value of 42.6). The M-E and S-E wines (i.e., white varieties) displayed slightly reduced b^* values up to 180 days of ageing that tended to increase to values close to those initially observed (T0). Both Tinta Negra wines, TNS and TND, exhibited a clear tendency to increase their b^* values, especially when they underwent *estufagem* prior to oak ageing. It is plausible that the b^* value increase is associated with the yellow pigments derived from Maillard reactions and acidic sugar degradation [13,39]. Furthermore, the L^* values (perceptual lightness) increase with age in TNS, and this tendency of the wine to become clearer is augmented by the *estufagem* ageing process. A decrease in the L^* values can be observed in Sercial wines. In Malvasia and TND wines, there were no evolution trends for this parameter during ageing.

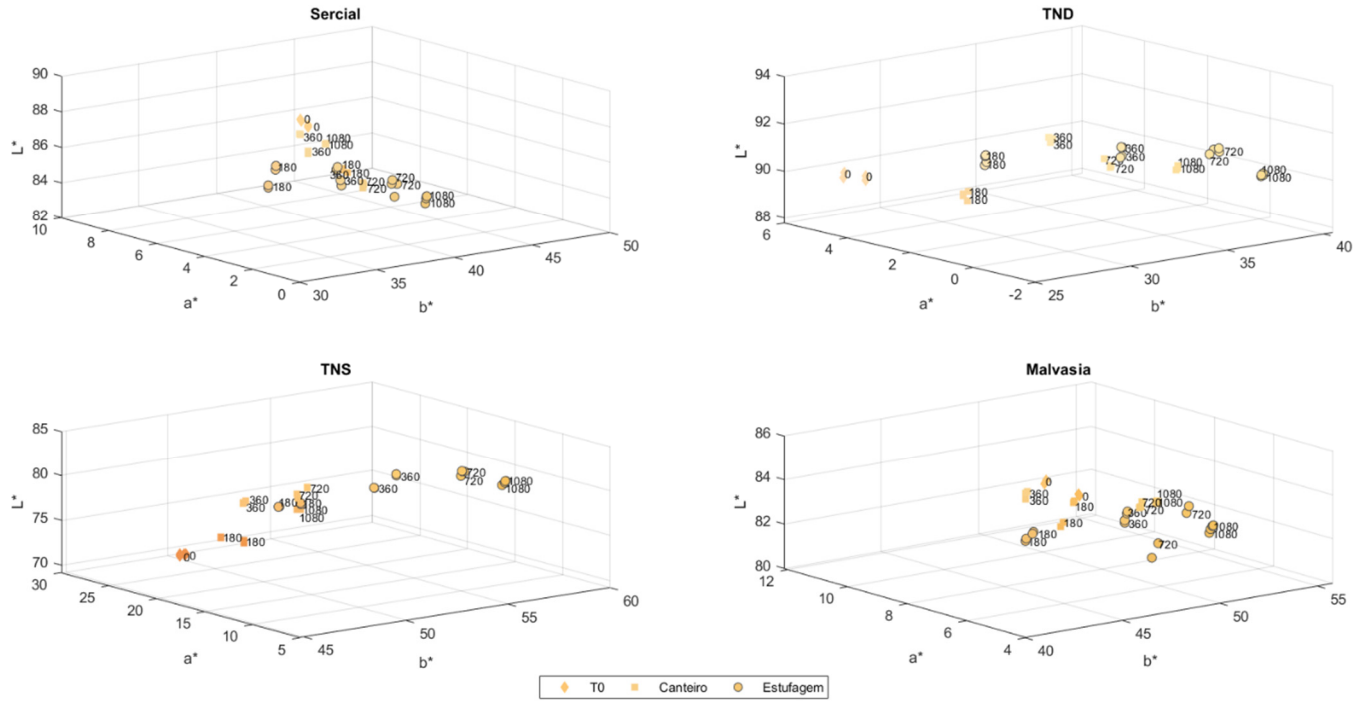

Figure 3. CIELAB coordinates of the Malvasia, Sercial, and Tinta Negra Sweet (TNS) and Tinta Negra Dry (TND) wines during *canteiro* vs. *estufagem* ageing for up to three years. The numbers labelled at each point correspond to the ageing days.

3.5. Correlation Study

The plot matrix of correlations among the pairs of variables studied (the four metabolites, colour parameters, and ageing time) according to the maturation process (*canteiro* or *estufagem*) are presented in Figures 4 and 5. The Spearman correlation coefficient was chosen to proceed with the analysis. The coefficient correlation values are presented in Figure 4 (*canteiro*) and Figure 5 (*estufagem*) and range from −1 to +1. Negative and positive values indicate that both variables tend to increase or decrease together. Additionally, the larger the absolute value of the coefficient, the stronger the relationship between the variables.

All metabolites increased significantly in both ageing systems, mostly within the first year. As shown in Figures 4 and 5, the evolution of furfural and HMF strongly correlates with the ageing time, regardless of the maturation process applied (i.e., *canteiro* or *estufagem*), presenting correlation coefficients (r) of 0.85 and 0.94 for furfural and 0.77 and 0.73 for HMF. The distribution levels of furfural and HMF are higher in wines subjected to *estufagem.* This result is not surprising since both compounds are recognised as heat-treatment markers [2]. Similarly, sotolon presented strong and moderate correlations of r = 0.79 and r = 0.65 with the ageing time when aged by the *canteiro* and *estufagem* processes, respectively. Despite *estufagem* having a stronger influence on sotolon's increase during wine ageing, especially in sweet wines (Section 3.1), this compound is more directly correlated with the ageing time under *canteiro* ageing.

The evolution trends of sotolon, furfural, and HMF evolution were strongly correlated (r = 0. 74–0.90) in both ageing processes. This result was somehow expected, considering that these compounds can originate from similar metabolic pathways [11–13]. Notably, the CIELAB coordinate *b** (yellow colour) correlated with these furanic compounds when the wines were *estufagem* aged, with r varying between 0.54 and 0.70 (Figure 5). These results are consistent with the fact that the development of these metabolites in wines has been previously related to thermal processing. Additionally, the yellow pigments are likely derived from non-enzymatic browning reactions, the Maillard reaction, and acid-catalysed sugar degradation [11–13].

EC evolution strongly correlated with ageing time in the *canteiro* (r = 0.88) and *estufagem* (r = 0.78) processes. Although EC has different formation pathways of the age markers monitored in this study, a strong correlation between EC evolution and furfural and HMF evolution (r = 0.66–0.85) was observed in both maturation processes. It is important to point out that the EC levels never surpassed the limit established by most markets (100 µg/L) [31] regardless of the three-year ageing process utilised.

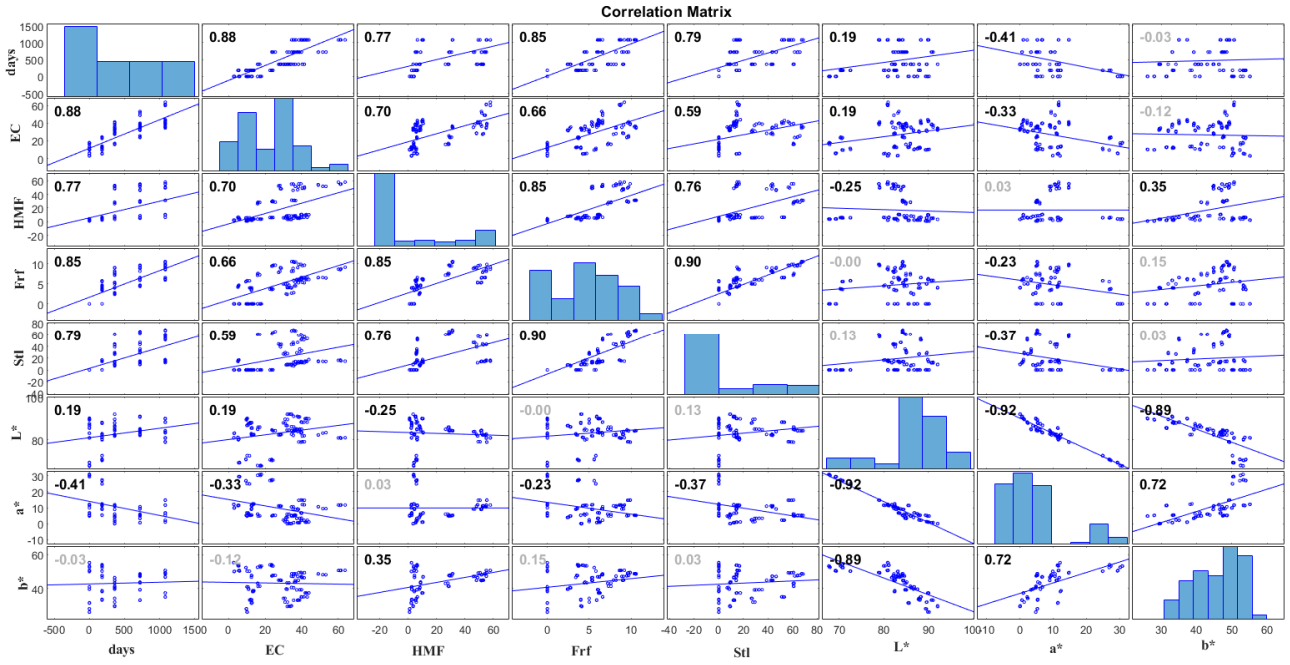

Figure 4. Plot matrix of correlations in *canteiro* among the pairs of variables studied: sotolon (Stl), furfural (Frf), HMF, EC, colour parameters (*L**, *a**, nd *b**), and ageing time (days). The Spearman correlation coefficient can range from −1 to +1.

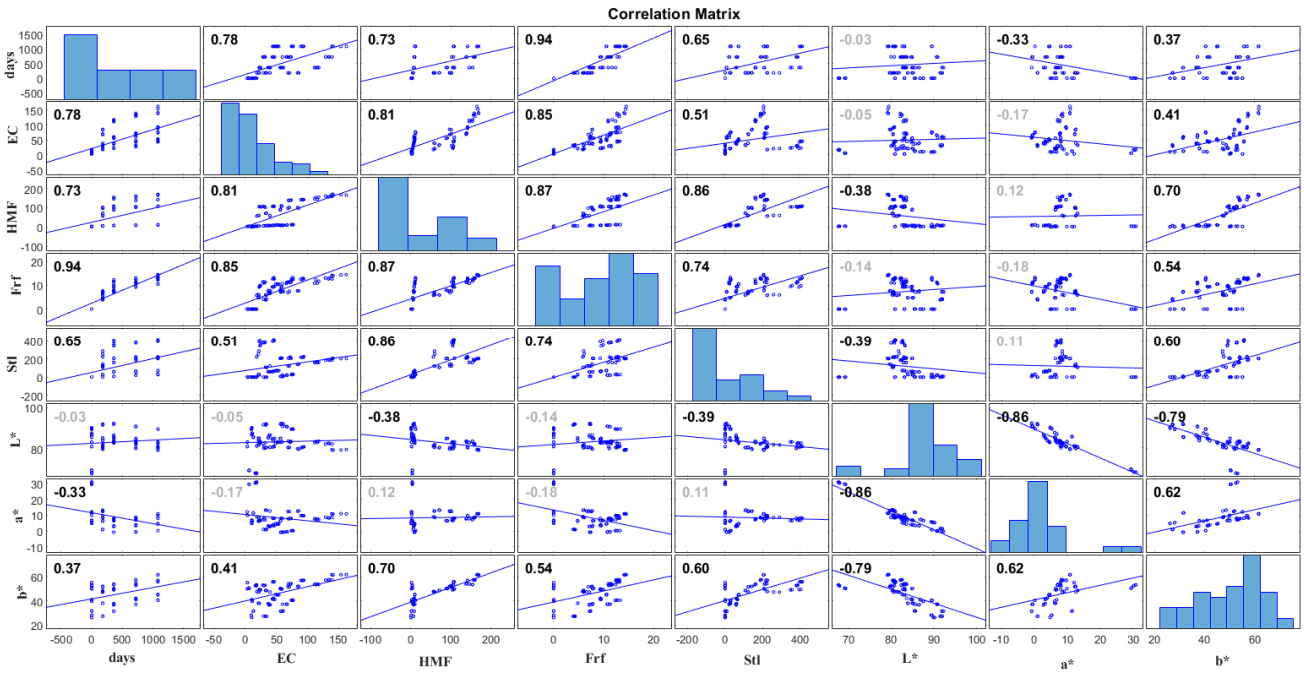

Figure 5. Plot matrix of correlations in *estufagem* among the pairs of variables studied: sotolon (Stl), furfural (Frf), HMF, EC, colour parameters (*L**, *a**, nd *b**), and ageing time (days). The Spearman correlation coefficient can range from −1 to +1.

4. Conclusions

This study suggests that *estufagem*, when applied under controlled conditions to young MWs, accelerates the formation rate of ageing-related quality metabolites, without adding additional risk to moderate consumption. HMF and sotolon formation rates were found to be strongly dependent on the wine style and ageing process, reaching levels up to 3.4- and 6.0-fold higher in *estufagem* ageing, respectively. Both seem to contribute to the aroma of sweet MWs, particularly those aged under *estufagem*, reaching OAVs up to 17.5. The furfural formation rate was not influenced by the wine style, but it was accelerated under *estufagem* ageing. The EC levels also increased with ageing time and elevated temperature exposition, reaching levels up to 2.3-fold higher when MWs were *estufagem* aged. Tinta Negra wines showed a higher predisposition to form this compound, but the limit established by most markets was never exceeded. However, greater attention should be devoted to its control in wines made from this red variety.

The correlation study showed that all metabolites correlated with ageing time in both maturation processes. Interestingly, EC evolution correlated with both furans despite different formation pathways being reported. The sotolon, furfural, and HMF evolution trends were strongly correlated with each other, reinforcing that they are derived from the same formation mechanisms.

This study provides important information that could be leveraged to develop more efficient methods and alternative solutions for detecting these metabolites to control the quality and safety of MWs and other fortified wines.

Author Contributions: Conceptualisation, V.P.; formal analysis, J.M.L., A.I.F. and A.C.P.; investigation, J.M.L., A.I.F. and M.P.; writing—original draft preparation, J.M.L. and A.I.F.; writing—review and editing, J.M.L., A.I.F. and V.P.; visualization, A.I.F. and A.C.P.; supervision, V.P.; project administration, V.P., M.P., F.A. and J.C.M.; funding acquisition, V.P., F.A. and J.C.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Regional Development Fund (ERDF) and by national funds (FCT/MCTES) under the projects IMPACT II (MADFDR-01-0190-FEDER-000010), IMPACT III (M1420-01-0247-FEDER-000024), AROMA (CENTRO-01-0145-FEDER-031568 and PTDC/EEI-EEE/31568/2017), and UIDP/50025/2020. Vanda Pereira (Post Doc), Ana C. Pereira (Post Doc) and João M. Leça (PhD student) are thankful to the *Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação* (ARDITI) for funding their grants in the scope of the project M1420-09-5369-FSE-000001. Ana Isabel Freitas (PhD student) is thankful to *Fundação para a Ciência e Tecnologia* (FCT) for the financial support through the grant SFRH/BD/145262/2019.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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