Chapter

Port Wine: Production and Ageing

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Abstract

Port wine is a traditional and worldwide renowned fortified wine produced in the Douro Demarcated Region (DDR) Northeast of Portugal by specific and traditional winemaking practices. The final quality and uniqueness of the different Port wine styles are highly dependent on the ageing process, namely time, temperature and oxygen levels that will ultimately dictate the type and extension of the chemical changes that occur during this process. These chemical changes occurring during the Port wine ageing process results in significant changes in colour and aroma according to the different ageing conditions employed for the different Port wine styles. This chapter intends to give a broad and generic overview of the known and potential chemical changes occurring during ageing of Port wine that are responsible for the changes in the sensory profile observed during the ageing process. Also, the known chromatic and aromatic characteristics of the different Port wine styles and the specific ageing processes, reductive or oxidative, are reviewed.

Keywords: Port wine, Douro Demarcated Region, ageing process, colour, aroma

1. Introduction

Port wine is a traditional fortified wine produced in the Douro Demarcated Region (Northeast of Portugal in the Douro Valley, **Figure 1**) under very specific conditions. There are several Port wine styles being related to the winemaking and ageing process and also to the ageing time, which enhances uniqueness to the wines and recognition throughout the world. The Douro Demarcated Region is located within the Douro River basin, surrounded by mountains, having a total area of approximately 250,000 hectares. This region has singular climate and soil characteristics for the production of selected grape varieties for the Port winemaking that contributes to the distinctive characteristics of Port wines and guarantee that these wines are irreproducible elsewhere. This area is divided into three naturally distinct sub-regions (**Figure 1**) concerning the climatic as well as socio-economic factors, "Baixo Corgo" (Lower Corgo), "Cima Corgo" (Upper Corgo), and "Douro Superior" (Upper Douro) [1].

Different grape varieties are used in the production of Port wine, being usually produced by their blend. There is evidence that the grape varieties determines the wine character, even after the extended ageing process. The grape varieties that may be cultivated in the Douro Demarcated Region are regulated by Decree-Law n°104/85, 10th of April, 1985 [2]. Accordingly, Port wine is produced only from the authorised grape varieties, being the principal red grape varieties recommended for Port wine production "Touriga Nacional", "Mourisco Tinto", "Bastardo", "Tinta

Figure 1.
Location of the Douro Demarcated Region in the northeast of Portugal and of the three sub-regions: "Baixo Corgo", "Cima Corgo" and "Douro Superior" according to [1].

Roriz", "Tinta Cão", "Tinta Amarela", "Tinta Barroca", "Touriga Franca" and "Tinta Francisca", since these grape varieties produce wines with stable colour, fruity aroma and sugar content, characteristics required to produce good quality Port wines. The white grape varieties used for White Port wine production are "Malvasia Fina", "Viosinho", "Donzelinho Branco", "Gouveio", "Rabigato", and "Códega" [3].

In the Port wine vinification process, the alcoholic fermentation is stopped according to the desired residual sugar content by the addition of a wine spirit known as "aguardente vínica" (with an alcohol content of about 77% (v/v)), to an alcohol content up to 18–22% (v/v) of the final product. Therefore, Port wine is a naturally sweet fortified wine since the natural sugar from the grapes is not completely transformed into alcohol. After the vinification process, Port wine is usually stored and aged in wood barrels of different sizes, from 2 years to many decades in accordance with the intended Port wine style. The Port wine ageing process can take place either in the Douro Valley or in Vila Nova de Gaia (Porto), in order to qualify for a Certificate of Origin from the "Instituto dos Vinhos do Douro e Porto" (IVDP).

2. Port wine production and styles

Port wine is subjected to an extensive set of legislation and regulations. According to the Decree-Law n°173/2009 of 3rd of August [4], the IVDP, located in Oporto city, has the responsibility of promoting and perform the quality control of Port wine, as well as the amount of Port wine that can be produced annually, regulating all the production process, and the protection of the denominations of origin Douro and Port and the geographical indication of the Douro Region. The panel of expert tasters of IVDP is responsible for the certification and approval of wines and wine spirits, as well as the granting of the guarantee seal [5].

In the Port wine vinification process, the alcoholic fermentation is stopped, between 6% and 9% (v/v) alcohol content, according to the Port wine style and sweetness desired. Therefore, the wine is runoff from the skins, and it is fortified with a wine spirit containing 77% (v/v) of ethanol to raise the alcohol concentration to 18–22% (v/v). The average proportions of wine spirit added are 115 L for each 435 L of fermenting wine. The wine spirit allowed to be used in Port wine production required rigorous quality standards regulated by the laboratories and panel of tasters of IVDP. The sensory characteristics evaluated by the panel are turbidity, colour, aroma, and taste. The analytical parameters (ethyl carbamate, total higher alcohols, acetaldehyde (ethanal), ethyl acetate, methanol, 2-butanol, 1-butanol, allylic acid, cyanidric acid, calcium, copper, iron, alcohol content, total acidity, and density) must be below of the allowed limits described in Regulation n° 84/2010 [6]. Contrasting with most other fortifying spirits, the wine spirit used in Port wine production is not highly rectified; therefore, it contains many flavourants, especially higher alcohols, and aldehydes and this fortification process results in a high concentration of acetaldehyde in these initial wines.

Port wine can be extra dry, dry, semi-dry, sweet or very sweet, according to the levels of unfermented sugars remaining (**Table 1**) that is dependent on the time of wine spirit addition to stop the alcoholic fermentation [1].

There are four main different styles of Port wine, designated as Tawny, Ruby, White and Rosé (**Figure 2**). Port wine intended for Tawny style are obtained from different wines in different stages of ageing. During the ageing in the wood barrels, the red colour of the wines gradually develops into tawny, medium tawny or light tawny, with an aroma of dried fruits and wood [1]. In this style, there are some special categories like Tawny Reserve, Tawny with Indication of Age (10, 20, 30 and 40 years) and "Colheita". This last category is an exception, as these wines are from a single vintage [7]. All these wines are ready to drink when they are bottled [1]. Port wines that belong to Ruby style are wines that the evolution of their deep red colour is limited and the fruity character is maintained [1]. Within this Port style, special categories

Sweetness	Specific gravity (g/cm^3, 20°C)	°Baumé (°Bé)	Sugar content (g/L)
Extra dry	<0.9980	0.0	<40
Dry	0.9980–1.0079	0.0–1.3	40–65
Semi-dry	1.0080–1.0179	1.4–2.7	65–85
Sweet	1.0180–1.0339	2.8–5.0	85–130
Very sweet	>1.0340	> 5.0	>130

Table 1.
Port wine classification according to their sugar content.

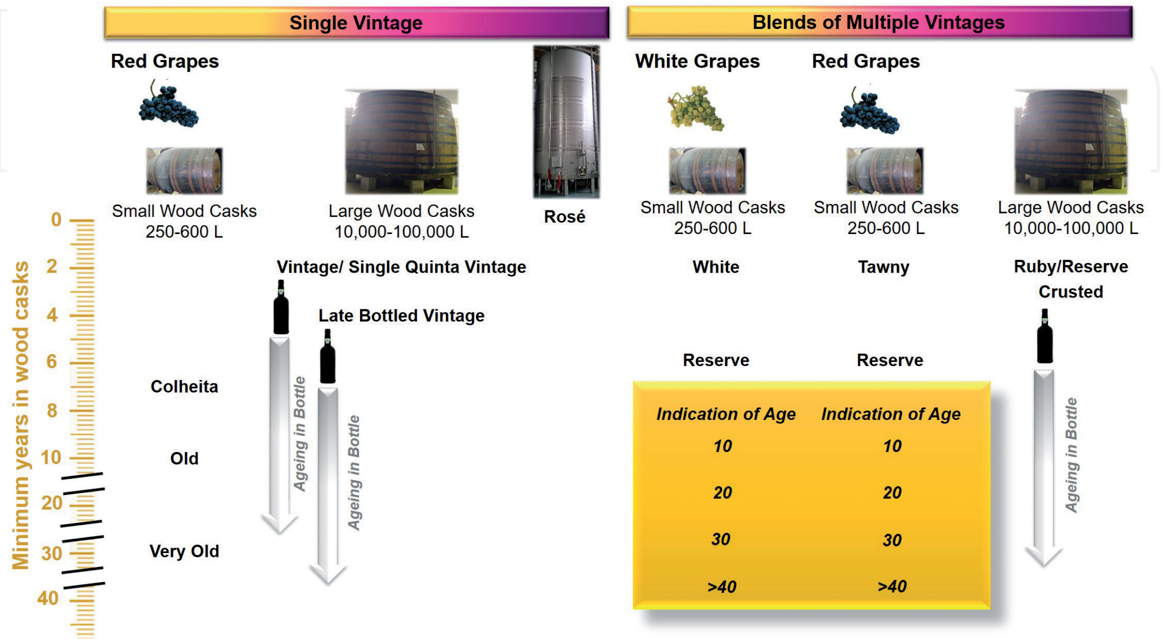

Figure 2.
Port wines styles and categories according to [1, 7].

can be found like Crusted, Reserve, Late Bottled Vintage (LBV) and Vintage [7]. Crusted Ports are high quality, very full-bodied, deep coloured wines, obtained by blending wines from numerous vintages, aged for a minimum of 2 years in wood barrels and then bottled and aged during further 3 years. The year in which the wine was bottled must be indicated on the bottle label [1, 7]. LBV is a special single harvest and it is bottled after 4 to 6 years from the harvest, with the previous ageing in vats (wood or stainless steel) and has a deep ruby red colour, extremely full body and rich in the mouth. To be recognised as Vintage, the wines must present an outstanding quality and with a very full bodied and have a deep colour. Vintage and LBV are good for storing since they age well in the bottle [1]. The types of Port wines made from red grape varieties vary in colour from deep purple to light gold, with transitional hues like tawny, golden tawny, golden and light gold. White Port wines differ according to shorter or longer periods of ageing and different degrees of sweetness. The colour of White Port wines varies from pale yellow, straw to golden white. However, when aged in wood barrels for many years, white Port wines develop, through natural oxidation, a golden hue that is very similar to that of a very old Tawny Port wine made from red grapes [1]. The special categories inside this style are similar to those of Tawny Port wines [7]. White Port wine is a Port wine style with increasing market expression. Nowadays it represents 33% of the total Port wine sales with a higher market value (+6.2%) when compared to Tawny Port wine, and nearly 80% of the production is exported [1]. Rosé Port wine is a recent Port wine style, first released in the market in 2008 by Croft, part of the Taylor Fladgate Partnership. It is technically a Ruby Port but fermented in a similar way to a Rosé wine, with limited grape skin maceration, thus producing the pink colour. Croft came up with Rosé Port as a way to introduce the pleasures of Port wine to a younger market. The colour of this type of Port wine may go from light ruby to pale salmon. This style is commonly described as a light and fresh style of Port wine that is very fruity and enjoyable [1]. Different ageing processes leads to numerous Port wines quality categories, presenting different colours (from white to deep purple), sweetness (sweet to dry) and a wide range of flavours. The Port wines styles and categories are summarised in **Figure 2**.

3. Port wine ageing process

The ageing process is an important period for this wine and includes storage, ageing in wood barrels or vat tanks and/or bottle ageing. When aged in old wood barrels their size depends on the Port wine style. Wine intended for Ruby and Vintage Port wine production will be aged in large wood barrels and that intended for Tawnies will be aged in small ones. The Tawny Port wine undergoes an oxidative ageing process, while Ruby and Vintage Port have a much less oxidative ageing termed reductive ageing process [1, 7].

Ruby, Reserve and LBV Port wines usually age in large wood barrels for two, three years or even six years (LBV) and have a deep red youthful colour and intense fruity flavours, evocative of cherry and blackberry. Tawny Port wines (10, 20, 30 and 40 years old) age for longer periods in small wood barrels and show nuttiness and aroma of butterscotch. White Port wines usually age for two or three years in large wood barrels. Traditionally, White Port wines are fermented with skin contact like Red Port wines; in this case, the wines are aged in conditions that results in its oxidation. Nevertheless, the trend is for a shorter maceration period, to obtain White Port wines with a pale colour and fresh aromas [1]. Like Red Port wines, most White Port wines are fortified when half of the grape sugar concentration has been fermented. Semi-dry and dry White Port wines are fortified later, or when alcoholic fermentation is finished [8].

The fortification process gives a high concentration of acetaldehyde to the wines. Acetaldehyde is probably responsible for the colour stability by favouring the production of anthocyanin-tannin polymers (discussed below) [9, 10]. The high sugar concentration retained tends to mask the bitterness of small flavanols, but not their astringency [8, 11]. Young Port wines are generally sweet, intensely red with a high concentration in tannins and with a fruity aroma. The colour, aroma and flavour of young Port wines are due to compounds from the grape, from the alcoholic fermentation and from the wine spirit used for the fortification. These wines need to age to develop the complex sensory attributes typically associated with the several Port wine styles. Port wines can be aged for a minimum of three years to a decade or more in old wood barrels to develop their character; normally it is aged in an old wood barrel, ranging from 525 or 600 L capacity up to 200,000 L. The type and length of the ageing process, as well as the capacity of the ageing barrel, and the oxygenation during racking, influenced the Port wine style that will be developed depending mainly on the wine style planned. Therefore, wines destined for Ruby and Vintage Port wines will be usually aged in large wood barrels, while those intended for the production of Tawny Port wines will be aged in small wood barrels (**Figure 2**). Racking is a very important operation during Port wine ageing and may be performed periodically. Slight fortification after each racking operation to adjust the alcoholic content up to 22% (v/v), compensating the volume lost via evaporation from the wood barrels [8].

The value of aged Tawny Port wine is linked to the characteristic aroma compounds developed during the ageing process in small wood barrels that allow the admission of oxygen. This oxidative ageing is influenced by factors such as oxygen levels, temperature, and pH. The high quality Tawny Port wines generally have an 'indication of age (10, 20, 30, or over 40 years old)' on the bottle, and are a blend of wines aged in wood barrels from different years. The age indicated on the label corresponds to a wine that has the sensory characteristics recognised by the IVDP of a wine aged in wood with 10, 20, 30, or over 40 years, obtained by blending wines with different ages. Tawny Port wines produced from a single vintage are referred to as 'Colheita' Port wines, aged in wood barrels for a minimum of 7 years [1].

Ruby Port wines have red colour, full-bodied structure and often still quite fruity in character when the wines are ready to drink. Ruby Port wines are aged between 3 to 5 years before blending and bottling in old large wood barrels known as "balseiros" of larger capacity, between 10.000 and 100.000 L, and do not usually have any wood-aged characteristics. The flavour modifies from an intensely fruity, even spirity character when the wines are very young to a rich fruity ruby wine after 3 to 5 years ageing in wood. They are used to age full-bodies and fruity wines such as Ruby, LBV and Vintage Port wine. These wines age more slowly than those aged in smaller wood barrels, retaining their structure and fresh fruity aromas that are the main characteristics of these wines. Some special Ruby Port wines (the so called Vintage Port wines) have a considerable bottle ageing process, giving lighter red wines, with often a very fruity character, despite having aged for two decades or more. Vintage Port wines are aged in wood barrels for two or three years, followed by a considerable ageing time in a bottle in the so called reductive ageing (10 to 50 years or more before consumption), and so it develops a different character from those wines aged exclusively in wood barrels. These wines remain fruity and with a red colour. Consequently, Vintage Port wines develop much of its distinctive bouquet from a long process of reductive ageing in bottle.

After the initial period in wood, LBV wines are aged in dark glass bottles in cool dark cellars with controlled temperature, ventilation and relative humidity. The vintage year is always indicated on the label [1].

White Port wine is made in the same way as red Port wines. However, there is a tendency to reduce the skin contact time, and even to ferment clarified grape juice at a lower temperature (18–20°C), to obtain wines with fruity aromas. The wines are aged in small size old wood barrels for a minimum of three years before its commercialisation depending on the desired White Port wine colour type.

3.1 Chemical changes and sensory development during Port wine ageing

3.1.1 Colour development

The colour of red and white Port wines is one of the main quality parameters of the different Port wines styles. For Port wines made from red grape varieties, the initial wine colour is mainly due to the anthocyanins extracted from grape skins during vinification. Nevertheless, in a young Port wine, the percentage of colour due to the so called polymeric pigments is already 23 to 30% indicating that changes in the compounds responsible for the colour have already started during the short alcoholic fermentation and wine spirit addition to stop the alcoholic fermentation. The red Port wine colour increases up to 80% during the first months of ageing depending on the concentration of free acetaldehyde present in the young wine. After 46 weeks of ageing, the polymeric pigments can make up 78 and 98% of the wine colour [12]. The colour evolution during ageing is explained by the involvement of anthocyanins in different equilibria in solution and their simultaneous transformation through various concurring chemical reactions to a range of other pigments, many of them still unknown (**Figure 3**).

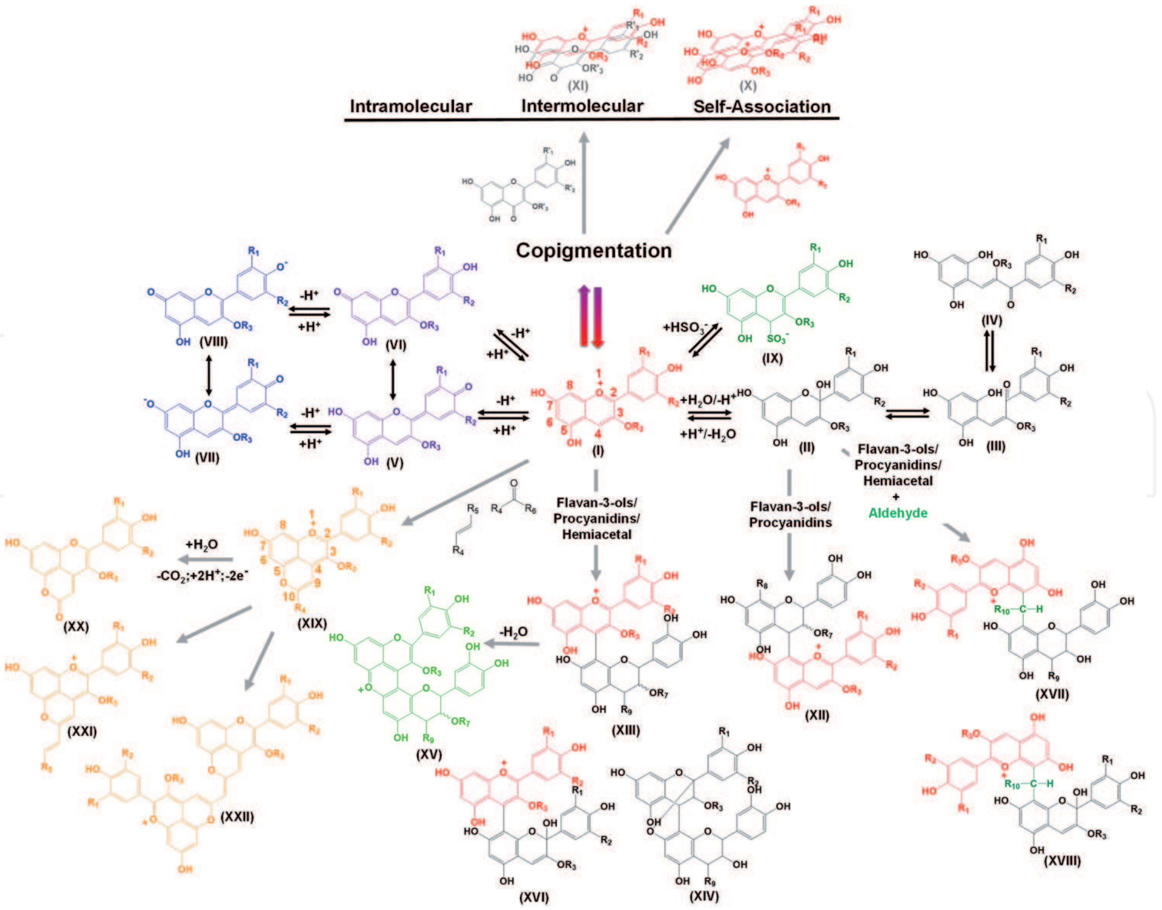

Figure 3.
*Colour evolution during ageing by the involvement of anthocyanins in different equilibria and their simultaneous transformation through various concurring chemical reactions to a range of other pigments (references are listed in **Table 2**).*

These changes are dependent on the wine composition like anthocyanin, flavonol and tannin concentrations, different processing parameters like temperature, oxygen level, pH and the presence of other compounds either produced during alcoholic fermentation, added during processing or formed during the ageing process. On the other hand, no studies have been reported about the colour changes occurring during White Port wine ageing.

Anthocyanins in aqueous solution, depending on the pH, occur in different forms present in equilibria [13–15]. At pH < 2, the red flavylium cation is the main structure present (I in **Figure 3**). With increasing pH, for values between 3 and 6, after hydration of the flavylium cation, the colourless hemiketal (II) structure is formed, this last being in equilibrium with the pale yellow *cis*-chalcone (III) through tautomerisation. This chalcone isomer is also in equilibrium with the *trans*-chalcone isomer (IV). With the pH increase, the flavylium cation is deprotonated to the corresponding violet neutral quinoidal bases (V and VI) that at higher pH yields the blue anionic quinoidal bases after further deprotonation (VII and VIII, **Figure 3**) [15]. When sulphur dioxide (SO_2) is present, there is observed reversible bleaching of anthocyanins that occurs due to the formation of the colourless anthocyanin-4-bisulphite adducts [16] (IX).

Considering all these equilibriums, at wine pH (3–4) these pigments would be expected to be present mainly in their non-coloured hemiketal form (II). However, the flavylium cation (I) is the main form present in young red wines. This is the result of its stabilisation by different copigmentation mechanisms such as self-association and interaction with other wine components [17–20]. In the copigmentation process, anthocyanins and other colourless organic compounds, such as flavonoids, amino acids, organic acids, polysaccharides, anthocyanins, or metallic ions, form molecular or complex associations [21]. The copigmentation is based in two effects [22]: (1) the formation of the π–π complex which causes changes in the spectral properties of the molecules in the flavylium ion, increasing the absorption intensity (hyperchromic effect) and its wavelength (bathochromic shift); and (2) the stabilisation of the flavylium form by the π complex displaces the equilibrium in such way that the red colour increases. This association also gives protection for the water nucleophilic attack in the 2 position of the flavylium cation [23] and for other species such as peroxides and sulphur dioxide in the 4 position [24, 25], so that the balance is displaced from hydrated forms towards the red flavylium cations. If the copigment is other anthocyanin, a self-association is formed (X); in the case of copigments with free electron pairs, an intermolecular copigmentation takes place (XI) finally, in the most complex case, the copigmentation can be carried out by a part of the structure itself (usually one of the aromatic acyl group substituents) (**Figure 3**).

During wine ageing, the concentration of monomeric anthocyanins starts to decrease leading to the formation of new anthocyanin derived pigments with different colour features and greater colour expression at high pH, important for the long-term colour stability of aged red wines [21]. The formation of most of the anthocyanin-derived pigments occurs in the first months of ageing, as the oxidative conditions in oak barrels favour their formation [26, 27]. Copigmentation has been hypothesised as the first mechanism involved in the formation of polymeric anthocyanin-derived pigments in red wines during ageing [19]. Numerous pigments have been characterised in wines and wine-like model solutions, and can be classified into three groups with respect to their formation pathways: 1) Direct condensation between anthocyanins and flavonols; 2) Condensation between anthocyanins and flavonols mediated by aldehydes, mainly acetaldehyde; and 3) Pyroanthocyanins (**Figure 3**). Although some of these pigments have only been detected in very small quantities in red wines, they have unique spectroscopic features that may, in some way, contribute together to the overall colour of aged red wines. In the first case, free anthocyanins can condense directly with flavan-3-ols and oligomeric proanthocyanins generating tannin-anthocyanins condensation products (T-A^+, XII) or anthocyanin-tannin condensation products (A^+-T, XIII) [8, 28–33]. The T-A^+ formation begins with the acid cleavage of the interflavanic bond of a procyanidin, giving a carbocation T^+ which reacts with the hydrated form of the anthocyanin (II). This mechanism leads to a colourless compound (T-AOH) which easily dehydrates to the coloured flavylium form T-A^+ [34]. In the formation of A^+-T pigments, nucleophilic addition of the flavanol takes place onto the flavylium form of the anthocyanin, yielding a colourless compound with the anthocyanin in flavene form. This flavene can be oxidised, resulting in a coloured flavylium A^+-T pigment (XIII) or in a colourless compound A(-O-)T with a type-A bond (XIV) [31] (**Figure 3**). As described for the monomeric anthocyanins, these pigments can also occur in a dynamic equilibrium among some molecular forms, mainly the quinoidal base, the flavylium cation and the hemiketal or carbinol pseudobase [30]. Both T-A^+ pigments and colourless A(-O-)T have been detected in wines [35]. Dimeric anthocyanins (XVI) consisting of one unit under flavylium cation and the other one under hydrated hemiketal form (A^+-AOH) were also characterised by mass spectrometry in wine like solutions [36] (**Figure 3**).

The A^+-T adducts can generate yellow-orange xanthylium pigments (XV) by further structural rearrangements. After the dehydration, a new heterocyclic pyran ring is formed and the xanthylium structure is generated [17, 37–40] (**Figure 3**). However, xanthylium pigments are also proposed to be formed directly from oligomeric flavan-3-ols [41, 42].

On the other hand, the acetaldehyde-mediated polymerisation between either only flavanols or with anthocyanins is the most well documented reaction in the literature [31, 37, 43–51]. Acetaldehyde is the main aldehyde (90%) present in wines as a result of yeast metabolism during the first stages of alcoholic fermentation, being also produced throughout the wine ageing process from ethanol oxidation [52]. In fortified wines like Port wines, this compound and other aldehydes (propionaldehyde, 2-methylbutyraldehyde, isovaleraldehyde, methylglyoxal, benzaldehyde) are present in higher amounts due to the addition of wine spirit (40–260 mg/L of acetaldehyde) to stop the alcoholic fermentation [53]. Ethyl-linked products, including ethyl-linked flavanols [54, 55] and ethyl-linked anthocyanin-flavanol pigments (XVII) [55] have been detected in wines (**Figure 2**). The formation of ethyl-linked anthocyanin oligomers (A^+-Et-AOH, XVIII) was also shown to occur both in model solution and in wine [56]. The ethyl-linked 8,8-malvidin-3-glucoside dimer was characterised by NMR under biflavylium cation forms [56]. However, physicochemical studies carried out on this pigment showed that the dimer under monoflavylium cation is the most abundant form at wine pH [57].

Another important group of anthocyanin derived pigments formed during ageing, also found in red Port wines, are the pyranoanthocyanins (XIX) (**Figure 3**). Pyranoanthocyanins are a group of anthocyanin-derived pigments [58, 59], which were first discovered in red wine by Cameira-dos-Santos et al. [60]. Pyranoanthocyanins are structurally characterised by the presence of a fourth ring between C-4 and the 5-hydroxyl group of an anthocyanin moiety, differing from each other on the type of group or molecule linked to the C-10 of the new ring [58, 61, 62]. The pyranic ring in pyranoanthocyanins provides protection against the nucleophilic attack from water or bisulphite, increasing their stability [63], making these compounds exceptionally stable pigments towards sulphite bleaching and pH variations. Both anthocyanin-flavanol derived pigments, direct ones and ethyl-linked ones, show less stability during ageing than pyranoanthocyanins. Through the reaction of anthocyanins with acetaldehyde [61, 63], pyruvic acid [58, 64], cinnamic acids [65, 66], acetoacetic acid [64], and procyanidins in the presence of acetaldehyde [67], several different classes of these pigments have been identified in the past decade such as vitisins [58, 59, 61, 68, 69], hydroxyphenyl-pyranoanthocyanins (pinotins) [59, 64, 70–72], methylpyranoanthocyanins [59, 73], vinylflavanol-pyranoanthocyanins [59], portisins [58, 59, 61, 66, 67, 74, 75], and more recently a new family of pyranoanthocyanin dimers [28, 73, 76, 77] (**Table 2**).

Pyruvic acid leads to the major pyranoanthocyanins determined in wines, i.e. carboxy-pyranoanthocyanins (R = COOH), sometimes referred to as vitisin A [58, 59, 61, 68, 69]. In red Port wines, it is the main pigment found during ageing. Due to its particular vinification process, the concentration of vitisin A is very high: 51.2 mg/L for Touriga Nacional Port wines, for example [78]. Indeed, wine fortification after alcoholic fermentation allows greater availability of pyruvic acid [79], which leads to reaching the highest contents shortly after fermentation and during the first year of ageing, followed by a slow decline [80]. After one year of ageing in barrels, the contents decrease by about 15–25% and about 70% after two years, whereas it is not so much important during bottle ageing (9–18%). Romero and Bakker [81] have demonstrated that the addition of pyruvic acid to finished Port wines from four different grape varieties resulted in an increase of malvidin-pyruvic acid adducts. It was also found that the concentration of anthocyanin-pyruvic acid adducts in wines was directly related to the original grape anthocyanin profile; the higher the initial anthocyanin precursor forms, the higher the concentration of corresponding adducts [81–83]. Morata et al. [84] have reported that the yeast strain used in the alcoholic fermentation (inoculated or not) also affects the production of malvidin-3-glucoside-pyruvate, existing a direct relation between the concentration of the pigment and the production of pyruvic acid by the yeast.

Moreover, the content of SO_2 in must can also influence the production of malvidin-3-glucoside-pyruvate, since SO_2 regulates the concentration of pyruvic acid through the formation of a weak bisulphite addition compound [85].

Romero and Bakker [68] have reported that malvidin-derived pyruvic acids adduct in model solutions provided approximately 11-fold (at pH 3) and 14-fold (at pH 2) more colour than grape anthocyanins.

Flavanol pyranoanthocyanins are formed by the cycloaddition between anthocyanins and 8-vinylflavanol adducts initially derived from the cleavage of ethyl-linked flavanol oligomers [46] or pigments [86, 87]. In red Port wines, pyranoanthocyanin-procyanidin dimers were identified in higher concentrations than the corresponding pyranoanthocyanin-catechins, representing up to 80% of the total pyranomalvidin-flavanols. This postulate is concordant with the fact that procyanidin dimers are more abundant than catechin monomers in grapes and wines from the Douro region [88, 89]. Furthermore, their concentrations decreased in older wines for both malvidin-3-glucoside derived-pigments (10.59 mg/L in 3 year aged wines, 9.16 mg/L in 4 year aged wines and 7.86 mg/L in 6 year aged wines) and associated coumaroyl pigments (6.62, 5.51 and 3.33 mg/L in 3, 4 and 6 year aged wines, respectively).

A second generation of pyranoanthocyanins can be formed by the reaction between a vitisin A and other metabolites. For example, oxovitisins (XX) are neutral yellowish pyranone structures involving the nucleophilic attack of water at the C-10 position of vitisin A [90].

Pyroanthocyanins	Precursors	References
R1 and R2 = OCH3; R3 = Glucose		
(I) In **Figure 3**		
Malvidin-3-glucoside (Oenin)		[13–15]
(XIX) In **Figure 3**		
R4 = H		
Non-substituted pyroanthocyanins (Vitisin B)	Oenin+acetaldehyde	[58–61, 68, 69]
R4 = COOH		
Carboxypyroanthocyanins (Vitisin A)	Oenin+pyruvic acid	[58–61, 68, 69]
R4 = CH_3		
Methylpyroanthocyanins	Oenin+acetoacetic acid or acetone	[59, 73]
R4 = $COCH_3$		
Acetylpyroanthocyanins	Oenin+diacetyl	[8, 28–33]
R4 = hydroxyphenyl		
Hidroxyphenylpyroanthocyanins	Oenin+*p*-coumaric acid or vinylphenol	[59, 64, 70–72]
R4 = dihydroxyphenyl		
Pinotin A	Oenin + caffeic acid or vinylcatechol	[59, 64, 70–72]
R4 = flavanol		
Flavanol-pyroanthocyanins	Oenin+vinylflavanols or flavan-3-ols + acetaldehyde	[59]
(XX) In **Figure 3**		
Pyranone-anthocyanins (oxovitisins)	Carboxypyroanthocyanins + water	[90]
(XXI) In **Figure 3**		
R6 = hydroxyphenyl		
Vinylphenyl-pyroanthocyanins (Portisin B)	Carboxypyroanthocyanins+hydroxycinnamic acids or vinylphenols	[58, 59, 61, 66, 67, 74, 75]
R6 = flavanol		
Vinylflavanol-pyroanthocyanins (Portisin A)	Carboxypyroanthocyanins + vinylflavanols or flavan-3-ols and acetaldehyde	[58, 59, 61, 66, 67, 74, 75]
(XXII) in **Figure 3**		
Pyroanthocyanins dimers	Carboxypyroanthocyanins + methylpyroanthocyanins	[28, 73, 76, 77]

Table 2.
Pyranoanthocyanins identified in wines and precursors.

In 2003 Mateus et al. [74] reported a new group of pyranoanthocyanins-vinylpyranoanthocyanins-which were named portisins (XXI), because of their occurrence in aged red Port wine [61, 67, 74, 75], **Figure 3**. The structure of these compounds consists of a pyranoanthocyanin moiety linked through a vinyl bridge to a flavanol or phenol unit. Their pathway of formation involves the carboxypyranoanthocyanins and vinylphenolic compounds. The first of these compounds reported in the literature arise from reaction of 8-vinylflavanol with carbon C-10 of the carboxypyranoanthocyanins, followed by loss of a formic acid group yielding the vinyl bridge. Portisins have been shown to have very high colouring capacity, much higher than that of their anthocyanin or pyruvic acid adduct counterparts [91–93]. Later, other portisins (B type) were detected in aged Port wines. In these, the flavanol moiety is replaced by a phenolic moiety with different hydroxylation and methoxylation patterns [61, 67, 74, 75]. These compounds were reported to result from the reaction of carboxypyranoanthocyanins with vinylphenols and cinnamic acids, following a mechanism similar to that of vinylflavanols and involving a further decarboxylation. However, the colour features of these portisins are different from those of the portisins discussed above because they have a λmax hypsochromically shifted from that of vinylpyranoanthocyanin-catechins, and are only slightly affected by the substitution pattern of the new phenolic ring (between 533 and 540 nm at aqueous pH 1) [91].

The condensation between A-type vitisins and methylpyroanthocyanins results in the formation of pyranoanthocyanin dimers (XXII), **Figure 3**. These turquoise blue pigments were found in a 9 year aged Port wine [73].

3.1.2 Aroma composition and sensory development

The volatile compounds present in Port wines have their origin on the grapes used, are produced during the alcoholic fermentation and being also added as part of the wine spirit used for Port wine production that contains trace volatile compounds such as esters (ethyl hexanoate, ethyl octanoate, ethyl decanoate) and terpenes (α-terpineol, linalool) that can affect the quality of the Port wines, contributing to a fruity, balsamic and spicy aroma [94]. In addition, wine spirits are rich in aldehydes such as acetaldehyde, propionaldehyde, isovaleraldehyde, isobutyraldehyde, and benzaldehyde [94]. The volatile profile of young Port wines is significantly different from that of aged Tawny Port wines or bottle-aged Port wines. Producers blend wines from several vintages and vineyards to produce wines with a consistent character. The final aroma character of the Port wine is to a considerable extent determined by the processes that take place during the oxidative ageing process of these wines, such as oxidation, carbohydrate degradation, formation and hydrolysis of esters, formation of acetals and to a lesser extent extraction of components from wood [11]. More than 200 volatile components have been detected in Port wines, 141 of which have been entirely or partially identified, however, the sensory importance of the various groups of volatile compounds does not entirely explain the sensory properties of Ruby or Tawny Port wines [95]. For the Ruby Port wine sensory profile, the attributes are 'Ruby', 'Persistence', 'Red fruits', 'Fruity flavour', 'Astringency' and 'Floral' were dominant, whereas in the White Port wine attributes like 'Honey', 'Sweet taste', 'Alcoholic sensation', 'Balance', 'Acid taste' and 'Moscatel' are the ones that better characterise these wines, Tawny Port wines are characterised by the attributes 'Dried fruits flavour', 'Dried fruits', 'Spices', 'Wood' and 'Sweet/Honey' [96, 97]. The Pink Port wines sensory attributes are characterised by the attributes 'Red fruit aroma', 'Body', 'Fruit aroma', 'Fruity flavour', 'Spicy sensation' and 'Persistence' [98].

Norisoprenoids have been found to contribute significantly to the aroma of young and aged Port wines [76, 99–101]. In a one year aged Port wine produced from Touriga Franca and Touriga Nacional grape varieties the norisoprenoid, 2,6,6-trimethylcyclohex-2-ene-1,4-dione, described as having sweet honey aroma, was identified by Rogerson et al. [102]. In a young Port wine produced from Tinto Cão and Tinta Barroca grape varieties, Rogerson et al. [103] identified the 1,3-dimethoxybenzene and 2-ene-1,4-dione. Falqué-Lopez et al. [104] characterised a one year aged Touriga Nacional monovarietal Port wine as having 'plum brandy', 'mulberry', 'cherry', 'wild fruits' and 'dry raisin' aromas and Guedes de Pinho et al. [105] identified linalool and linalyl acetate as being the responsible for the bergamot descriptor.

Ferreira et al. [100] have studied the influence of several factors on the levels of norisoprenoid in Port wines such as dissolved oxygen levels, free sulphur dioxide concentration, pH, and time/temperature of ageing. These authors observed that temperature and pH had a major influence on norisoprenoids levels and oxygen saturation reduced these compounds.

The concentration of several norisoprenoids increases during ageing, as for example β-ionone and β-damascenone in Vintage Port wines, and vitispirane, 2,2,6-trimethylcyclohexanone (TCH) and 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) in Tawny Port wines [99]. Ferreira and Pinho [99] showed that the occurrence of β-damascenone, β-ionone, TCH, TDN, and vitispirane was distinct in young or aged Port wines. It was observed that in wood barrel ageing TDN, vitispirane, and TCH increased, however, the concentration of β-ionone and β-damascenone decreased. Freitas et al. [106] described that TCH was responsible for the "rock-rose-like" aroma. According to several authors, in Port wines due to the short fermentation time precursors of norisoprenoids such as carotenoids, β-carotene, lutein, neoxanthin and violaxanthin can be present [104, 107, 108]. Carotenoids are the precursor of norisoprenoids and in Port wine carotenoids persist after the vinification process [108]. Grape varieties used for Port wine production are rich in certain carotenoids and viticultural practices, such as bunch shading [108, 109] and grapevine water status [110] can influence the concentration of carotenoids in the grape berries.

Acetals, derived from glycerol and acetaldehyde, also appear to be involved in the flavour of aged Tawny Port wines [111]. The levels of aldehydes and methyl ketones increase during the oxidative ageing of Port wines. The major aliphatic aldehyde is acetaldehyde with a clear trend of increasing with the time of storage in wood barrels. Glycerol is present in wines in large amounts, in particular with concentration from 4 to 8 g/L in Port wines and therefore the formation of acetal can be high. At wine pH, four isomers are formed by condensation of glycerol and acetaldehyde: *cis*- and *trans*-5-hydroxy-2-methyl-1,3-dioxane and *cis*- and *trans*-4-hydroxymethyl-2-methyl-1,3-dioxolane. These four acetals have been studied in more detail in order to understand their impact on wine aroma and if these substances can be used as indicators of Port wines age with oxidative ageing conditions. These four isomers are found in Port wine at high concentrations. Nevertheless, this reaction is strongly dependent on free sulphur dioxide levels. When there is no free sulphur dioxide, the level of four isomers increases with the extent of ageing. On the other hand, when sulphur dioxide combines with acetaldehyde, the acetals cannot be formed because of the formation of the acetaldehyde-bisulphite adduct. The concentrations of the four acetals increases consistently with age due to the constant increase of acetaldehyde content and the nonexistence of free sulphur dioxide during Port wine storage. The acetal with the highest intensity aroma described as sweet and Port-like is *trans*-5-hydroxy-2- methyl-1,3-dioxane and the aroma threshold limit of the total concentration of the four acetals was determined as 100 mg/L [111]. Many acetals have been isolated from Tawny Port wines, but their contribution to the oxidised character of the wine is unclear [8].

Port wines with extensive wood-ageing have higher concentrations of diethyl and other succinate esters that contribute to the Port wine bouquet. Oak lactones (β-methyl-γ-octalactone isomers) and other oxygen-containing heterocycles have also been isolated. Some of the latter are furan derivatives, such as dihydro-2-(3H)-furanone and may contribute to a sugary oxidised bouquet [8]. Esters of 2-phenylethanol may contribute to the fruity, sweet bouquet of Port wines, and diacetyl can contribute to its caramel odour [99]. Some aldehydes and ketones are associated with the oxidative aged Port wines, conferring "rancio" odour to wines [112, 113].

However, in wood barrel aged Port wine the 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) seems to be the most significant volatile compound [112, 113]. Some works suggested that sotolon contributes to the characteristic barrel aged aroma of Port wines [114], being the fundamental molecule to understand the "perceived age" of Port wines. The levels of sotolon were measured in "Colheita" and Tawny categories and were shown that it increases with ageing time being present in a range of concentration of some dozen μg/L in a young wine, to about 100 μg/L in wines with 10 years ageing, and to about 1000 μg/L in Port wines older than 50 years [112–114]. Albeit being a compound with an apparently important role in Port wine aged aroma, the mechanism of sotolon formation in wine is not yet fully understood. However, different pathways have been proposed, such as aldol condensation between α-ketobutyric acid and acetaldehyde [115–117] (Pathway 1 – **Figure 4**) and the reaction between ethanol and ascorbic acid [118] (Pathway 2 – **Figure 4**). The formation of sotolon in Port wines is dependent on the temperature and oxygen levels [112, 113], which are crucial parameters during the oxidative ageing of Tawny Port wines. The sensory threshold limit of sotolon was determined as 19 μg/L, which is above the amount present in Port wines older than 10 years [112, 113].

Young Port wines show higher levels of volatile sulphur compounds than aged Port wines [119]. Sulphur compounds, such as 2-mercaptoethanol, 2-(methylthio) ethanol, ethyl 3-(methylthio) propionate, 3-(methylthio)-1-propanol, *cis*-(odourless), and *trans*-2-methyltetrahydrothiophen-3-ol, 3-(ethylthio)-1-propanol, 4-(methylthio)-1-butanol, dimethyl sulfone, benzothiazole, 3-(methylthio)-1-propionic acid and N-3-(methylthiopropyl) acetamide are not present or are present in lower concentrations in aged Tawny Port wines when compared to young Tawny Port wines [119].

The changes observed in the volatile composition of Port wines during the oxidative ageing results in a complex, oxidised character defined as nutty, nuts,

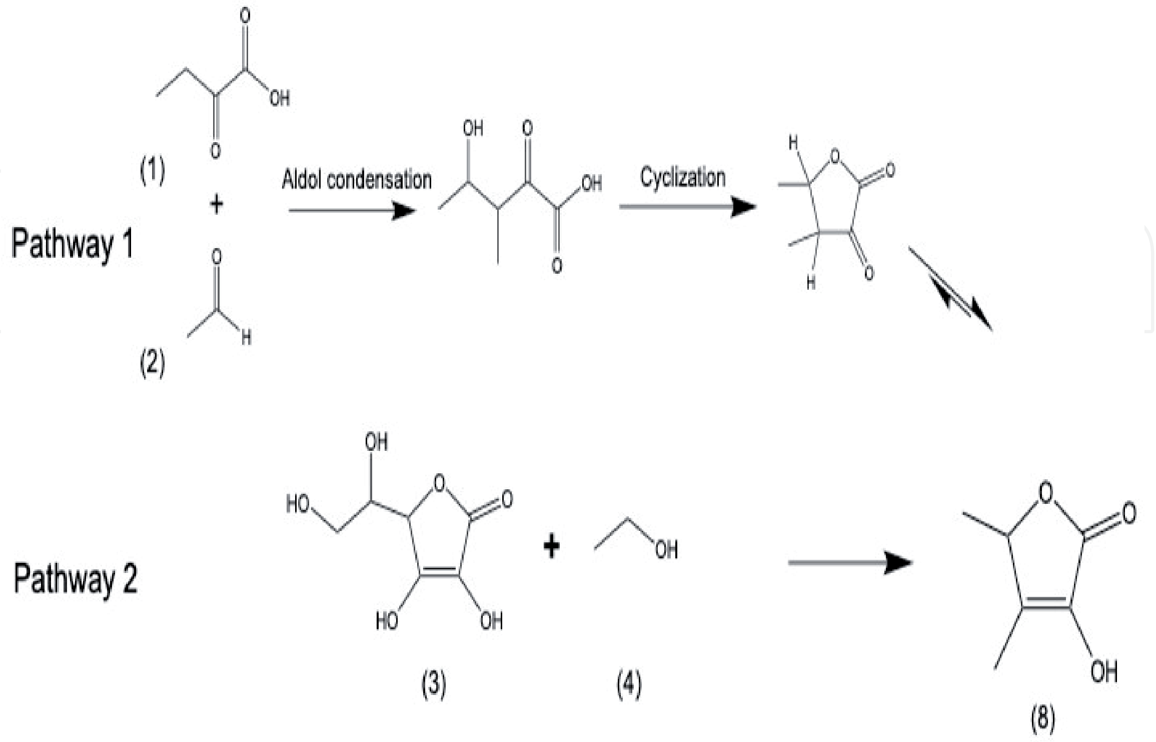

Figure 4.
Pathway 1: Reaction between α-ketobutyric acid (1) and acetaldehyde (2); pathway 2: Reaction between ascorbic acid (3) and ethanol (3). These reactions can lead to the formation of sotolon (8) according to [115–118].

raisins and crisp apples with a slightly oaky note' giving an impression of dryness. According to Falqué et al. [104] and Ferreira et al. [120] floral, bergamot-like, violet or jasmine notes are present in young Port wines that are changed during the ageing process in wood barrels. Freitas et al. [106] and Ferreira et al. [121] have described the flavours developed during ageing of Port wines as woody, burnt, dry fruit, nutty, and spicy.

4. Conclusions

Port wine is one of the most famous and old fortified wine in the world, produced in the Douro Demarcated Region (Portugal), a region with a singular "terroir". It is a traditional product with more than 250 years old and commercialised all around the world for many centuries. However, more knowledge is still needed in order to understand and control its composition and its evolution during the wine ageing process. Port wine presents a complex physicochemical matrix that results from complex and concurrent chemical reactions that occur during the ageing process significantly changing its sensory profile. Different Port wine styles are obtained using different ageing processes, related to different oxygen levels, temperature and sulphur dioxide variations. The two main Port wine styles are the Ruby and Tawny Port wines, with the first style being obtained by a reductive ageing process resulting in a ruby colour and fruity aroma, while Tawnies Port wine styles shows a brown colour and an intense dry fruit aroma. The colour of the different Port wines styles is one of their main quality parameters. For the Port wines produced with red grapes, colour changes are related to the changes in the anthocyanins composition during the ageing process. The colour attributes of the Port wines made with white grapes is still largely unknown. Sotolon plays an important role in aged Port wines aroma obtained by oxidative ageing, due to its low olfactory threshold and pleasant and potent aroma. Sotolon levels increase during Port wine oxidative ageing. The intense government legislation and specific production rules protect this important product produced in a world protected region (Unesco) in order to reduce adulterations or even imitations. However, more studies are still needed to deepen our knowledge in order to understand and control the reactions involved in Port wine ageing process that contribute to its uniqueness.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] IVDP, Instituto dos Vinhos do Douro e Porto [Internet]. 2020. Available from: http://www.ivdp.pt/.

[2] Decree-Law n°104/85, 10th of April. Ministério da Agricultura. Diário da República, 1ª série, no. 83.

[3] Portaria n° 195/1985 of 10th April. Ministério da Agricultura, do desenvolvimento rural e das pescas. Diário da República, 1° série-B, no. 91.

[4] Decree-Law n°173/2009 of 3rd of August. Ministério da Agricultura, do desenvolvimento rural e das pescas. Diário da República, 1ª série, no. 148.

[5] Moreira N, Guedes de Pinho P. Port Wine. In: Jackson RS, editor. Advances in Food and Nutrition Research. Vol. 63. Academic Press, 2011. p. 119-146. DOI: 10.1016/B978-0-12-384927-4.00005-1.

[6] Regulation n° 84/2010 of 8th of February. Diário da República, 2ª série, no. 26.

[7] Regulation n° 242/2010 of 15th March. Instituto dos Vinhos do Douro e do Porto, I. P. Diário da República, 2ª série, no. 51.

[8] Jackson RS. Wine Science, Principles, Practice, Perception. 4th ed. Academic Press; 2014. 776 p. DOI: 10.1016/B978-0-12-381468-5.00011-7.

[9] Saucier C, Lopes P, Mirabel M, Guerra C, Glories Y. Tannin-anthocyanin interactions: influence on wine colour. In: Waterhouse AL, Kennedy JA, editors. Red Wine Colour: Revealing the Mysteries. ACS Symposium Series 886; 2004. p. 265-273. DOI: 10.1021/bk-2004-0886.ch016

[10] Timberlake CF, Bridle P, Interactions between anthocyanins, phenolic compounds and acetaldehyde and their significance in red wines. American Journal of Enology and Viticulture. 1976; 27: 97-105.

[11] Clarke RJ, Bakker J. Wine Flavour Chemistry. 1st ed. Blackwell Publishers; 2004. 336 p.

[12] Bakker J, Timberlake CF, The mechanism of colour changes in ageing port wine. American Journal of Enology and Viticulture. 1986; 37: 288-292.

[13] Brouillard R, Lang J, The hemiacetal-*cis*-chalcone equilibrium of malvin, a natural anthocyanin. Canadian Journal of Chemistry.1990; 68: 755-761.

[14] Santos H, Turner DL, Lima JC, Figueiredo P, Pina FS, Macanita AL, Elucidation of the multiple equilibria of malvin in aqueous-solution by onedimensional and 2-dimensional NMR. Phytochemistry. 1993; 33:1227-1232.

[15] Brouillard R, Delaporte B, Chemistry of Anthocyanin Pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. Journal of the American Chemical Society.1977; 99: 8461-8468.

[16] Berke B, Chèze C, Vercauteren J, Deffieux G, Bisulfite addition to anthocyanins: Revisited structures of colorless adducts. Tetrahedron Letters. 1998; 39:5771-5774. doi: 10.1016/S0040-4039(98)01205-2.

[17] Liao H, Cai Y, Haslam E, Polyphenol Interactions. Anthocyanins: Co-Pigmentation and Colour Changes in Red Wines. Journal of the Science of Food and Agriculture.1992; 59 (3): 299-305.

[18] Trouillas P, Sancho-García JC, De Freitas V, Gierschner J, Otyepka M, Dangles O, Stabilizing and modulating

color by copigmentation: insights from theory and experiment. Chemical Reviews. 2016; 116: 4937-4982.

[19] Brouillard R, Dangles O, Anthocyanin molecular interactions: The first step in the formation of new pigments during wine aging?. Food Chemistry. 1994; 51 (4): 365-371.

[20] Gonzalez-Manzano S, Duenas M, Rivas-Gonzalo J C, Escribano-Bailon MT, Santos-Buelga C, Studies on the copigmentation between anthocyanins and flavan-3-ols and their influence in the colour expression of red wine. Food Chemistry. 2009; 114 (2): 649-656.

[21] Boulton R, The copigmentation of anthocyanins and its role in the color of red wine: A critical review. American Journal of Enology and Viticulture. 2001; 52 (2): 67-87.

[22] Dangles O, Brouillard R, Polyphenol interactions. The copigmentation case: Thermodynamic data from temperature variation and relaxation kinetics. Medium effect. Canadian Journal of Chemistry. 1992; 70 (8): 2174-2189.

[23] Matsufuji H, Otsuki T, Takeda T, Chino M, Takeda M, Identification of reaction products of acylated anthocyanins from red radish with peroxylradicals. Journal of Agricultural and Food Chemistry. 2003; 51(10): 3157-3161.

[24] García-Viguera C, Bridle P, Influence of structure on colour stability of anthocyanins and flavylium salts with ascorbic acid. Food Chemistry. 1999; 64(1): 21-26.

[25] Mazza G, Brouillard R, Recent developments in the stabilization of anthocyanins in food products. Food Chemistry. 1987; 25(3): 207-225.

[26] Atanasova V, Fulcrand H, Cheynier V, Moutounet M, Effect of oxygenation on polyphenol changes occuring in the course of wine-making. Analytica Chimica Acta. 2002, 458:15-27.

[27] Alcalde-Eon C, EscribanoT, Santos-Buelga C, Rivas-Gonzalo JC, Changes in the detailed pigment composition of red wine during maturity and ageing: A comprehensive study. Analytica Chimica Acta. 2006, 563(1):238-254.

[28] Nave F, Teixeira N, Mateus N, De Freitas V, The fate of flavanol–anthocyanin adducts in wines: Study of their putative reaction patterns in the presence of acetaldehyde. Food Chemistry. 2010, 121: 1129-1138.

[29] Salas E, Le Guernevé C, Fulcrand H, Poncet-Legrand C, Cheynier V, Structure determination and colour properties of a new directly linked flavanol-anthocyanin dimer. Tetrahedron Letters. 2004, 45: 8725-8729.

[30] Remy S, Fulcrand H, Labarbe B, Cheynier V, Moutounet M, First confirmation in red wine of products resulting from direct anthocyanin-tannin reactions. Journal of the Science of Food and Agriculture, 2000; 80: 745-751.

[31] Salas E, Fulcrand H, Meudec E, Cheynier V, Reactions of anthocyanins and tannins in model solutions. Journal of Agricultural and Food Chemistry. 2003, 51: 7951-7961.

[32] Salas E, Atanasova V, Poncet-Legrand C, Meudec E, Mazauric JP, Cheynier V, Demonstration of the occurrence of flavanol-anthocyanin adducts in wine and in model solutions. Analytica Chimica Acta. 2004; 513(1): 325-332. DOI: 10.1016/j.aca.2003.11.084.

[33] He F, Pan Q-P, Shi Y, Duan C-Q, Chemical synthesis of proanthocyanidins in vitro and their reactions in aging wines. Molecules. 2008; 13: 3007-3032.

[34] Macz-Pop GA, Gonzalez-Paramas AM, Perez-Alonso JJ, Rivas-Gonzalo JC, New flavanol-anthocyanin condensed pigments and anthocyanin composition in guatemalan beans (Phaseolus spp.). Journal of Agricultural and Food Chemistry, 2006; 54: 536-542.

[35] Morata A, Gómez-Cordovés MC, Calderón F, Suárez JA, Effects of pH, temperature and SO_2 on the formation of pyranoanthocyanins during red wine fermentation with two species of *Saccharomyces*. International Journal of Food Microbiology, 2006; 106: 123-129.

[36] Lu Y, Foo LY, Unusual anthocyanin reaction with acetone leading to pyranoanthocyanin formation. Tetrahedron Letters, 2001; 42: 1371-1373.

[37] Santos-Buelga C, Francia-Aricha EM, De Pascual-Teresa S, Rivas-Gonzalo JC, Contribution to the identification of the pigments responsible for the browning of anthocyaninflavanol solutions. European Food Research and Technology, 1999; 209: 411-415.

[38] Baranowski ES, Nagel CW, Kinetics of malvidin-3-glucoside condensation in wine model systems. Journal of Food Science, 1983; 48: 419-429.

[39] Somers TC, The phenolic nature of wine pigments. Phytochemistry, 1971; 10: 2175-2186.

[40] Dueñas M, Fulcrand H, Cheynier V, Formation of anthocyanin-flavanol adducts in model solutions. Analytica Chimica Acta, 2006; 563:15-25.

[41] Fulcrand H, Cheynier V, Oszmiansky J, Moutounet M, An oxidised tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. Phytochemistry, 1997; 46: 223-227.

[42] Jurd L, Somers TC, The formation of xanthylium salts from proanthocyanidins. Phytochemistry, 1970; 9: 419-427.

[43] Rivas-Gonzalo J C, Bravo-Haro S, Santosbuelga C, Detection of compounds formed through the reaction of malvidin-3-monoglucoside and catechin in the presence of acetaldehyde. Journal of Agricultural and Food Chemistry, 1995; 43: 1444-1449.

[44] Francia-Aricha E M, Guerra M T, Rivas-Gonzalo J C, Santos-Buelga C, New anthocyanin pigments formed after condensation with flavanols. Journal of Agricultural and Food Chemistry, 1997; 45: 2262-2266.

[45] Es-Safi N, Fulcrand H, Cheynier V, Moutounet M, Competition between (+)-catechin and (−)-epicatechin in acetaldehyde-induced polymerization of flavanols. Journal of Agricultural and Food Chemistry, 1999; 47:2088-2095.

[46] Es-Safi N, Fulcrand H, Cheynier V, Moutounet M, Studies on the acetaldehyde induced condensation of (−)-epicatechin and malvidin 3-O-glucoside in a model solution system. Journal of Agricultural and Food Chemistry, 1999; 47:2096-2102.

[47] Escribano-Bailon T, Alvarez-Garcia M, Rivas-Gonzalo J.C, Heredia FJ, Santos-Buelga C, Color and stability of pigments derived from the acetaldehyde-mediated condensation between malvidin-3-*O*-glucoside and (+)-catechin. Journal of Agricultural and Food Chemistry, 2001; 49: 1213-1217.

[48] Remy-Tanneau S, Guerneve C L, Meudec E, Cheynier V, Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometries. Journal

of Agricultural and Food Chemistry, 2003; 51: 3592-3597

[49] Pissarra J, Mateus N, Rivas-Gonzalo J C, Santos-Buelga C, De Freitas V, Reaction between malvidin 3-glucoside and (+)-catechin in model solutions containing different aldehydes. Journal of Food Science, 2003; 68: 476-481

[50] Pissarra J, Lourenço S, González-Paramás AM, Mateus N, Santos-Buelga C, Silva AS, De Freitas V. Structural characterization of new malvidin-3-glucoside-catechin aryl/alkyl pigments. Journal of Agricultural and Food Chemistry, 2004; 52:5519-5526.

[51] Saucier C, Guerra C, Pianet I, Laguerres M, Glories Y, (+)-Catechin-acetaldehyde condensation products in relation to wine-ageing. Phytochemistry, 1997; 46:229-234.

[52] Liu SQ, Pilone GJ, An overview of formation and roles of acetaldehyde in winemaking with emphasis on microbiological implications. International Journal of Food Science & Technology, 2000; 35(1):49-61.

[53] Pissarra JI, Lourenço S, Machado JM, Mateus N, Guimaraens D, de FreitasV, Contribution and importance of wine spirit to the port wine final quality - initial approach. Journal of the Science of Food and Agriculture, 2005; 85: 1091-1097.

[54] Saucier C, Little D, Glories Y, First evidence of acetaldehyde-flavanol condensation products in red wine. American Journal of Enology and Viticulture, 1997; 48: 370-373

[55] Cheynier V, Fulcrand H, Sarni P, Moutounet M, Application des techniques analytiques à l'étude des composés phénoliques et de leurs réactions au cours de la vinification. Analusis, 1997; 25: M14-M21.

[56] Atanasova V, Fulcrand H, Le Guerneve C, Cheynier V, Moutounet M, Structure of a new dimeric acetaldehyde malvidin 3-glucoside condensation product. Tetrahedron Letters, 2002; 43: 6151-6153.

[57] Atanosova V, Fulcrand H, Le Guernevé C, Dangles O, Cheynier V, PolyphenolCommunications.Marrakech, 2002; Vol. 2, p 417.

[58] Fulcrand H, Benabdeljalil C, Rigaud J, Cheynier V, Moutounet M, A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. Phytochemistry, 1998; 47: 1401-1407

[59] de Freitas V, Mateus N, Formation of pyranoanthocyanins in red wines: a new and diverse class of anthocyanin derivatives. Analytical and Bioanalytical Chemistry, 2011; 401:1463-1473

[60] Cameira-dos-Santos PJ, Brillouet JM, Cheynier V, Moutounet M, Detection and partial characterisation of new anthocyanin derived pigments in wine. Journal of the Science of Food and Agriculture, 1996; 70:204-208.

[61] Bakker J, Timbarlake CF, The isolation and characterisation of new colour stable anthocyanins occurring in some red wines. Journal of Agricultural and Food Chemistry. 1997; 45: 35-43. DOI: doi.org/10.1021/jf960252c.

[62] Benabdeljalil C, Cheynier V, Fulcrand H, Hakiki A, Mosaddak M, Moutounet M, Evidence of new pigments resulting from reaction between anthocyanins and yeast metabolites. Sciences des Aliments, 2000; 20: 203-220

[63] He J, Santos-Buelga C, Silva AMS, Mateus N, Freitas V, Isolation and structural characterization of new anthocyanin-derived yellow pigments in aged red wines. Journal of Agricultural

and Food Chemistry, 2006; 54(25): 9598-9603. DOI: 10.1021/jf062325q

[64] Fulcrand H, Santos P-JC, Sarni-Manchado P, Cheynier V, Favre-Bonvin J, Structure of new anthocyanin-derived wine pigments. Journal of the Chemical Society, Perkin Transactions 1. 1996; 7: 735-739.

[65] Schwarz M, Wabnitz TC, Winterhalter P, Pathway leading to the formation of anthocyanin vinylphenol adducts and related pigments in red wines. Journal of Agricultural and Food Chemistry. 2003; 51(12): 3682-3687. DOI: 10.1021/jf0340963

[66] Rentzsch M, Schwarz M, Winterhalter P, Hermosin-Gutierrez I, Formation of hydroxyphenyl-pyranoanthocyanins in Grenache wines: Precursor levels and evolution during aging. Journal of Agricultural and Food Chemistry. 2007; 55(12): 4883-4888. DOI: 10.1021/jf0702491

[67] Mateus N, Oliveira J, Santos-Buelga C, Silva AMS, Freitas, V, NMR structure characterization of a new vinylpyranoanthocyanin-catechin pigment (a portisin). Tetrahedron Letters. 2004; 45(17): 3455-3457. DOI: doi.org/10.1016/j.tetlet.2004.03.007

[68] Romero C, Bakker J, Interactions between grape anthocyanins and pyruvic acid, with effect of pH and acid concentration on anthocyanin composition and color in model solutions. Journal of Agricultural and Food Chemistry, 1999; 47: 3130-3139.

[69] Bakker J, Bridle P, Honda T, Kuwano H, Saito N, Terahara N, Timberlake CF, Identification of an anthocyanin occurring in some red wines. Phytochemistry, 1997; 44: 1375-1382.

[70] Mateus N, Pascual-Teresa S, Rivas-Gonzalo JC, Santos-Buelga C, Freitas, V, Structural diversity of anthocyanin-derived pigments in port wines. Food Chemistry. 2002; 76: 335-342. DOI: 10.1016/S0308-8146(01)00281-3

[71] Schwarz M, Jerz G, Winterhalter P, Isolation and structure of pinotin A, a new anthocyanin derivative from pinotage wine. Vitis. 2003; 42(2): 105-106.

[72] Hakånsson AE, Pardon K, Hayasaka Y, De Sa M, Herderich M, Structures and colour properties of new red wine pigments. Tetrahedron Letters, 2003; 44: 4887-4891.

[73] Oliveira J, Azevedo J, Silva MAS, Teixeira N, Cruz L, Mateus N, de Freitas VJ Pyranoanthocyanin dimers: a new family of turquoise blue anthocyanin-derived pigments found in port wine. Journal of Agricultural and Food Chemistry. 2010; 58: 5154-5159.

[74] Mateus N, Silva AMS, Rivas-Gonzalo JC, Santos-Buelga C, Freitas V, A new class of blue anthocyanin-derived pigments isolated from red wines. Journal of Agricultural and Food Chemistry. 2003; 51(7): 1919-1923. DOI: doi.org/10.1021/jf020943a

[75] Mateus N, Oliveira J, Pissarra J, González-Paramás AM, Rivas-Gonzalo JC, Santos-Buelga C, Silva AMS, Freitas V, A new vinylpyranoanthocyanin pigment occurring in aged red wine. Food Chemistry. 2006; 97(4); 689-695. DOI: 10.1016/j.foodchem.2005.05.051

[76] Oliveira J, Santos-Buelga C, Silva AMS, Freitas V, Mateus N, Chromatic and structural features of blue anthocyanin-derived pigments present in Port wine. Analytica Chimica Acta. 2006; 563: 2-9. DOI: 10.1016/j.aca.2005.11.027

[77] Chassaing S, Isorez G, Kueny-Stotz M, Brouillard R, En route to color-stable pyranoflavylium pigments-a systematic study of the reaction between 5-hydroxy-4-methylflavylium

salts and aldehydes. Tetrahedron Letters, 2008; 49: 6999-7004.

[78] Mateus N, Silva A M S, Vercauteren J, de Freitas V, Occurrence of anthocyanin-derived pigments in red wines. Journal of Agricultural and Food Chemistry, 2001; 49: 4836-4840.

[79] Whiting GC, Coggins PA, Organic acid metabolism in cider and perry fermentations. III. Keto-acids in cider-apple juices and ciders. Journal of the Science of Food and Agriculture, 1960; 11:705-709.

[80] Mateus N, Freitas V, Evolution and Stability of Anthocyanin-Derived Pigments during Port Wine Aging. Journal of Agricultural and Food Chemistry. 2001; 49: 5217-5222. DOI: 10.1021/jf0106547.

[81] Romero C, Bakker J, Anthocyanin and colour evolution during maturation of four port wines: effect of pyruvic acid addition. Journal of the Science of Food and Agriculture. 2000; 81: 252-260. DOI: 10.1002/1097-0010(20010115)81:2<252: AID-JSFA810>3.0.CO;2-5

[82] Romero C, Bakker J, Effect of storage temperature and pyruvate on kinetics of anthocyanin degradation, Vitisin A derivative formation, and colour characteristics of model solutions. Journal of Agricultural and Food Chemistry, 2000; 48: 2135-2141. DOI: 10.1021/jf990998l

[83] Monagas M, Núñez V, Bartolomé B, Gómez-Cordovés C, Anthocyanin-derived pigments in Graciano, Tempranillo, and Cabernet Sauvignon wines produced in Spain. American Journal of Enology and Viticulture, 2003; 54: 163-169.

[84] Morata A, Gómez-Cordovés MC, Colomo B, Súarez JA, Pyruvic acid and acetaldehyde production by different strains of *Saccharomyces cerevisiae* with vitisin A and B formation in red wines. Journal of Agricultural and Food Chemistry, 2003; 51:7402-7409.

[85] Asenstorfer RE, Markides AJ, Iland PG, Jones GP, Formation of vitisin A during red wine vinification and maturation. Australian Journal of Grape and Wine Research, 2003; 9:40-46

[86] Mateus N, Silva MAS, Santos-Buelga C, Rivas-Gonzalo JC, De Freitas V, Identification of anthocyanin-flavanol pigments in red wines by NMR and mass spectrometry. Journal of Agricultural and Food Chemistry, 2002; 50: 2110-2116.

[87] Cruz L, Teixeira N, Silva MAS, Mateus N, Borges J, De Freitas V, Role of vinylcatechin in the formation of pyranomalvidin-3-glucoside-(+)-catechin. Journal of Agricultural and Food Chemistry, 2008; 56: 10980-10987.

[88] De Freitas V A P, Glories Y, Monique A, Developmental Changes of Procyanidins in Grapes of Red *Vitis vinifera* Varieties and Their Composition in Respective Wines. American Journal of Enology and Viticulture, 2000; 51: 397-403.

[89] Mateus N, Marques S, Gonçalves AC, Machado J M, De Freitas V, Proanthocyanidin composition of red *Vitis vinifera* varieties from the Douro Valley during ripening: influence of cultivation altitude. American Journal of Enology and Viticulture, 2001; 52: 115-121.

[90] He J, Oliveira J, Silva AM, Mateus N, Freitas V, Oxovitisins: a new class of neutral pyranone-anthocyanin derivatives in red wines. Journal of Agricultural and Food chemistry. 2010; 58(15): 8814-8819. DOI: 10.1021/jf101408q

[91] Oliveira J, de Freitas V, Silva MAS, Mateus N, Reaction between hydroxycinnamic acids and

anthocyanin-pyruvic acid adducts yielding new portisins. Journal of Agricultural and Food Chemistry, 2007; 55: 6349. DOI: 10.1021/jf070968f

[92] Mateus N, Proença S, Ribeiro P, Machado J M, de Freitas V, Grape and wine polyphenolic composition of red *Vitis vinifera* varieties concerning vineyard altitude. Ciencia y Tecnología Alimentaria, 2001; 3: 102-110.

[93] Mateus N, Oliveira J, Haettich-Motta M, de Freitas V, New family of bluish pyranoanthocyanins. Journal of Biomedicine and Biotechnology, 2004; 5: 299.

[94] Rogerson FSS, Freitas VAP, Fortification spirit, a contributor to the aroma complexity of port. Journal of Food Science. 2002; 67: 1564-1569. DOI: 10.1111/j.1365-2621.2002.tb10323.x

[95] Williams AA, Lewis MJ, May HV, The Volatile Flavour Components of Commercial Port Wines. Journal of the Science of Food and Agriculture. 1983; 34: 311-319. DOI: 10.1002/jsfa.2740340316

[96] Vilela A, Monteiro B, Correia E, Sensory profile of Port wines: Categorical Principal Component Analysis, an approach for sensory data treatment. Ciência e Técnica Vitivinícola. 2015; 30:1-8.

[97] Vilela A, Ferreira R, Nunes F, Correia E, Creation and Acceptability of a Fragrance with a Characteristic Tawny Port Wine-Like Aroma. Foods, 2020; 9, 1244; doi:10.3390/foods9091244.

[98] Monteiro B, Vilela A, Correia E, Sensory profile of pink port wines: Development of a flavour lexicon. Flavour and Fragrance Journal. 2014; 29: 50-58.

[99] Ferreira ACS, Pinho PG, Nor-isoprenoids profile during port wine ageing—influence of some technological parameters. Analytica Chimica Acta. 2004; 513: 169-176. DOI: 10.1016/j.aca.2003.12.027

[100] Ferreira ACS, Monteiro J, Oliveira C, Pinho PG, Study of major aromatic compounds in port wines from carotenoid degradation. Food Chemistry. 2008; 110: 83-87. DOI: 10.1016/j.foodchem.2008.01.069

[101] Mendes-Pinto MM, Carotenoid breakdown products the—norisoprenoids—in wine aroma. Archives of Biochemistry and Biophysics. 2009, 483(2): 236-245. DOI: 10.1016/j.abb.2009.01.008

[102] Rogerson FSS, Castro H, Fortunato N, Azevedo Z, Macedo A, Freitas VAP, Chemicals with sweet aroma descriptors found in Portuguese wines from the Douro region: 2,6,6-trimethylcyclohex-2-2,6,6-trimethylcyclohex-2-ene-1,4-dione and diacetyl. Journal of Agricultural and Food Chemistry. 2001; 49: 263-269. DOI: 10.1021/jf000948c

[103] Rogerson FSS, Azevedo Z, Fortunato N, Freitas VAP, 1,3-Dimethoxybenzene, a newly identified component of port wine. Journal of the Science of Food and Agriculture. 2002; 82: 1287-1292. DOI: 10.1002/jsfa.1182

[104] Falqué E, Ferreira AC, Hogg T, Guedes-Pinho P, Determination of aromatic descriptors of Touriga Nacional wines by sensory descriptive analysis. Flavour and Fragrance Journal. 2004, 19(4): 298-302. DOI: 10.1002/ffj.1355

[105] Guedes de Pinho P, Falqué E, Castro M, Silva HO, Machado B, Ferreira AS, Further insights into the floral character of Touriga Nacional wines. Journal of Food Science. 2007, 72(6): S396-S401. DOI: 10.1111/j.1750-3841.2007.00405.x

[106] Freitas VAP, Ramalho PS, Azevedo Z, Macedo A, Identification of some volatile descriptors of the rock-rose like aroma of fortified red wines from Douro Demarcated Region. Journal of Agricultural and Food Chemistry. 1999, 47: 4327-4331. DOI: 10.1021/jf9901035

[107] Guedes de Pinho P, Ferreira ACS, Mendes-Pinto M, Benitez JG, Hogg TA, Determination of carotenoid profiles in grapes, musts, and fortified wines from Douro varieties of *Vitis vinifera*. Journal of Agricultural and Food Chemistry. 2001, 49(11): 5484-5488. DOI: 10.1021/jf010515p

[108] Mendes-Pinto MM, Ferreira ACS, Caris-Veyrot C, Pinho PG, Carotenoid, chrorophyll and chlorophyll-derived compounds in grapes and port wines. Journal of Agricultural and Food Chemistry. 2005; 53: 10034-10041. DOI: 10.1021/jf0503513

[109] Oliveira C, Ferreira AC, Costa P, Guerra J, Pinho PG, Effect of some viticultural parameters on the grape carotenoid profile. Journal of Agricultural and Food Chemistry. 2004; 52: 4178-4184. DOI: 10.1021/jf0498766

[110] Oliveira C, Ferreira ACS, Pinto MM, Hogg T, Alves F, Pinho PG, Carotenoid compounds found in grapes and their relationship to plant water status. Journal of Agricultural and Food Chemistry. 2003; 51: 5967-5971. DOI: 10.1021/jf034275k

[111] Ferreira ACS, Barbe J-C, Bertrand A, Heterocyclic acetals from glycerol and acetaldehyde in port wines: Evolution with aging. Journal of Agricultural and Food Chemistry. 2002, 50: 2560-2564. DOI: 10.1021/jf011391j

[112] Ferreira ACS, Hogg T, Guedes de Pinho P, Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. Journal of Agricultural and Food Chemistry. 2003; 51: 1377-1381. DOI: 10.1021/jf025847o

[113] Ferreira ACS, Barbe JC, Bertrand A, 3-Hydroxy-4,5-dimethyl-2(5H)-furanone: A Key Odorant of the Typical Aroma of Oxidative Aged Port Wine. Journal of Agricultural and Food Chemistry. 2003, 51: 4356-4363. DOI: 10.1021/jf0342932

[114] Martins RC, Monforte AR, Ferreira AS, Port wine oxidation management: A multiparametric kinetic approach. Journal of Agricultural and Food Chemistry. 2013; 61(22): 5371-5379. DOI: 10.1021/jf4005109

[115] Guichard E, Pham TT, Etievant P, Quantitative-determination of sotolon in wines by high-performance liquid-chromatography. Chromatographia. 1993, 37(9-10): 539-542. DOI: 10.1007/BF02275793

[116] Pham TT, Guichard E, Schlich P, Charpentier C, Optimal Conditions for the Formation of Sotolon from alpha-Ketobutyric Acid in the French "Vin Jaune". Journal of Agricultural and Food Chemistry. 1995, 43(10): 2616-2619. DOI: 10.1021/jf00058a012

[117] Pons A, Lavigne V, Landais Y, Darriet P, Dubourdieu D, Identification of a Sotolon Pathway in Dry White Wines. Journal of Agricultural and Food Chemistry. 2010, 58: 7273-7279. DOI: 10.1021/jf100150q

[118] Konig T, Gutsche B, Hartl M, Hubscher R, Schreier P, Schwab W. (3- Hydroxy-4,5-dimethyl-2(5H)-furanone (Sotolon) causing an off-flavor: Elucidation of its formation pathways during storage of citrus soft drinks. Journal of Agricultural and Food Chemistry. 1999, 47(8): 3288-3291. DOI: 10.1021/jf981244u

[119] Ferreira ACS, Rodrigues P, Hogg T, Pinho PG, Influence of Some Technological Parameters on the

Formation of Dimethyl Sulfide, 2-Mercaptoethanol, Methionol, and Dimethyl Sulfone in Port Wines. Journal of Agricultural and Food Chemistry. 2003, 51: 727-732. DOI: 10.1021/jf025934g

[120] Ferreira ACS, Falqué E, Castro M, Silva HO, Machado B, Pinho PG, Identification of key odorants related with high quality Touriga Nacional wine. Developments in Food Science. 2006, 43: 217-220. DOI: 10.1016/S0167-4501(06)80052-X

[121] Ferreira ACS, Avila I, Pinho PG. Sensorial impact of sotolon as the “perceived age” of tawny port wines. In: Frey C, Rouseff R, editors. Natural Flavors and Fragrances. Vol. 908. ACS Symposium Series; 2005. p. 141-159. DOI: 10.1021/bk-2005-0908.ch010