Impact of Forced-Aging Process on Madeira Wine Flavor

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The aim of this study was to determine the optimal temperature and baking time to obtain a Madeira wine considered typical by an expert panel. For this purpose simultaneous descriptive analyses of typical Madeira wines were performed, and seven descriptors were selected: “dried fruit”, “nutty”, “musty”, “baked”, “oak”, “mushroom”, and “brown sugar”. Up to 10 odor-active zones were the most frequently cited by the members of the GC-olfactometry panel as corresponding to the panel’s descriptors. The odor importance of each of the zones reported by the GC-O analysis was ranked by AEDA. Three odor zones were identified as common to both Malvasia and Sercial wines and had retention indices (RI) of 1993 (“brown sugar” and “toasted”), 2151 (“brown sugar”), and 2174 (“nutty”, “dried fruits”); sotolon was identified as responsible for this last aroma. Several molecules were selected to be quantified on baked wines on the basis of AEDA results and expected Maillard volatiles, such as sotolon, furfural, 5-methylfurfural, 5-ethoximethylfurfural, methional, and phenylacetaldehyde. It was observed that typicality scores were positively correlated with the concentrations of sotolon and sugar and baking time and negatively with the fermentation length.

KEYWORDS: Madeira wine; volatile compounds; sensorial analysis; AEDA-GC-O and GC-MS

INTRODUCTION

Madeira wine is a fortified wine with an ethanol content of 18–20% made on the Atlantic island that gives it its name. The Canteiro category of Madeira wines refers to those produced from grapes from a single harvest (1). Maturation of these wines is performed in oak casks in warm cellars for a greater or lesser period of time. Following this period, wines are bottled and labeled with the harvest year. In some Madeira wine companies, fortification is followed by a baking process known as “estufagem”, during which wines are submitted to temperatures of around 50 °C for a minimum period of 90 days (1, 2). After this procedure, wines are placed in oak casks for a minimum of 3 years. The baking procedure can be seen as a “forced aging” process, and it is the first step in the introduction of technology into Madeira wine production.

There are five main varieties of Vitis vinifera grapes from which Madeira wine is produced: Boal, Malvasia, Sercial, and Verdelho (white grape varieties) and Tinta Negra Mole (red grape variety). According to the extent of the fermentation process, and consequently the sugar content, Madeira wines can be divided into four basic categories: Sercial, usually fermented until 25 g/L of residual sugar, giving a dry wine; Boal, fermented half-way until 65 g/L of residual sugar, producing a medium dry wine; Verdelho, fermented until 90 g/L of residual sugar, giving a medium sweet wine; and Malvasia, traditionally not fermented, giving a sweet wine of 110 g/L of residual sugar. The traditional Madeira wine classification based on sugar contents does not correspond to OIV designations (3).

The vinification process of Madeira wine is particular: wines can be sweet or dry, and vinification can be processed by traditional or modern processes. In the traditional process, in the case of sweet wines, little or no fermentation occurs, leading to a sweet wine that is basically grape juice with alcohol fortification. In traditional dry wine, the fermentation procedure is carried out to full length and the alcohol fortification is made after complete fermentation (1). Modern vinifications are carried out in same way; however, for sweet wines fermentation occur for only 2 day (before alcohol fortification), and for dry wines...
fermentations are stopped by alcohol addition, before complete fermentation, similar to port wine vinification (4). It is clear that the resulting wines are very different. In fact, the sensorial profile of each type of vinification wine is particular for each category. Differences obtained are caused by the grape varieties and fermentation, by the forced-aging process related to baking temperature and time, and by the storage temperature and time (5).

Some previous works were performed to characterize Madeira wine aroma (6−8). Hence, several chemical compounds, such as monoterpenols, norisoprenoid aldehydes, alcohol acetates, acetalts, esters, furanic and pyranic compounds, and lactones, have been identified, and their concentrations in wines were related to some specific sensorial characteristics of Madeira wines (2, 5, 9, 10). In these studies, the formation of sotolon has been related to the levels of sugars (2). These results can be explained by a flavor generation reaction between a reducing sugar and an amino group-containing substance (11, 12), the Maillard mechanism. This mechanism can be divided into the Amadori or Heyns rearrangement or into the Strecker degradation and melanoidin formation. Amadori or Heyns rearrangement products can generate cyclic species, such as furfurals, pyrazines, or thiazoles; the Strecker degradation of the amino acids, in the presence of dicarbonylic compounds, formed by sugar retroaldolization, can form volatile aldehydes, such as methional and phenylacetaldehyde. Finally, almost any intermediary of the Maillard reaction can condense with other compounds, producing flavoring molecules or, ultimately, melanoidins (browning substances and roast/burnt flavoring compounds) (11, 12). The full extent of the contributions of biochemical and chemical reactions to the typicity is largely unknown.

The aims of this study were, first, to identify key odorants of Madeira wine and to assess their impact on its typicity; second, to elucidate the chemical processes on the generation of volatiles that contribute to the generation of volatiles responsible for typicity; and, third, to determine the optimal baking temperature and time regimen to employ in the modern configuration of Madeira wine production.

**MATERIALS AND METHODS**

**Reagents.** Chemicals and standards were obtained from Sigma-Aldrich (a high-purity grade, > 99.0%). Dichloromethane, anhydrous sodium sulfate, and ethanol were obtained from Merck, Darmstadt, Germany.

**Wine Material.** Several unbaked Madeira wines from the 2003 and 2004 harvests were submitted to three isothermal regimens (30, 45, and 55 °C) and different baking times, up to a maximum of 120 days (Figure 1). Wines were produced from two grape varieties, Malvasia and Sercial, with two levels of residual sugar: Malvasia traditional, 120 g/L; Malvasia modern, 84 g/L; Sercial modern, 17 g/L; and Sercial traditional, not detected.

**Sensory Analysis.** Descriptive analysis was performed according to the method described by Rainey (13). The main objectives were (i) to screen if the panel was able to detect differences between samples produced by different techniques at the same temperatures; (ii) to rank seven wine samples for the degree of “typical character” they exhibit; (iii) to rank six wines according to their “typical character” (three Sercial and three Malvasia wines previously selected as the most typical); and (iv) to measure the reliability of the sensory results obtained.

**Selected Panel Description.** Fifteen trained volunteers (9 males, 6 females, smokers and nonsmokers, aged 36−65 years) participated.

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**Table 1. Attributes Used for Training Sessions and Composition of Reference Standards**

<table>
<thead>
<tr>
<th>aroma</th>
<th>composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>dried fruit</td>
<td>mixed dried fruits (raisins + prune + figs)</td>
</tr>
<tr>
<td>nutty</td>
<td>mixed nuts (walnuts + almonds)</td>
</tr>
<tr>
<td>musty</td>
<td>verbally defined as “a damp basement”</td>
</tr>
<tr>
<td>cocoa</td>
<td>cocoa flavor (hot chocolate)</td>
</tr>
<tr>
<td>vanilla</td>
<td>water solution of vanilla extract</td>
</tr>
<tr>
<td>cherry</td>
<td>mixture of cherry preserves and maraschino cherry extract</td>
</tr>
<tr>
<td>citrus</td>
<td>tea spoon of orange/lemon natural extracts and grapefruit juice</td>
</tr>
<tr>
<td>coffee</td>
<td>fresh brewed coffee</td>
</tr>
<tr>
<td>rum</td>
<td>dark rum</td>
</tr>
<tr>
<td>oak</td>
<td>American oak chips in water solution</td>
</tr>
<tr>
<td>mushroom</td>
<td>fresh mushroom</td>
</tr>
<tr>
<td>brown sugar</td>
<td>burnt brown sugar</td>
</tr>
</tbody>
</table>

---

*All of the standards were presented in individual plastic corked tubes.

**Available only during training sessions.**
Sensory characteristics. To minimize adaptation, a 30 s break occurred between trials, and panelists were instructed to take additional breaks as they desired. The use of dark glasses is necessary to prevent the influence of wine color in the tests.

**Triangle Tests.** The triangle test was chosen as it allows distinguishing among three samples which has the different sensory characteristic. As it is a discrimination test, it is better for the detection of small differences between samples than it is the intensity rate test (14). Two triangle test sessions were conducted for both Sercial and Malvasia wines, to determine if there were detectable differences between the “traditional” and “modern” wines, under two different sets of conditions. For each essay six wines were presented corresponding to the three temperatures 30, 45, and 55 °C under study.

**Ranking Protocol.** The sensory impact of baking temperature on the “typical character” of Madeira was evaluated by ranking in a meaningful scale. According to the grape variety, baking temperature, and vinification process, three sets were constituted. Each set was presented at three different sessions in a random order and ranked on a scale from 1 to 7, 1 being the wine with the most “typical character” and 7, the wine with the least “typical character”. Each set was composed of seven samples, six unknown samples and one sample wine unanimously recognized by the panel as typical Madeira wine (reference sample), for both categories. All samples were presented in a random order (based on a William’s Latin square), coded (with a three-digit number) in transparent 170 mL (6.5 oz) tulip-shaped wine glasses, which were covered with plastic Petri dish lids. The panelists have to order all of the samples.

**Extraction of Wine Volatiles.** The extraction procedure was based on the method described previously (16). Briefly, 50 mL samples of baked Madeira wines were spiked with 50 µL of 3-octanol in a hydroalcoholic solution (1:1, v/v) at 422 mg/L as the internal standard and 5 g of anhydrous sodium sulfate (higher ionic strength, increases extractability). The wine was extracted twice with 5 mL of dichloromethane. The two organic phases obtained were blended and dried over anhydrous sodium sulfate. Two milliliters of this organic extract was concentrated to 0.4 mL under a nitrogen stream.

**Gas Chromatography—Mass Spectrometry (GC-MS).** Extracts were analyzed using a Varian CP-3800 gas chromatograph equipped with a Varian Saturn 2000 mass selective detector and Saturn GC-MS workstation software version 5.51. The column used was Stabilwax-DA (60 m × 0.25 mm, 0.25 µm) fused silica (Restek). The injector port was heated to 220 °C. The split vent was opened after 30 s. The carrier gas was helium C-60 (Gasin), at 1 mL/min, constant flow. The oven temperature was 40 °C (for 1 min), then increased at 2 °C/min to 220 °C, and held for 30 min. All mass spectra were acquired in the electron impact (EI) mode. The ion trap detector was set as follows: The transfer line, manifold, and trap temperatures were, respectively, 230, 45, and 170 °C. The mass range was m/z 33–350, with a scan rate of 6 scans/s. The emission current was 50 µA, and the electron multiplier was set in relative mode to autotune procedure. The maximum ionization time was 25000 ms, with an ionization storage level of m/z 35. The injection volume was 1 µL, and the analysis was performed in full-scan mode.

Identification was achieved from comparisons of mass spectra obtained from the sample with those from pure standards injected in the same conditions by comparing the Kovats indices and the mass spectra present in the NIST 98 MS Library Database or in the literature (15).

**Gas Chromatography—Olfactometry (GC-O).** Two microliters of the extract was injected into the GC equipped with an olfactometric detector. Chromatographic conditions were the following: Hewlett-Packard HP 5890 gas chromatograph; column BP-21 (50 m × 0.25 mm, 0.25 µm) fused silica (SGE); hydrogen (5.0, Gasin); flow, 1.2 mL/min; injector temperature, 220 °C; oven temperature, 40 °C for 1 min programmed at a rate of 2 °C/min to 220 °C, maintained during 30 min; splitless time, 0.5 min; split flow, 30 mL/min.

The make-up gas employed on the olfactometric device (SGE) was air (80% N2; 20% O2) (Gasin). Two streams were used; one was bubbled in water (noise moister), the other was applied at the exit of the GC column to lower the temperature of the effluent.
The descriptors collection on GC-O was performed by five trained persons (laboratory students) and was repeated several times. The descriptors retained were those that obtained the higher number of citations, considering each member of the panel (16).

Aroma Extract Dilution Analysis (AEDA). The relative importance of each of the different odor zones was evaluated by AEDA (17). Two milliliters of the extract was concentrated to 1:10 under a nitrogen stream. Then, 2 µL of the concentrated dichloromethane extract was separated on a capillary column. The odor-active regions and the odor qualities were assigned by five assessors (GC-O). The extract was stepwise diluted with dichloromethane (1 + 1 by volume), and the odor zones were re-evaluated. The process stopped when no aromas were detected by the assessor.

RESULTS AND DISCUSSION

The production of Madeira wine usually involves exposure to mildly high temperatures (up to 50 °C) and humidity levels of >70% (18). Madeira wine is usually produced by a baking regimen, with temperature oscillations during the thermal process. The final product has a characteristic flavor profile very different from that of other commercial wines. Hence, a study of the key odorants in Madeira wine was performed. For this purpose a GC-O/AEDA was done using wines from isothermal baking procedures and also nonbaked wine to enhance knowledge about the effects of the baking procedure in wine sensorial descriptors. The most relevant descriptors from the reference wine (“typical character”) and from either the Malvasia and Sercial selected baked wines (MT 45 °C and SM 45 °C) are shown in Tables 2 and 3, respectively. To accomplish this objective, the temperature and time of the baking process were also studied.

Simultaneous descriptive analyses of typical Madeira wines were performed by the expert panel, and seven descriptors were selected: “dried fruit”, “nutty”, “musty”, “baked”, “oak”, “mushroom”, and “brown sugar”. The descriptors “baked”, “brown sugar”, and “nutty” suggested the presence of compounds formed by Maillard reaction. In fact, for example, “nutty” (as we will see later this paper) is related to the presence of sotolon. Blank et al. (19) showed the involvement of Maillard

<table>
<thead>
<tr>
<th>wine</th>
<th>score</th>
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<th>KI = 1455</th>
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<td>1148</td>
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</table>

Table 4. Concentration of Volatile Compounds According to Temperature of Baking

Table 5. Concentration of Volatile Compounds According to Baking Time

The descriptors collection on GC-O was performed by five trained persons (laboratory students) and was repeated several times. The descriptors retained were those that obtained the higher number of citations, considering each member of the panel (16).

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reaction when they studied the formation of sotolon from glyoxal and amino acid (different concentrations and relative concentrations). Temperature and time are usually linked because they control, together, the kinetic reactions. High temperatures can
produce roasted or burned flavor, mainly by the formation of melanoidins (the final product of Maillard reaction). If enough time is given to the reaction, it can theoretically reach completion (formation of melanoidins) even if the reaction temperature is not very high. Therefore, the balance of these two variables can have a huge influence in the production of flavoring substances.

Deoxyosones are intermediates of the Amadori reaction, being part of the enolization and transamination reactions. They can be formed depending on the enolization that occurs: 1,2-enolization yields 3-deoxyosone, and 2,3-enolization yields 1-deoxyosone. The 1,2-enolization pathway is known to be the main pathway, as cited by Tressl et al. and Martins and van Boekel (12, 20). Furthermore, the 3-deoxyosone pathway is more probable at pH < 5 and the 1-deoxyosone pathway at pH > 7. As proposed by Weenen (21), flavor substances generated in early Maillard reaction can be divided into two classes from a chemical point of view: those that are formed by cyclization/condensation of the deoxyosones or those that are formed after the carbohydrate fragmentation. Products formed by cyclization/condensation of the intermediate deoxyosones include 5-hydroxymethylfurfural (HMF), 5-methylfurfural (5-MF), and furfural, and they are often formed in high or very high yields. Products formed after carbohydrate fragmentation include pyrazines and thiazoles and are formed in low or very low yields. Our results showed that at higher baking temperatures higher levels of these volatiles were formed (Table 4). Our results showed also that at the same baking temperature (45 °C), the longer is the period of baking, more volatiles are produced (Table 5).

According to Pripis-Nicolau et al. and Marchand et al. (22–24), dicarbonyl compounds can react with amino acid in conditions similar to the ones we encounter in wine samples. In earlier works Hashiba (25) detected some Amadori compounds in Japanese white wines, which suggest that these compounds can be formed even in an unfavorable environment.

Blank et al. (19) proposed a mechanism of formation of sotolon and Strecker aldehydes from isoleucine (amino acid) and from its lactone. The optimal pH condition for this formation is 5. Sotolon formation depends on temperature, favored by high temperatures and also by high concentrations of dicarbonylic compounds (26). It was also suggested that Strecker-inactive carbonyl compounds (such as propionaldehyde and phenylacetaldehyde) are also able to transform the lactone form of isoleucine into sotolon, by an alternative pathway (19).

All baked wines were then ranked according to their typicity by the expert panel (Figures 2–5). Ranking data were then converted into scores (27). First, the panel chose the wines baked at 45 °C during 3 months as the most similar comparing to the reference of typical Madeira wine. To establish the ideal time of baking Malvasia and Sercial wines were baked for 4 months at 45 °C (Figure 1). The sensory evaluation results gave the highest scores to the samples baked at 45 °C during 120 days for both Sercial and Malvasia (Table 5 and Figure 6). An important remark should be made: the Malvasia sample chosen by the panel was considered to be the wine most similar to the typical Madeira wine standard, and it has the highest residual sugar content of all wines evaluated by the panel. This indicates that the presence of sugar can be a clue factor taking to explain the preference of the expert panel.

Following the wine ranking step, the identification of key odorants of Madeira most typical wines (reference wine) was made using GC-O and AEDA procedures (Table 2). GC-O gave a flavor description of each wine sample and AEDA the global aroma impact of each flavor compound.

Up to 10 odor-active zones were the most frequently cited by the members of the GC-O panel as corresponding to the panel’s descriptors. Some descriptors are common to both Malvasia and Sercial wines, and others are specific to Malvasia or Sercial wines. The odor importance of each odorant zone reported by the GC-O analysis was ranked by AEDA (Table 3).

The dilution factors (DF) observed for the six odor zones specific for Malvasia wine were 16, 16, 128, 128, 256, and 64, respectively, for the retention indices 1352 (“nutty”), 1884...
("honey" and "brown sugar"), 2009 ("dry fruits" and "coffee"), 2023 ("burned sugar" and "coffee"), 2062 ("brown sugar"), and 2116 ("burned sugar"). The dilution factors observed for the three odor zones specific for Sercial wine were 8, 512, and 4, respectively, for the retention indices 1993 ("toasted"), 1415 ("toasted"), and 2174 ("nutty" and "dried fruits") (Table 3).

According to the observed dilution factors of the reference wine, Malvasia wine and Sercial wine, the RI of sotolon was 2174, and it was identified as responsible for the highest impact flavor, corresponding to "nutty" or "dried fruits" flavor. Sotolon has also been considered to be responsible for the aged Porto wine typical flavor (26, 28, 29).

Several molecules were then selected to be quantified on baked wines on the basis of AEDA results and expected Maillard volatiles, such as sotolon, furfural, 5-methylfurfural, 5-ethoxyethylfurfural, methional, and phenylacetaldehyde (Table 4). A positive correlation was also observed between baking time and paneltypicity scores (Table 5 and Figure 6). According to the present data the perceived quality increased for long periods of baking process, for both Malvasia and Sercial wines. Baking time has a higher impact on the typicity of Malvasia wines than of Sercial wines.

When data from Table 5 and Figure 6 are taken into consideration, typicity scores were positively correlated with the concentration of sotolon. These remarks clarify the importance of sotolon in the typicity of Madeira wines, reinforcing the results obtained by GC-O and AEDA analyses. It was also shown that the Maillard reaction had an important role in Madeira wine flavor formation as was previously suggested. Figure 6 shows also that the magnitude of biochemical reactions on the wine scores, occurring, for example, during alcoholic fermentation, is reduced with the time of baking process. Despite the grape variety, the difference between Malvasia traditional and Sercial modern is due to the fermentation process. In this case, the difference between both curves represents the alcoholic fermentation impact on wine scores. As can be seen, the difference between both curves diminishes during the baking procedure, so it can be inferred that the baking process reduces the impact of the fermentation flavor compounds; the AEDA results also have shown that the baked Madeira wine typical flavor is mainly due to the presence of volatile compounds formed during the normal or forced-ageing process. Flavor compounds formed during the forced-ageing process have a much greater impact on Madeira wine quality than the primary and secondary flavors, which have little impact on the global wine flavor. Madeira wine flavor is clearly due to the high levels of "aged marker compounds". It was also observed that shorter fermentation extent (and thus higher sugar concentration) corresponds to more typical wine. Sotolon is the compound that has the highest impact on typical Madeira wine flavor. Levels of sotolon can be used to assemble wines according to typicity character.

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