

Impact of Forced-Aging Process on Madeira Wine Flavor

HUGO OLIVEIRA E SILVA,[†] PAULA GUEDES DE PINHO,^{*,§} BEATRIZ P. MACHADO,[†]
 TIM HOGG,[†] J. C. MARQUES,[#] JOSÉ S. CÂMARA,[#] F. ALBUQUERQUE,[‡] AND
 ANTONIO C. SILVA FERREIRA^{*,†}

Escola Superior de Biotecnologia, Universidade Católica Portuguesa, R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal, REQUIMTE/Serviço de Toxicologia, Faculdade de Farmácia da Universidade do Porto, R. Aníbal Cunha 164, 4050-047 Porto, Portugal, Departamento de Química, Universidade da Madeira, Campus da Penteada, 9000-390 Funchal, Portugal, Madeira Wine Company, Rua dos Ferreiros 191, 9000-082 Funchal, Portugal

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-ethoxymethylfurfural, methional, and phenylacetaldehyde. It was observed that typicity 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 halfway 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

* Corresponding authors [fax 351-2-22003977; telephone 351 222078922; e-mail (A.C.S.F.) ferreira@mail.esb.ucp.pt, (P.G.d.P.) pguedes@ff.up.pt].

[†] Universidade Católica Portuguesa.

[§] Universidade do Porto.

[#] Universidade da Madeira.

[‡] Madeira Wine Co.

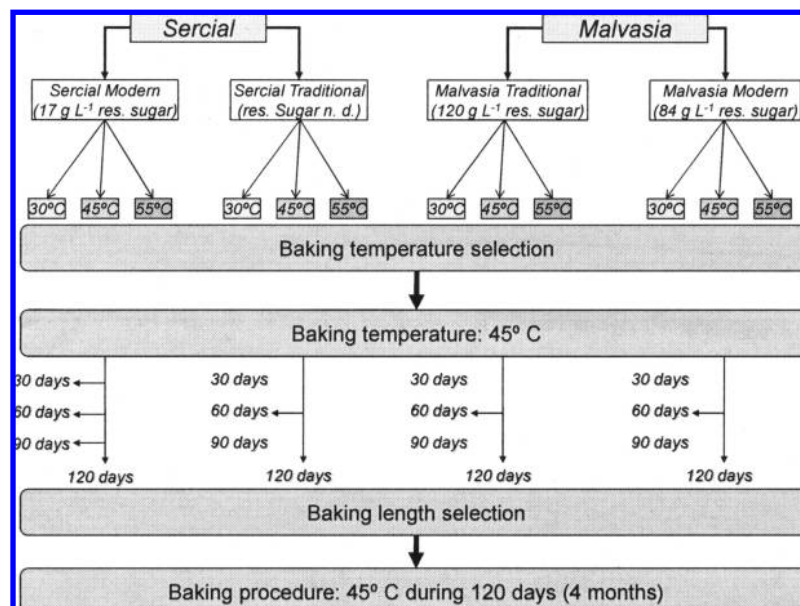


Figure 1. Experimental protocol.

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, acetals, 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.

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

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

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

^b Available only during training sessions.

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 nonbaked 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.

To establish the ideal time of baking, Malvasia [Malvasia with modern vinification (MM) and Malvasia with traditional vinification (MT)] and Sercial [Sercial with modern vinification (SM) and Sercial with traditional vinification (ST)] wines were baked during 4 months at 45 °C in inox contents of 200 L with control temperature.

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.

Table 2. Retention Indices (RI), Sensory Descriptors, and Dilution Factors (DF) Obtained by Gas Chromatography–Olfactometry and Aroma Extract Dilution Analysis of a Dichloromethane Extract of the Reference Wine (“Typical Character”)

Madeira Wine (Reference)		
RI	descriptor	DF
1057	pine	8
1063	pine	32
1063	ethanol	32
1082	banana	8
1096	pine	32
1356	woody	16
1413	honey	8
1468	citric	8
1480	baked vegetable	32
1611	woody	8
1647	honey	1
1651	honey	32
1791	honey	16
1947	wood, honey	128
2015	toasted	8
2134	chocolate	64
2158	dry fruits, nutty	1024

Table 3. Retention Indices (RI), Sensory Descriptors, and Dilution Factors (DF) Obtained by Gas Chromatography–Olfactometry and Aroma Extract Dilution Analysis of the Dichloromethane Extracts of the Selected Malvasia and Sercial Madeira wines

Selected Malvasia (M) and Sercial (S) Wines			
RI	descriptor	DF	grape variety
1352	nutty	16	M
1415	toasted	8	S
1453	baked vegetable	512	S
1884	honey	16	M
1993	toasted	256	M + S
2009	dry fruits, coffee	128	M
2023	burned sugar, coffee	128	M
2030	spicy, toasted	4	S
2062	burned sugar	256	M
2116	burned sugar	64	M
2151	burned sugar	16	M + S
2174	dry fruits, nutty	256	M + S

Training Sessions. Fifteen judges were selected on the basis of previous acuity tests and sensory performances (14, 15). The majority of the judges had Madeira wine tasting experience, and they participated in previous descriptive Madeira wine sensory tests.

To train the panel for the present study, four sessions were held. In each one two samples of commercial wines were presented with 12 reference aroma standards (Table 1) to aid panelists in identifying and remembering the sensory attributes found in the evaluated wines (15). The aroma standards were provided only during the training sessions.

Sensory Evaluation Sessions. Prior to sample evaluation, panelists received instruction regarding the evaluation procedure in both written and verbal formats. The following written instructions were placed on the ballot: “Smell the samples from left to right. Two of the samples are identical. Determine which one is the odd sample. You may re-smell samples. If no difference is apparent, you must guess”. Panelists, standing in front of the tray, were presented with three sets of three samples simultaneously, two of them from one production process treatment and the other from another vinification process. To minimize visual comparison of samples and eliminate side-by-side comparisons, the volume presented was reduced to 10 mL per glass and the panelists were instructed to keep samples capped until use, removing caps only to obtain the sample and to disregard visual cues. Although it is not possible for panelists to “disregard” cues, attention was focused to other sensory characteristics. To minimize adaptation, a 30 s break occurred

between triads, 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 μ L 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 \times 0.25 mm, 0.25 μ m) 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 scan/s. The emission current was 50 μ A, and the electron multiplier was set in relative mode to autotune procedure. The maximum ionization time was 25000 μ s, with an ionization storage level of m/z 35. The injection volume was 1 μ L, 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 (16).

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 \times 0.25 mm, 0.25 μ m) 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 (nose moister), the other was applied at the exit of the GC column to lower the temperature of the effluent.

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

wine ^b	baking temp (°C)	compd concn ^a (8 µg/L)					
		KI = 1654	KI = 1455	KI = 1584	KI = 1977	KI = 2156	KI = 2308
		Phal	Mal	5-MF	5-EF	Sot	HMF
SM	30	53	nd	1	0.5	17	1
	45	53	nd	8	3	89	9
	55	69	16	33	61	48	80
ST	30	54	nd	1	0.3	nd	1
	45	59	15	6	2	66	5
	55	75	24	33	16	71	15
MM	30	39	6	3	1	29	8
	45	21	9	8	19	127	58
	55	27	14	49	293	76	439
MT	30	34	1	3	6	19	2
	45	44	1	6	10	163	61
	55	45	2	67	419	81	688

^a KI, Kovats retention index; Phal, phenylacetaldehyde; Mal, methional; 5-MF, 5-methylfurfural; 5-EF, 5-ethoxymethylfurfural; Sot, sotolon; HMF, hydroxymethylfurfural. Analysis performed in duplicate. ^b SM, Sercial modern; ST, Sercial traditional; MM, Malvasia modern; MT, Malvasia traditional.

Table 5. Concentration of Volatile Compounds According to Baking Time

wine ^b	score	baking length (days)	compd concn ^a (8 µg/L)					
			KI = 1654	KI = 1455	KI = 1584	KI = 1977	KI = 2156	KI = 2308
			Phal	Mal	5-MF	5-EF	Sot	HMF
SM	-0.35	30	10	3	2	0.5	7	0.5
	2.22	60	13	5	3	0.7	8	1.2
	2.44	90	16	6	5	2	10	1.2
	4.46	120	21	10	5	2	13	1.8
ST	-0.47	30	34	20	0.9	0.3	1	0.3
	-4.67	60	40	25	1	0.4	1	0.4
	-8.27	90	29	25	2	0.4	1	0.5
	-5.04	120	28	17	3	0.4	2	0.6
MM	-9.45	30	26	6	4	1	42	6
	-1.69	60	32	9	4	3	49	12
	-0.67	90	42	14	7	4	124	25
	-3.98	120	68	18	12	8	169	61
MT	-3.99	30	46	1	2	1	12	23
	0.3	60	53	1	3	4	24	52
	1.74	90	59	2	5	7	126	87
	3.97	120	60	2	43	108	399	1148

^a KI, Kovats retention index; Phal, phenylacetaldehyde; Mal, methional; 5-MF, 5-methylfurfural; 5-EF, 5-ethoxymethylfurfural; Sot, sotolon; HMF, hydroxymethylfurfural. Analysis performed in duplicate. ^b SM, Sercial modern; ST, Sercial traditional; MM, Malvasia modern; MT, Malvasia traditional.

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

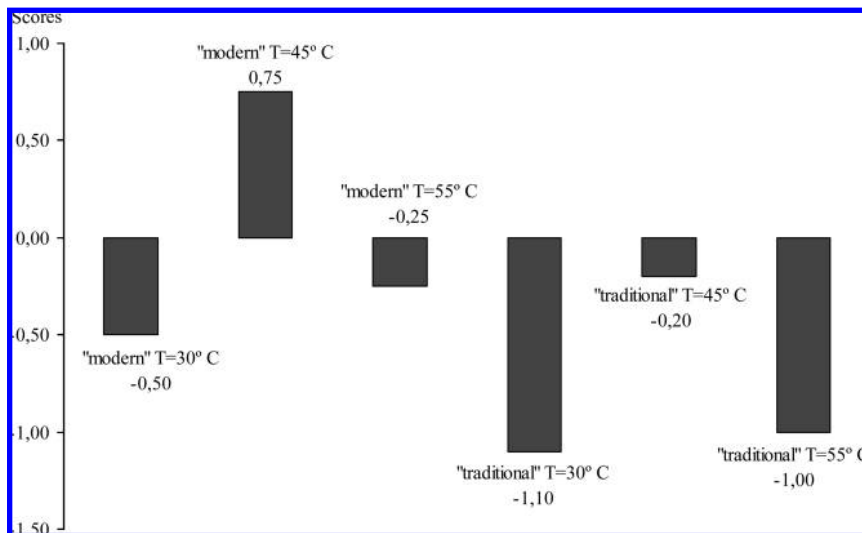


Figure 2. Scores obtained for Sercial wine samples, baked at 30, 45, and 55 °C during 3 months.

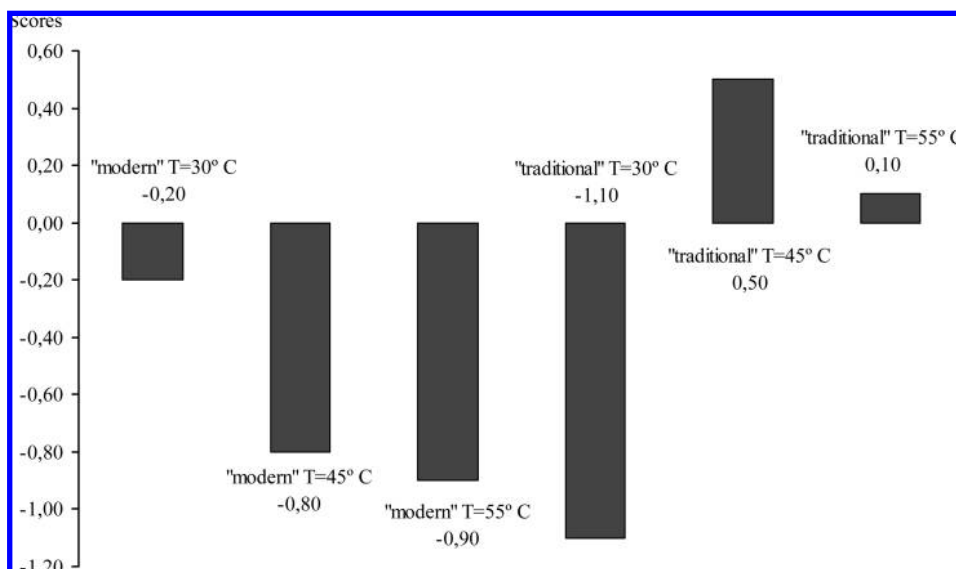


Figure 3. Scores obtained for Malvasia wine samples, baked at 30, 45, and 55 °C during 3 months.

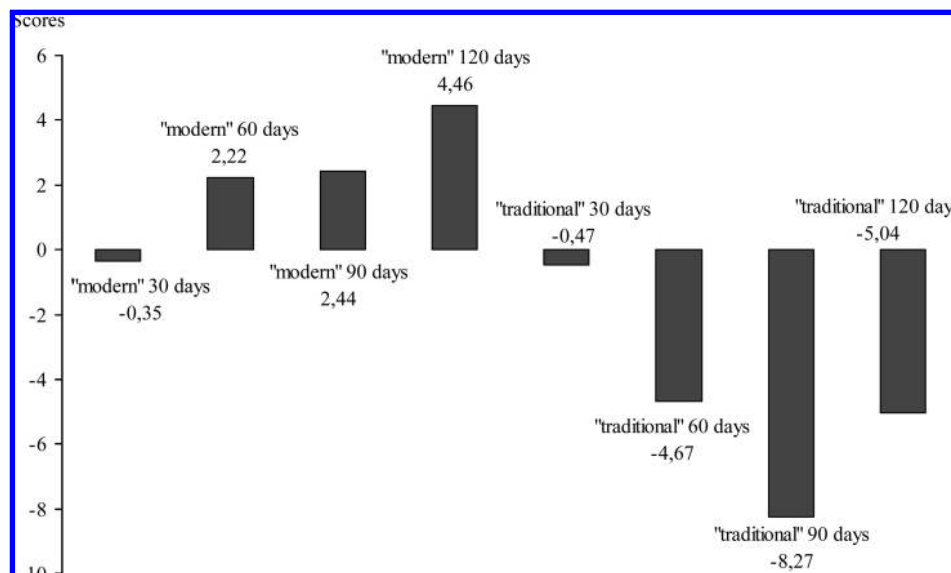


Figure 4. Scores obtained for Sercial wine samples, baked at 45 °C during 4 months.

reaction when they studied the formation of sotolon from glyoxal and amino acid (different concentrations and relative concentra-

tions). Temperature and time are usually linked because they control, together, the kinetic reactions. High temperatures can

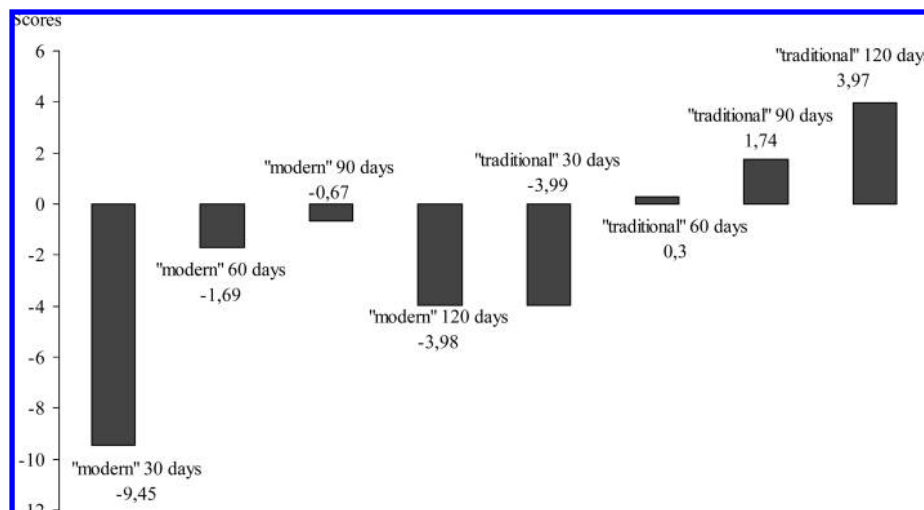


Figure 5. Scores obtained for Malvasia wine samples, baked at 45 °C during 4 months.

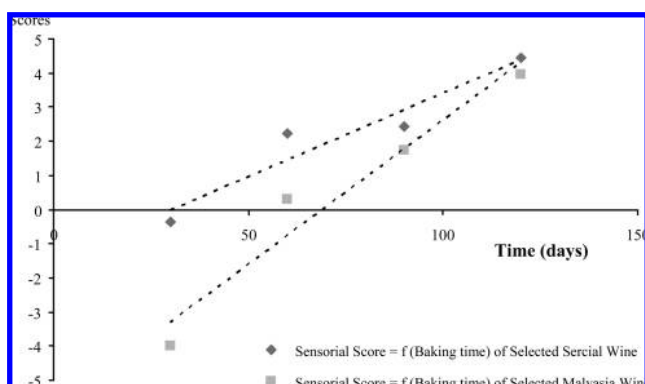


Figure 6. Relationship between baking time and sensory scores.

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 dicarbonyl 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 1415 ("toasted"), 1453 ("baked", not a panel's descriptor), and 2030 ("spicy" and "toasted").

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" 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-ethoxymethylfurfural, methional, and phenylacetaldehyde (Table 4). A positive correlation was also observed between baking time and panel typicity 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-aging process. Flavor compounds formed during the forced-aging 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.

LITERATURE CITED

- http://www.madeirawineguide.com/.
- Câmara, J. S.; Marques, J. C.; Alves, M. A.; Silva Ferreira, A. C. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone levels in fortified Madeira wines: relationship to sugar content. *J. Agric. Food Chem.* **2004**, *52*, 6765–6769.
- Boulton, R. B.; Singleton, V. L.; Bisson, L. F.; Kunkee, R. *Principles and Practices of Wine Making*; Chapman and Hall: London, U.K., 1995; Vol. 1.
- Silva Ferreira, A. C.; Rodrigues, P.; Hogg, T.; Guedes de Pinho, P. Influence of some technological parameters on the formation of dimethyl sulfide, 2-mercaptoethanol, methionol and dimethyl sulfone in Port wines. *J. Agric. Food Chem.* **2003**, *51*, 727–732.
- Câmara, J. S.; Marques, J. C.; Alves, M. A.; Silva Ferreira, A. C. Heterocyclic acetals in Madeira wines. *Anal. Bioanal. Chem.* **2003**, *373*, 1221–1224.
- Campo, E.; Ferreira, V.; Escudero, A.; Marques, J. C.; Cacho, J. Quantitative gas chromatography-olfactometry and chemical quantitative study of the aroma of four Madeira wines. *Anal. Chim. Acta.* **2006**, *563*, 180–187.
- Camara, J. S.; Alves, M. A.; Marques, J. C. Changes in volatile composition of Madeira wines during their oxidative ageing. *Anal. Chim. Acta* **2006**, *563*, 188–197.
- Silva Ferreira, A. C.; Reis, S.; Rodrigues, C.; Oliveira, C.; Guedes de Pinho, P. Simultaneous determination of ketoacids and dicarbonyl compounds, key Maillard intermediates on the generation of aged wine aroma. *J. Food Sci.* **2007**, *72* (5), 314–318.
- Câmara, J. S.; Herbert, P.; Marques, J. C.; Alves, M. A. Varietal flavor compounds of four grape varieties producing Madeira wines. *Anal. Chim. Acta* **2004**, *513*, 203–207.
- Câmara, J. S.; Alves, M. A.; Marques, J. C. Analysis of Madeira wines using solid phase microextraction–GC/MS. *Adv. Mass Spectrom.* **2001**, *15*, 943.
- Hodge, J. E. Chemistry of browning reactions in model systems. *J. Agric. Food Chem.* **1953**, *1*, 928.
- Tressl, R.; Nittka, C.; Kersten, E. Formation of isoleucine-specific Maillard products from [1-¹³C]-D-glucose and [1-¹³C]-D-fructose. *J. Agric. Food Chem.* **1995**, *43*, 1163–1169.
- Rainey, B. A. Importance of reference standards in training panelists. *J. Sensory Stud.* **1986**, *1*, 149–154.
- Issanchou, S.; Hossenlopp, J. Les mesures hédoniques: méthodes, portées et limites In *Plaisirs et Préférences Alimentaires*; Giachetti, I., Ed.; Polytechnica: Paris, France, 1992; pp 49–75.
- Lawless, H. T.; Heymann, H. *Sensory Evaluation of Food, Principles and Practices*; Aspen: Fredericksburg, MD, 1998.
- Silva Ferreira, A. C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, *51*, 1377–1381.
- Ullrich, F.; Grosch, W. Identification of the most intense volatile flavour compounds formed during autoxidation of linoleic acid. *Z. Lebensm. Unters. Forsch.* **1987**, *184*, 277–282.
- Hurley, C.; Jackson, A.; Johnson-Gilbert, E. *André Dominé—Wine*, 5th ed.; Könemann: 2004; pp 688–689.
- Blank, I.; Lin, J.; Fumeaux, R.; Welti, D. H.; Fay, L. B. Formation of 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolone) from 4-hydroxy-L-isoleucine and 3-amino-4,5-dimethyl-3,4-dihydro-2(5H)-furanone. *J. Agric. Food Chem.* **1996**, *44*, 1851–1856.
- Martins, S. I. F. S.; van Boekel, M. A. J. S. Key intermediates in early stage Maillard reaction: kinetic analysis. *Int. Cong. Ser.* **2002**, *1245*, 469–470.
- Weenen, H. Reactive intermediates and carbohydrate fragmentation in Maillard chemistry. *Food Chem.* **1998**, *62* (4), 393–401.
- Marchand, S.; de Revel, G.; Bertrand, A. Approaches to wine aroma: release of aroma compounds from reactions between cysteine and carbonyl compounds in wine. *J. Agric. Food Chem.* **2000**, *48*, 4890–4895.
- Pripis-Nicolau, L.; de Revel, G.; Bertrand, A. Formation of flavor components by the reaction of α -amino acids and carbonyls components in mild conditions. *J. Agric. Food Chem.* **2000**, *48*, 3761–3766.
- Marchand, S.; de Revel, G.; Vercauteren, J.; Bertrand, A. Possible mechanism of involvement of cysteine in aroma production in wine. *J. Agric. Food Chem.* **2002**, *50*, 6160–6164.
- Hashiba, H. Isolation and identification of Amadori compounds from miso, white wine and sake. *Agric. Biol. Chem.* **1978**, *42* (9), 1727–1731.

- (26) Silva Ferreira, A. C.; Avila, I.; Guedes de Pinho, P. Sensorial impact of sotolon as the "perceived age" of tawny port wines In *Trends in Natural Flavors and Fragrances, Chemistry, Analysis and Production*; Frey, C., Rouseff, R. L., Eds.; American Chemical Society: Washington, DC, 2005; pp 141–159.
- (27) Larmond, E. *Laboratory Methods for Sensory Evaluation of Food*; Publication 1637; Research Branch, Canada Department of Agriculture, 1982; pp 63.
- (28) Guedes de Pinho P.; Silva Ferreira A. C. Role of Strecker aldehydes on beer flavor stability *Flavour Science Recent Advances and Trends, Proceedings of the 11th Weurman Flavour*

Research Symposium; Wender, B., Petersen, M., Eds.; Elsevier Science: Amsterdam, The Netherlands, 2006; pp 529–532.

- (29) Silva Ferreira, A. C.; Barbe, J. C.; Bertrand, A. B. 3-Hydroxy-4,5-dimethyl-2(5H)-furanone: a key odorant of the typical aroma of oxidative aged Porto wine. *J. Agric. Food Chem.* **2003**, *51*, 1373–1376.

Received for review July 15, 2008. Revised manuscript received October 13, 2008. Accepted October 14, 2008.

JF802147Z