

Study of the Formation Mechanisms of Some Volatile Compounds during the Aging of Sweet Fortified Wines

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Sweet fortified wines, traditionally aged under strong oxidation conditions, have a characteristic aroma. An experimental laboratory study investigated the aging of red and white sweet fortified wines under various conditions. The formation of various molecules, previously identified as characteristic of the aroma of this type of wine, was monitored by analysis. The development of these compounds during accelerated aging was affected by oxidation and the color of the wine. Among the molecules studied, sotolon [3-hydroxy-4,5-dimethyl-2(5H)-furanone] was one of the few molecules present in concentrations above the perception threshold, in both red and white wines. Buildup was strongly affected by the presence of oxygen in white wine subjected to accelerated aging. (Ethoxymethyl)furfural, formed from 5-(hydroxymethyl)furfural, and furfural, derived from sugars, are also involved in the aroma of sweet fortified white wines aged in oxygen-free conditions. The substances most characteristic of accelerated aging of sweet fortified red wines were 5-(hydroxymethyl)furfural, acetylformoin, and hydroxymaltol, the formation of which is affected by oxidation, and dihydromaltol, formed in the absence of oxidation.

Keywords: *Sweet fortified wines; aromas; oxidative aging; formation mechanisms*

INTRODUCTION

Sweet fortified wines have traditionally been aged in used barrels, often partly empty, in cellars with variable temperatures. Due to their alcohol content (15–18%), yeast is not involved in the aging process of sweet fortified wines, unlike sherry or Vin Jaune from the Jura (Martin et al., 1992; Dubois et al., 1976). Oxygen is assumed to play a major role in the many chemical reactions occurring during aging. The wine is put into used barrels (20–80 years old) of various sizes (225, 660, and 1500–5000 L) to facilitate the penetration of oxygen, whereas the aromatic contribution of the oak wood was often considered relatively unimportant.

A great deal of research has already been carried out on wine aging and oxidation (Ribéreau-Gayon, 1933). Oxidation results in organoleptic modifications, generally described as “oxidized” in dry wines, “rancio” in sweet fortified red wines, and “maderized” in sweet fortified white wines (Kayser and Demolon, 1900, 1909). Berthelot (1863, 1864) was the first to report the presence of acetaldehyde and associate it with the phenomenon of oxidation. Many authors have studied the oxidation reaction of ethanol into ethanal, as well as the role of the latter in the development of the wine (Mathieu, 1905; Trillat, 1908; Laborde, 1917; Peltier, 1932). Cantarelli (1967) showed the importance of the carbonyl group of aldehydes as well as the role of amino

acids in the formation of the “rancid” odor in maderized wines. In the case of dry wines, these phenomena lead to the deterioration of the bouquet during aging. On the other hand, oxidation phenomena are generally considered to be favorable, or even indispensable, for the proper development of the aroma of sweet fortified wines.

In our research into the formation mechanisms of the molecules responsible for the characteristic aroma of old sweet fortified wines, we drew inspiration from the work of Deibner and Bernard (1956). These authors mentioned the role of Maillard reactions in the formation of the aroma of sweet fortified wines following heat treatment, but they were unable to identify the respective compounds. Our study investigated both white and red wines, as the phenolic compounds present in red wines have well-known antioxidant properties (Semichon, 1905; Laborde, 1910; Moureu and Dufraisse, 1922, 1924; Ribéreau-Gayon, 1933) and were thus likely to affect the development of the aroma during aging.

Using modern techniques, such as gas-phase chromatography combined with olfactory detection, mass spectrometry, and infrared spectrometry (Cutzach et al., 1998a,b), we have recently identified various volatile and odorous compounds found in sweet fortified wines during aging. In this work, we have studied the formation of some volatile compounds during experimental laboratory aging of sweet fortified wines. We have determined that the formation of aroma components may sometimes be affected by the presence of the phenolic compounds responsible for the color of red wines.

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MATERIALS AND METHODS

Standard Volatile Compounds. The following substances were supplied by Sigma-Aldrich Chimie (Saint Quentin Fallavier, France) with a purity of at least 97%, measured by gas-phase chromatography: ethyl levulinate, ethyl furoate, diethyl glutarate, cyclotene, γ -octalactone, maltol, 1*H*-pyrrole carboxaldehyde, pantolactone, γ -nonalactone, furaneol, sotolon, triethyl citrate, 5-(hydroxymethyl)furfural (HMF), furfural, 5-methylfurfural, and furfuryl alcohol.

Homofuraneol (sugarone) and norfuraneol were supplied by Givaudan Roure (Barneveld, The Netherlands) with a purity of 98%.

The compounds 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP), hydroxymaltol, ethoxybutyrolactone, furyl hydroxymethyl ketone, 2,5-furandicarbaldehyde, and ethoxymethylfurfural were synthesized, using the previously described methods (Cutzach et al., 1998b).

The synthesis of 5-hydroxy-2-methyl-1,3-dioxanes and 4-(hydroxymethyl)-2-methyl-1,3-dioxolanes was based on the process described by Maillard (1971). To toluene (150 mL) were added glycerol (69 g), ethanal (43 g), sodium sulfate (70 g), and *p*-toluenesulfonic acid (300 mg), and the mixture was refluxed for 10 h. The reaction was complete and all of the glycerol disappeared from the reaction mixture. After evaporation of the toluene under a stream of nitrogen, the crude residue analyzed by CPG/SM showed a 66% pure mixture of dioxanes and dioxolanes, the remainder being the residual solvent. The following dioxanes and dioxolanes were obtained: *cis*-5-hydroxy-2-methyl-1,3-dioxane (20%); *cis*-4-(hydroxymethyl)-2-methyl-1,3-dioxolane (22%); *trans*-4-(hydroxymethyl)-2-methyl-1,3-dioxolane (15.5%); and *trans*-5-hydroxy-2-methyl-1,3-dioxane (8.5%).

The method used to determine the concentrations of volatile compounds in wine was described in a previous paper (Cutzach et al., 1998b).

Assessing the Aromatic Impact of a Substance. The contribution of a volatile compound to the aroma of a wine was estimated according to its aromatic index: $I = c/s$, where c was the concentration of the substance in the wine considered and s its perception threshold, determined in a synthetic standard solution. The perception thresholds of the various molecules considered were determined in previous work (Cutzach et al., 1998b; Chatonnet, 1995). A molecule was considered to participate actively in a wine's aroma if its aromatic index I was at least 1.

Laboratory Conditions for the Accelerated Aging of Sweet Fortified Wines. The use of controlled conditions in the laboratory makes it possible to accelerate the oxidation–reduction processes that occur slowly under normal conditions during the aging of these wines. A young (1996) sweet fortified white wine, made from White Grenache and Macabeu, and a young (1995) sweet fortified red wine, made from Black Grenache, were placed in an incubator at 37 ± 2 °C.

Accelerated Aging of a Young Sweet Fortified White Wine in the Presence of Oxygen. The samples were aged in the presence of oxygen. The wine (400 mL) was distributed among six 750 mL bottles, leaving some space at the top. The bottles were stoppered with nonairtight corks. Each bottle was kept in the incubator for a precise period of time. Samples were analyzed every 3 months. Sensory analysis was carried out systematically, as well as by measuring the concentrations of the volatile compounds.

Accelerated Aging of a Young Sweet Fortified White Wine in the Absence of Oxygen. The same wine was placed in glass ampules (3×400 mL), sealed to ensure the absence of oxygen, placed in the incubator at 37 °C for 12 months, and analyzed as above.

Accelerated Aging of a Young Sweet Fortified Red Wine in the Presence and in the Absence of Oxygen. The experimental procedure was identical to that described for the white wines.

RESULTS

The initial characteristic aroma of a young sweet fortified white wine subjected to accelerated aging in the presence of oxygen changed considerably over time. On aging at 37 °C, it gradually developed increasingly complex aromas, more and more like those of old sweet fortified white wines. After 1 year, the aroma of the wines aged with oxidation was similar to that of a sweet fortified wine >6 years old, with nuances of nuts, dried apricots, jam, and roast coffee (Torres et al., 1991; Cutzach et al., 1998a,b). The same wine aged in the absence of oxygen developed differently. The nutty nuances were less intense, the wine was not very forthcoming, and the aroma was closed, almost reduced, unlike the normal character of an old sweet fortified wine.

The sweet fortified red wine, aged under the same conditions for 6 months, acquired a prune aroma in the presence of oxygen and reduction characteristics in the absence of air. After 6 months of accelerated aging, most of the volatile compounds present in both the red and white wines increased. However, there were some differences in the case of compounds that require large-scale oxidation (Tables 1–3).

The compounds characteristic of the volatile fraction of sweet fortified wines aged in the incubator were divided into five categories, according to the effect of oxidation and grape variety on their development.

The first included ethyl furoate, maltol, sugarone, γ -nonalactone, pantolactone, and γ -octalactone. These compounds increased little during aging in the incubator. Their formation did not seem to be affected either by oxidation or by the color of the wine.

The second category included furfural and norfuraneol, which vary only due to oxidation. In both the white and red wines aged in the incubator, the increase in furfural was almost twice as large in the presence of oxygen. Oxygen had the opposite effect on norfuraneol.

The formation of the following compounds in the third category, that is, furyl hydroxymethyl ketone, Furaneol, and dihydromaltol, depended only on the color (red or white). Sweet fortified white wine subjected to accelerated aging had the highest concentrations of furyl hydroxymethyl ketone and Furaneol. Furaneol disappeared from the red wine after 6 months in the incubator. On the other hand, dihydromaltol would seem to be a typical molecule in sweet fortified red wines aged in the incubator, where it was found in greater quantities than in white wines, irrespective of the aging conditions.

Those molecules for which formation was affected both by the color of the grape variety and by the presence of oxygen were placed in the last two categories.

The fourth category includes those volatile compounds that were formed in larger quantities in red wine subjected to accelerated aging. HMF and acetylformoin always increased more in red wines aged in the incubator than in white wines. The increase was even more marked in red wines aged without oxygen. On the other hand, the hydroxymaltol content in red wines was always higher in the presence of oxygen.

Finally, the fifth, and largest, category includes those compounds present in much higher concentrations in the white wines aged in the incubator and affected by oxidation. Oxygen promotes the formation of 5-methylfurfural, 2,5-furandicarbaldehyde, ethyl levulinate, di-

Table 1. Evolution of Volatile Compounds of a Red Sweet Natural Wine during Various Conditions of Aging

volatile compound ($\mu\text{g/L}$)	wine storage				
	0 months	with oxygen		without oxygen	
		3 months	6 months	3 months	6 months
furan derivatives					
furfural	2000	14440	21736	9598	11993
5-methylfurfural	34	215	240	226	143
(hydroxymethyl)furfural (HMF)	5000	74240	99478	49037	149131
5-(ethoxymethyl)furfural	4	18	26	23	26
2,5-furandicarbaldehyde	50	130	202	93	160
esters					
ethyl levulinate	18	59	58	39	32
ethyl furoate	10	23	22	22	19
diethyl glutarate	22	58	44	56	38
enolic derivatives					
dihydromaltol ^a	4	34	18	52	15
maltol	18	50	46	61	23
cyclotene	2	4	3	5	2
acetylformoine ^b	72	128	280	226	444
Furaneol	29	34	0	51	0
homofuraneol	5	3	1.6	3	0.86
norfuranol	110	92	86	580	347
sotolon	5	50	120	13	12
DDMP ^c	3150	8844	5040	13172	5040
hydroxymaltol	240	4409	6160	2366	628
lactones					
γ -nonalactone	53	68	50	79	44
pantolactone	498	320	513	746	553
γ -octalactone	1.5	3	1.4	3	2
ethoxybutyrolactone	55	987	711	177	99
aldehydes and ketones					
furyl hydroxymethyl ketone	102	431	661	770	639
1 <i>H</i> -pyrrolecarboxaldehyde	7	38	53	25	25
dioxanes and dioxolanes					
<i>cis</i> -5-hydroxy-2-methyl-1,3-dioxane	1500	12096	14298	1582	2690
<i>cis</i> -4(hydroxymethyl)-2-methyl-1,3-dioxolane	175	1915	3113	221	431
<i>trans</i> -4(hydroxymethyl)-2-methyl-1,3-dioxolane	110	1276	2255	127	186
<i>trans</i> -5-hydroxy-2-methyl-1,3-dioxane	316	2375	2913	322	596

^a Maltol equivalent. ^b Furaneol equivalent. ^c 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one. Boldface: concentration higher than perception threshold.

ethyl glutarate, cyclotene, sotolon, DDMP, ethoxybutyrolactone, and 1*H*-pyrrolecarboxaldehyde, as well as dioxanes and dioxolanes. On the other hand, there is always a greater increase in (ethoxymethyl)furfural in the absence of oxygen.

DISCUSSION

The amounts of almost all of the components analyzed in this work, identified as characteristic of sweet fortified wines (Cutzach et al., 1998a,b), increased during the accelerated aging process. The formation of some of these compounds was clearly affected by oxidation and wine color.

Formation Independent of Color and Oxidation.

Ethyl furoate, maltol, and homofuraneol arise from Maillard reactions. Ethyl furoate is certainly produced by the esterification reaction between ethanol and furoic acid. This acid, produced by Maillard reactions (Vernin et al., 1982), does not require the presence of oxygen for its formation. It was, therefore, confirmed that its ester should be formed independently of oxidation. Maltol, also produced by Maillard reactions, develops irrespective of color and oxidation. The same is true of homofuraneol and sugarone [2-ethyl-5-methyl-4-hydroxy-3(2*H*)-furanone], which are derived by Maillard reactions from phosphorylated pentoses (Blank et al., 1996).

The other three compounds that were formed independently of grape color and oxidation were lactones. Pantolactone, or dihydro-4,4-dimethyl-3-hydroxy-2(3*H*)-furanone, identified and isolated in Californian and

Spanish sheries (Webb et al., 1967), is formed during alcoholic fermentation due to the action of a reductase produced by yeast acting on a ketopantoyl lactone (King et al., 1972) (Figure 1).

The nutty aroma characteristic of relatively old sweet fortified red wines was associated with γ -octalactone and γ -nonalactone (Cutzach et al., 1998a). However, in view of their perception threshold, they would not seem to have any direct influence on the aroma of sweet fortified red wines.

Formation of Compounds due to Oxidation.

Furfural may be produced by the breakdown of pentoses and/or Maillard reactions. However, concentrations were always higher in sweet fortified wines subjected to accelerated aging in the presence of oxygen. This indicated that the breakdown of sugars, activated by oxygen, was the most prevalent way in which furfural was formed in sweet fortified wines.

On the other hand, the norfuranol [4-hydroxy-5-methyl-3(2*H*)-furanone] content of the wines decreased in an oxidized environment. This molecule may come from ribose-5-phosphate derived from ribonucleotides (Tonsbeek et al., 1969). The oxidation breakdown products of this furanone are not known.

Formation of Compounds Affected Only by Grape Color.

Furyl hydroxymethyl ketone, a molecule with very little odor, may be formed by the breakdown of glucose or fructose (Feather and Harris, 1973; Popoff, 1976; Cutzach et al., 1998) (Figure 2) and also following Maillard reactions involving systems such as fructose-

Table 2. Evolution of Volatile Compounds of a White Sweet Natural Wine during Various Conditions of Aging

volatile compound ($\mu\text{g/L}$)	wine storage							
	0 months	with oxygen				without oxygen		
		3 months	6 months	12 months	3 months	6 months	12 months	
furan derivatives	897	5000	9000	27000	4000	6000	25000	
furfural	18	81	233	704	53	97	1100	
5-methylfurfural	261	301	889	505	330	465	576	
(hydroxymethyl)furfural (HMF)	3600	9000	39000	24000	14000	39000	54000	
5-ethoxymethylfurfural	1	12	28	100	28	100	430	
2,5-furandicarbaldehyde	16	45	186	363	26	85	645	
esters								
ethyl levulinate	5	37	56	183	12.5	17	89	
ethyl furoate	5	13	16	56	15	19	54	
diethyl glutarate	12	55	70	119	34	43	67	
enolic derivatives								
dihydromaltol ^a	13	2	25	57	3	15	123	
maltol	6	8	14	37	14	13	42	
cycloten	0.4	0.9	3	21	0.8	1.3	26	
acetylformoine ^b	70	173	185	173	148	143	518	
Furaneol	623	384	194	35	257	201	62	
homofuraneol	9	0.3	1	0	6	8	2	
norfuranol	166	6	52	13	318	322	212	
sotolon	4	75	157	207	15	17	4	
DDMP ^c	2300	1800	10000	3000	2600	3500	3800	
hydroxymaltol	900	320	2000	82	150	700	63	
lactones								
γ -nonlactone	24	23	26	16	22	22	8	
pantolactone	366	539	620	943	145	413	812	
γ -octalactone	1.3	2.5	1.5	2	1.7	1.5	0.4	
ethoxybutyrolactone	32	542	1587	1498	69	169	107	
aldehydes and ketones								
furyl hydroxymethyl ketone	41	191	437	815	273	490	2010	
1 <i>H</i> -pyrrolecarboxaldehyde	1	10	280	38	6	15	44	
dioxanes and dioxolanes								
<i>cis</i> -5-hydroxy-2-methyl-1,3-dioxane	700	15000	24000	48000	1500	1800	4000	
<i>cis</i> -4-(hydroxymethyl)-2-methyl-1,3-dioxolane	100	3700	5500	10000	300	370	800	
<i>trans</i> -4-(hydroxymethyl)-2-methyl-1,3-dioxolane	200	2500	3700	6500	200	230	500	
<i>trans</i> -5-hydroxy-2-methyl-1,3-dioxane	180	3700	5400	11000	450	500	930	

^a Maltol equivalent. ^b Furaneol equivalent. ^c 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one. Boldface: concentration higher than perception threshold.

alanine or fructose- γ -aminobutyric acid (Vernin et al., 1982). The increase in furyl hydroxymethyl ketone in sweet fortified white wine aged in the incubator was twice as great as that in sweet fortified red wine.

Furaneol [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] was a characteristic molecule of white wines aged in the incubator, as it disappeared from red wines after 6 months of aging. This molecule may be formed from pentoses or hexoses by Maillard reactions (Blank et al., 1996), but also by the breakdown of DDMP produced from hexoses (Kim et al., 1996). Furaneol may also be converted into acetylformoine (Figure 3).

On the other hand, in sweet fortified red wine aged in the incubator, the increase in dihydromaltol was over twice that in white wines. Dihydromaltol is partially broken down in sweet fortified white wines in the presence of oxygen and seems to be protected from oxidation in red wines.

Formation of Compounds Affected by Grape Color and Oxidation. *Sweet Fortified Red Wines.* The heat breakdown of pentoses and hexoses (Hurrel, 1982) and Maillard reactions produced the odorless compound 5-(hydroxymethyl)furfural (HMF). This molecule seems to be stable in the absence of oxygen in sweet fortified red wines subjected to accelerated aging. In the presence of oxygen, HMF may produce 5-methylfurfural and 2,5-furandicarbaldehyde, following a dehydration reaction facilitated by the acidic environment (Baltes, 1988) (Figure 4).

Acetylformoin [2,4-dihydroxy-2,5-dimethyl-3(2*H*)-furanone] was produced from pentoses or hexoses by Mail-

lard reactions (Blank et al., 1996) and also by the breakdown of DDMP from the hexoses (Kim et al., 1996). It formed more easily in sweet fortified red wines aged in the incubator in the absence of air, where it seemed to be stable. It was particularly sensitive to oxidizing breakdown reactions. Furthermore, the development of this molecule would seem to be opposite from that of Furaneol in sweet fortified wines subjected to accelerated aging (Figure 3).

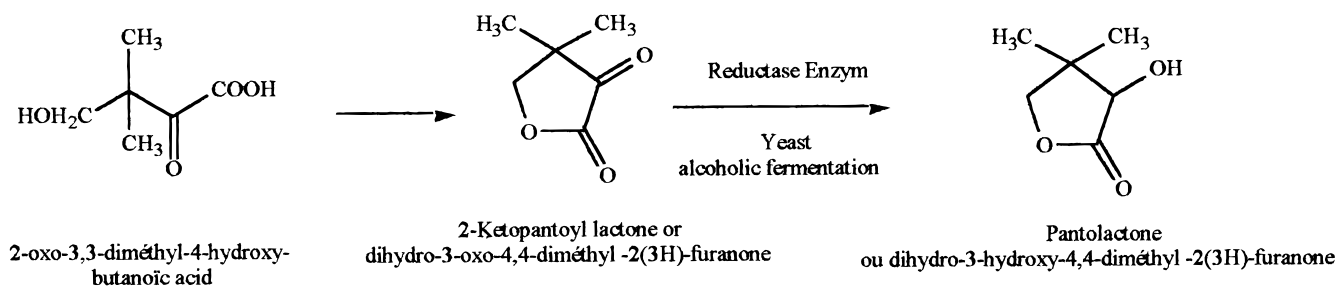
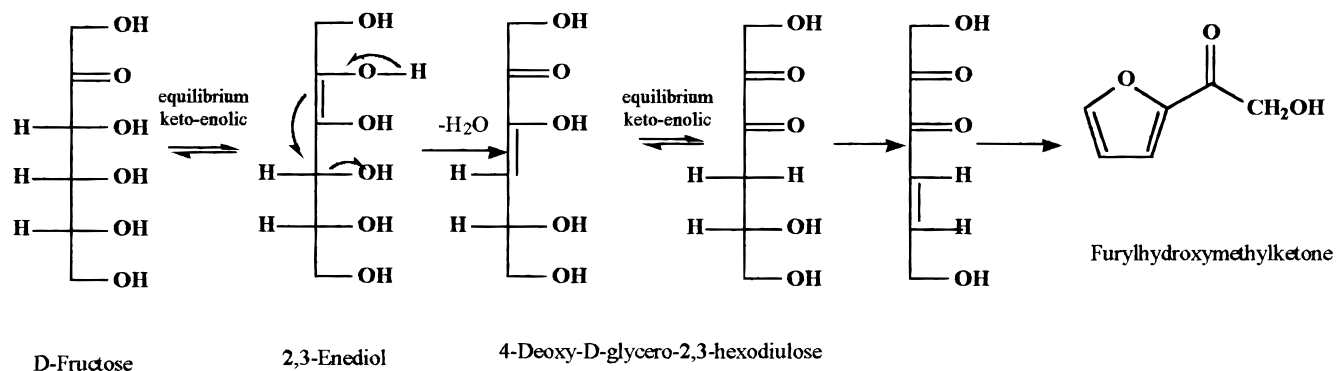
On the other hand, greater quantities of hydroxymaltol were formed in sweet fortified red wines aged in the incubator in the presence of oxygen (Table 3).

Sweet Fortified Red Wines. In sweet fortified white wines aged in the incubator, only 5-(ethoxymethyl)furfural developed in the absence of air. HMF reacted with ethanol to form 5-(ethoxymethyl)furfural (Figure 5). Dehydration was catalyzed in the acid environment. Temperature played a major role in determining the outcome of the reaction. In the previous work, the oldest wines analyzed were reds that had been aged in bottles (without oxygen) for many years. As more 5-(ethoxymethyl)furfural accumulated in wines aged in the incubator in the absence of air, it was logical that this molecule should be found in higher concentrations in older sweet fortified red wines than in the young sweet fortified red wines. In the sweet fortified white wines aged in the incubator, the quantities found were above the perception threshold (Cutzach et al., 1998b) after 1 year of aging in the presence of oxygen and after 6 months in the absence of air.

Table 3. Comparison of Increased Concentration Factors of Volatile Compounds between White and Red Sweet Natural Wines during Accelerated Aging

volatile compound ($\mu\text{g/L}$)	with oxygen		without oxygen	
	white wine, 6 months	red wine, 6 months	white wine, 6 months	red wine, 6 months
furan derivatives				
furfural	10	11	7	6
5-methylfurfural	13	7	5	4
(hydroxymethyl)furfural (HMF)	11	20	11	30
5-ethoxymethylfurfural	28	6	100	6
2,5-furandicarbaldehyde	11	4	5	3
esters				
ethyl levulinate	11	3	3	1.8
ethyl furoate	3.2	2.2	3.8	1.9
diethyl glutarate	6	2	3	1.7
enolic derivatives				
dihydromaltol ^a	2	4.5	1.1	3.75
maltol	2.3	2.5	2.16	1.3
cycloten	7.5	1.5	3.25	1
acetylformoine ^b	2.6	3.9	2	6.1
Furaneol	0.31	very small	0.32	very small
homofuraneol	0.11	0.32	0.88	0.172
norfuranol	0.31	0.78	2.86	3.15
sotolon	39	24	4	2
DDMP ^c	4	1.6	1.5	1.6
hydroxymaltol	2	26	0.77	2.6
lactones				
γ -nonalactone	1.1	0.94	0.92	0.83
pantolactone	1.69	1.03	1.1	1.11
γ -octalactone	1.15	0.93	1.15	1.33
ethoxybutyrolactone	49	13	5	1.8
aldehydes and ketones				
furyl hydroxymethyl ketone	11	6.5	12	6.3
1 <i>H</i> -pyrrolecarboxaldehyde	280	7.6	15	3.6
dioxanes and dioxolanes				
<i>cis</i> -5-hydroxy-2-methyl-1,3-dioxane	34	9	2.6	1.8
<i>cis</i> -4(hydroxymethyl)-2-methyl-1,3-dioxolane	55	18	3.7	2.5
<i>trans</i> -4(hydroxymethyl)-2-methyl-1,3-dioxolane	18	20	1.15	1.7
<i>trans</i> -5-hydroxy-2-methyl-1,3-dioxane	30	9	2.8	1.9

^a Maltol equivalent. ^b Furaneol equivalent. ^c 2,3-Dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one.

**Figure 1.** Possible formation pathway of pantolactone.**Figure 2.** Possible formation of furyl hydroxymethyl ketone from the degradation of D-fructose (Feather and Harris, 1973).

On the other hand, 2,5-furandicarbaldehyde showed a more marked increase in sweet fortified white wines subjected to accelerated aging in the presence of oxygen. This compound has little odor and is produced mainly

by Maillard reactions (Baltes et al., 1988) but may also derive from the dehydration of HMF in an acid environment.

The formation of ethyl levulinate and diethyl glut-

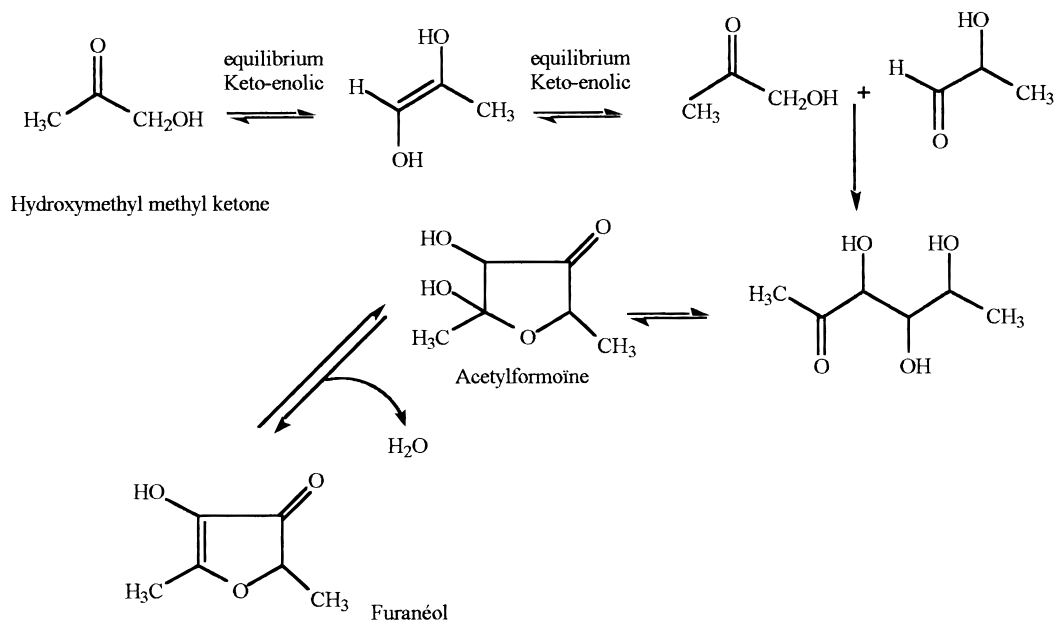


Figure 3. Possible formation pathway for acetylformoine and Furaneol (Kim et al., 1996).

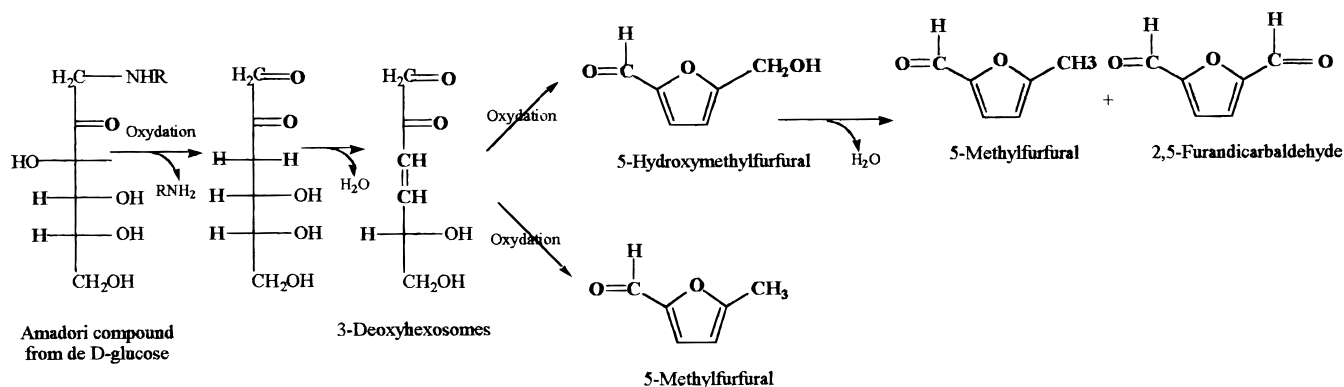


Figure 4. Possible formation and degradation of HMF (Baltes, 1988).

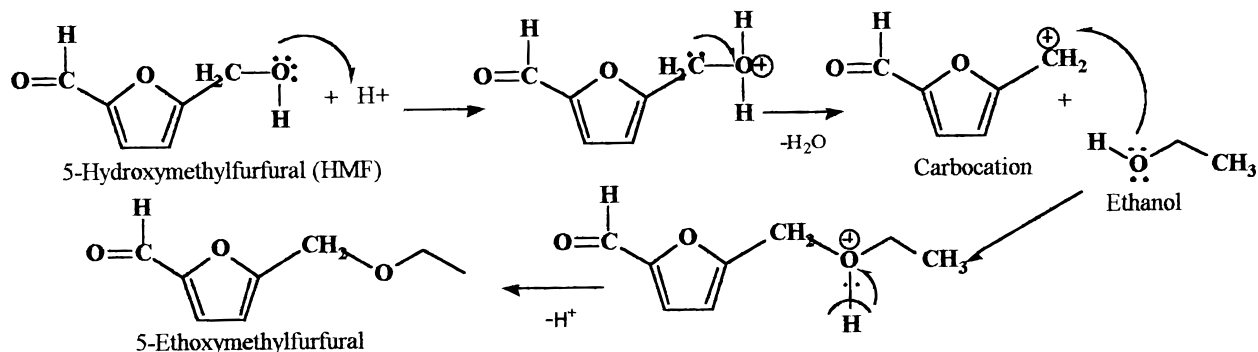


Figure 5. Possible formation of 5-ethoxymethylfurfural from HMF.

arate seems to be promoted in sweet fortified white wines aged in the incubator in the presence of oxygen. Ethyl levulinate is formed from levulinic acid and ethanol. Levulinic acid may be formed by the heat breakdown of D-glucose, 2-deoxy-D-erythro-pentose, furfuryl alcohol (Shu et al., 1995) (Figure 6), or HMF (Feather and Harris, 1973) in an acid environment. Levulinic acid, as well as HMF and furyl hydroxymethyl ketone, was the main compound resulting from the heat breakdown of sugars (Popoff, 1976; Hodge, 1967). Ethyl glutarate may result from the esterification reaction between ethanol and pentanedioic acid. The latter may

also be formed when sugars are broken down by heat in the presence of oxygen.

Cyclotene (2-hydroxy-3-methyl-2-cyclopenten-1-one) was formed by Maillard reactions and may also derive from lactic aldehyde (Baltes, 1988) formed from D-fructose (Baltes, 1988) or DDMP (Kim et al., 1996). The formation of cyclotene in sweet fortified white wine starts slowly, reaching its maximum value after 12 months of aging in the incubator (Table 2). The presence of oxygen seems to have an influence on the quantities formed. In this case, cyclotene was mainly formed from

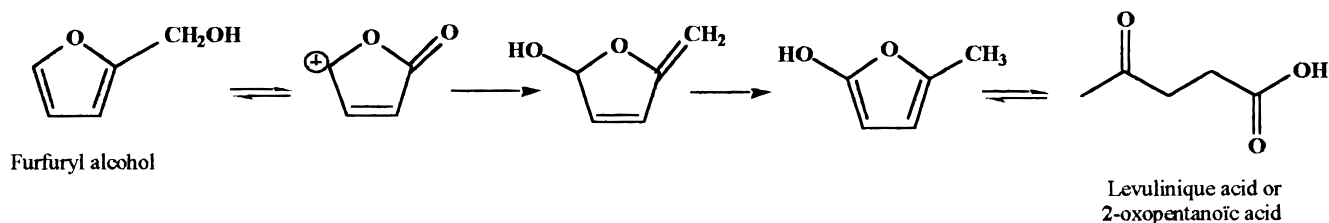


Figure 6. Possible formation of levulinic acid from furfuryl alcohol (Feather and Harris, 1973).

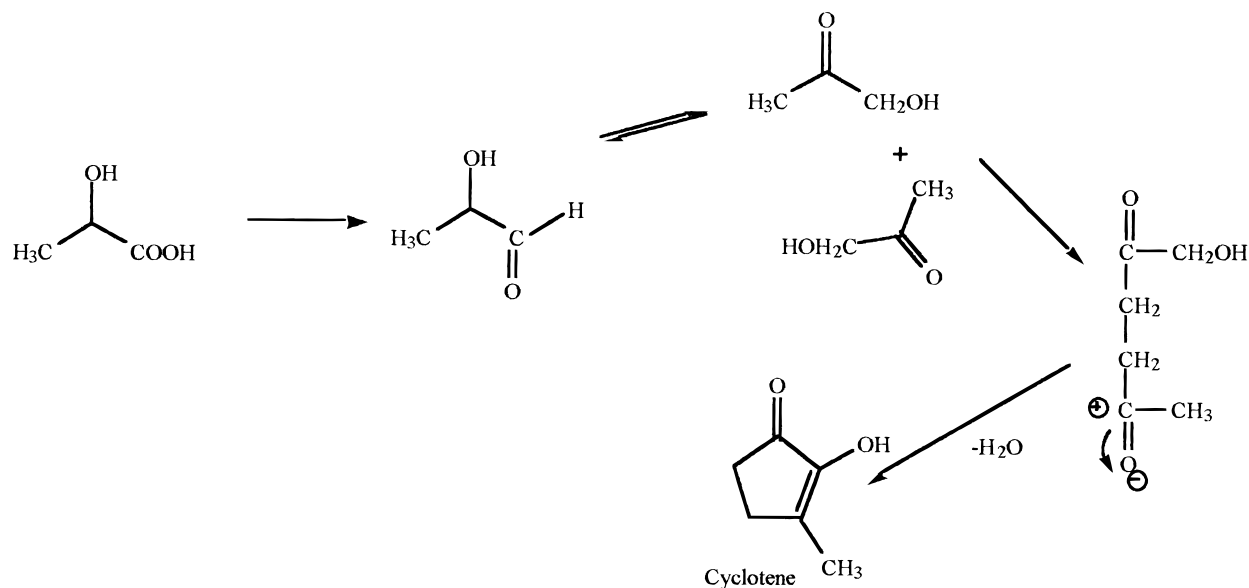


Figure 7. Possible formation of cyclotene from lactic acid (Baltes, 1988).

lactic aldehyde (Figure 7), resulting from oxidation and/or the breakdown of fructose.

The formation kinetics of DDMP were very different from those of hydroxymaltol. The DDMP content of sweet fortified white wines subjected to accelerated aging continued to increase, whereas the concentration of hydroxymaltol decreased. The reaction mechanisms for the formation of these products were described by Tressl et al., (1978). DDMP is well-known as a major product of Maillard reactions involving glucose and fructose (Nishibori et al., 1993, 1994; Kim et al., 1996). There was a greater increase in DDMP in sweet fortified white wines aged in the incubator, whereas the greatest accumulation of hydroxymaltol occurred in red wines in the presence of oxygen (Table 3).

The ethoxybutyrolactone content of sweet fortified wines increased during accelerated aging, whatever the conditions. However, the increase was always more marked in the presence of oxygen and in white wine (Table 3).

The presence of oxygen promoted the formation of ethoxybutyrolactone from the precursor, ethyl 4-oxobutyrate, formed during alcoholic fermentation (Muller et al., 1972). In an oxidizing environment, ethyl 4-oxobutyrate oxidizes into a highly unstable hemiacetal ester, which is rapidly converted into ethoxybutyrolactone (Figure 8).

The concentration of sotolon [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone] increased in a linear manner over time during aging. After 12 months, it reached a value >20 times higher than its perception threshold in sweet fortified white wine subjected to accelerated aging (Table 3). The concentrations measured in an oxygenated environment were clearly higher than those meas-

ured in the absence of air in sweet fortified white wines. After 6 months of aging, the increase factor for sotolon in sweet fortified white wine was 39, whereas it reached only 24 in sweet fortified red wine. It seems, therefore, that the presence of antioxidant polyphenolic compounds, which combine preferentially with acetaldehyde in an acid environment, slow the formation of sotolon in red wines to a considerable extent.

This highly odorous molecule is formed chemically by the aldol condensation of α -ketobutyric acid (Figure 9) produced from threonine, with acetaldehyde resulting from the oxidation of ethanol (Sulser et al., 1967; Pham et al., 1995). During oxidative aging, ethanol is converted into acetaldehyde (Cantarelli, 1967; Wildenrad et al., 1974), thus allowing the formation of sotolon. It is important to note that the sweet fortified wines were always aged in an oxidizing environment without a yeast "flor".

Finally, increases in the dioxane and dioxolane contents were also accentuated in sweet fortified white wines aged in the incubator in the presence of oxygen. Concentrations of these compounds in wine are known to increase with age (Muller et al., 1978; Williams et al., 1978). The *cis*- and *trans*-5-hydroxy-2-methyl-1,3-dioxane and *cis*- and *trans*-4-hydroxymethyl-2-methyl-1,3-dioxolane contents increased during the aging of both red and white sweet fortified wines, particularly in the presence of oxygen. However, the concentrations measured in sweet fortified red wines were clearly lower than those in sweet fortified white wines.

Dioxanes and dioxolanes are produced by the reaction of acetaldehyde with glycerol. During aging, under the influence of strong oxidation, ethanol is converted into acetaldehyde, which is required for the formation of

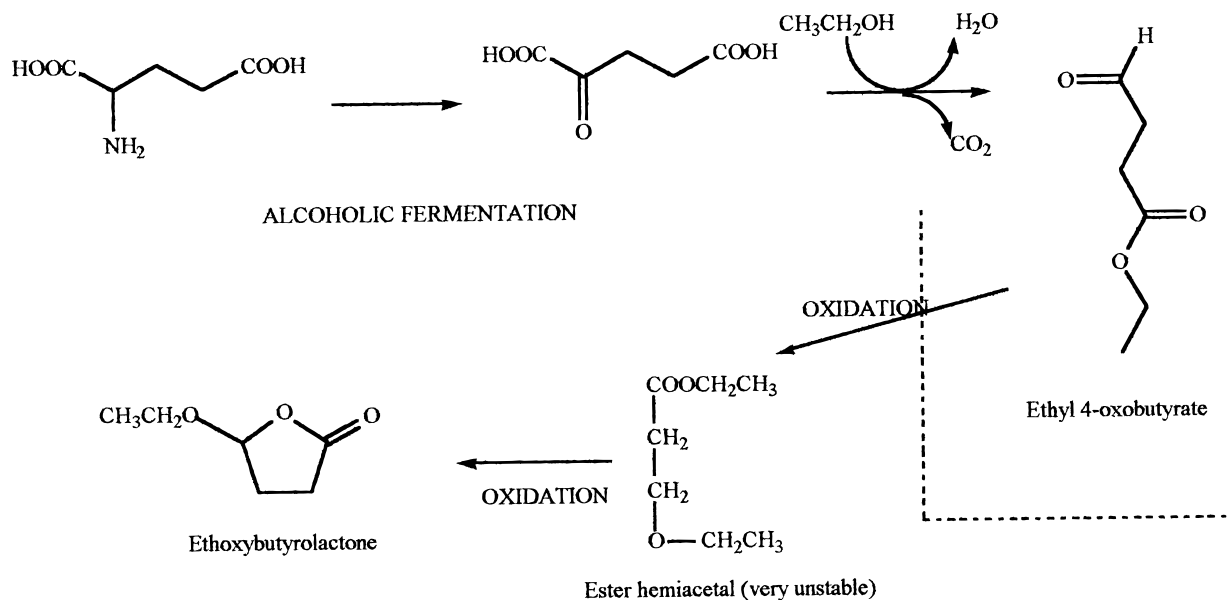


Figure 8. Possible formation of ethoxybutyrolactone from 4-oxobutyrate (Webb et al., 1967).

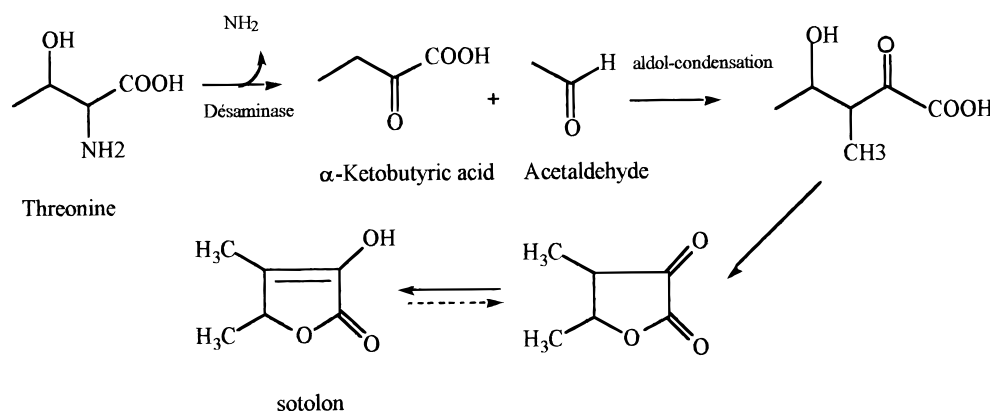


Figure 9. Formation of sotolon from α-ketobutyric acid (Pham et al., 1995).

dioxanes and dioxolanes (Muller et al., 1978; Williams et al., 1978). The presence of polyphenolic compounds was, once again, responsible for the low dioxane and dioxolane content in sweet fortified red wines, as they slowed the oxidation reactions and combined easily with acetaldehyde.

Unlike those analyzed by Ferreira et al. (1997), these compounds, analyzed by gas-phase chromatography coupled with olfactory detection or added to wines, do not have any particular odor. They are chemical markers for oxidative aging but have no effect on aroma or flavor.

CONCLUSION

Accelerated aging tests, conducted in an incubator in the presence or absence of air, make it easier to study the formation of the various characteristic molecules in the volatile fraction of red and white sweet fortified wines. Many of the molecules studied were formed via Maillard reactions. The average temperatures and the high quantity of sugars facilitated the triggering of this type of reaction, which does not require the presence of oxygen. The formation of some molecules resulting from Maillard reactions was unaffected by air or grape color.

The formation of other compounds, on the other hand, may be considerably affected (positively or negatively) by oxidation and wine color.

For the first time, by measuring the concentrations of several volatile and odorous compounds, this work provides an objective illustration of the differences in the development of the aromas of red and white wines during accelerated aging.

We have highlighted the characteristic molecules in red and white sweet fortified wines. For example, sotolon, a rare molecule always present in quantities above its perception threshold, was typical of the oxidation of sweet fortified red wines aged in the incubator. Similarly, the furyl hydroxymethyl ketone content was always higher in sweet fortified red wines subjected to accelerated aging. Variations in concentrations of this molecule seem to be solely dependent on wine color. On the other hand, HMF, always more abundant in sweet fortified red wines, was formed continuously from hexoses by Maillard reactions. It has no noticeable aroma in wine. On the other hand, when it breaks down due to oxidation, the 5-(ethoxymethyl)-furfural formed is a typical component of the aroma developed by sweet fortified white wines during aging.

After aging for the same length of time, sweet fortified red wines were definitely much less oxidized than sweet fortified white wines. Differences in the development and concentrations of volatile and odorous substances were clearly affected by the presence of wine poly-

phenols, due to their antioxidant properties and their capacity to react with some aldehydes.

The development of compounds characteristic of the volatile fraction of sweet fortified wines produced and aged under normal wine-making conditions will be examined in a later study.

LITERATURE CITED

- Baltes, W. Investigation on thermal aroma formation. In *Frontiers of Flavor*, Proceedings of the 5th International Flavor Conference, Porto Karras, Chalkidiki, Greece, July 1–3, 1987; Elsevier Science Publishers: Amsterdam, The Netherlands, 1988; pp 575–584.
- Baltes, W.; Mevissen, L. Model reactions on roast aroma formation. VI. Volatile reaction products from the reaction of phenylalanine with glucose during cooking and roasting. *Z. Lebensm. Unters. Forsch.* **1988**, *187*, 209–214.
- Berthelot. Action de l'oxygène sur les vins. *C. R. Acad. Sci.* **1863**, *57*, 398, 795, 963.
- Berthelot. Action de l'oxygène sur les vins. *C. R. Acad. Sci.* **1864**, *57*, 80, 292.
- Blank, I.; Fay, L. B. Formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 4-hydroxy-2(or 5)-ethyl-5(or 2)-methyl-3(2H)-furanone through Maillard reaction based on pentose sugars. *J. Agric. Food Chem.* **1996**, *44*, 531–536.
- Cantarelli, C. Etude de la madérisation et de sa prévention dans les vins blancs. Fermentations et vinifications. *2nd Symposium International d'Oenologie*, Bordeaux-Cognac; 1967; pp 391–407.
- Chatonnet, P. Incidences du bois de chêne sur la composition chimique et les qualités organoleptiques des vins—Applications technologiques. Thèse de D.E.R., Université de Bordeaux II, 1991, No. 2, 224 pp.
- Chatonnet, P. Influence des procédés de tonnellerie et des conditions d'élevage sur la composition et la qualité des vins élevés en fûts de chêne. Thèse de Doctorat, Université de Bordeaux II, 1995, No. 338, 268 pp.
- Cutzach, I.; Chatonnet, P.; Henry, R.; Dubourdieu, D. Identification of volatile compounds with a "toasty" aroma in heated oak used in barrelmaking. *J. Agric. Food Chem.* **1997**, *45*, 2217–2224.
- Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Etude de l'arôme des vins doux naturels non muscatés. 1^{ère} Partie: Analyse qualitative de l'arôme des vins doux naturels de Banyuls et Rivesaltes rencontré au cours de leur vieillissement. *J. Int. Sci. Vigne Vin.* **1998a**, *32* (2), 99–110.
- Cutzach, I.; Chatonnet, P.; Henry, R.; Pons, M.; Dubourdieu, D. Etude de l'arôme des vins doux naturels non muscatés. 2^{ème} Partie: Dosage de certains composés volatils intervenant dans l'arôme des vins doux naturels au cours de leur vieillissement. *J. Int. Sci. Vigne Vin.* **1998b**, *34* (2), 211–221.
- Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Rôle du sotolon dans l'arôme des vins doux naturels. Influence des conditions d'élevage et de vieillissement. *J. Int. Sci. Vigne Vin.* **1998c**, *34* (2), 223–233.
- Cutzach, I.; Chatonnet, P.; Henry, R.; Dubourdieu, D. Identifying new volatile compounds in toasted oak. *J. Agric. Food Chem.* **1999**, in press.
- Deibner, L.; Benard, P. Recherche sur la maturation des vins doux naturels. I. Influence du traitement thermique prolongé à l'abri de l'air. *Ann. Technol.* **1956**, *3*, 357–376.
- Dubois, P.; Rigaud, J.; Dekimpe, J. Identification de la diméthyl-4,5 tetrahydrofuranedione-2,3 dans le vin Jaune du Jura. *Lebensm. Wiss. Technol.* **1976**, *9*, 366–368.
- Feather, M. S.; Harris, J. F. Dehydration reactions of carbohydrates. *Adv. Carbohydr. Chem. Biochem.* **1973**, *28*, 161–224.
- Ferreira, A. C. S.; Barbe, J. C.; Bertrand, A. Heterocycles acetals from glycerol and ethanal in port wine. Evolution with oxidative aging. In *Application de la Chimie Analytique aux Vins et Spiritueux*, 1st Symposium In vino analytica scientia; Bordeaux, 1997; pp 411–414.
- Hodge, J. E. Origin of flavor in food: nonenzymatic browning reactions. In *The Chemistry and Physiology of Flavor*; Schultz, H. W., Ed.; AVI Publishing: Westport, CT, 1967; Vol. 22, pp 465–485.
- Hurrell, R. F. Maillard reaction. In *Food Flavours*; Morton, I. D., Macleod, A. J., Eds.; Elsevier Scientific Publishers: New York, 1982; Part A, pp 399–438.
- Kayser, Demelon. Influence de l'aération sur la formation des produits volatils dans la fermentation alcoolique. *C. R. Acad. Sci.* **1900**.
- Kayser, Demelon. Influence de l'aération sur la formation des produits volatils dans la fermentation alcoolique. *Rev. Vitic.* **1909**, *1*, 61.
- Kim, M. O.; Baltes, W. On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one in the Maillard reaction. *J. Agric. Food Chem.* **1996**, *44*, 282–289.
- King, H.; Wilken, D. R. Separation and preliminary studies on 2-ketopantoyl lactone and 2-ketopantoic acid reductases of yeast. *J. Biol. Chem.* **1972**, *247* (12), 4096–4098.
- Laborde. Etude sur les matières tannoïdes du vin, matière colorante et oenotannin. *Rev. Vitic.* **1910**, *1* (33), 206–211, 238–242.
- Laborde. Contribution à l'étude des aldéhydes du vin. *Ann. Inst. Pasteur* **1917**, *30*, 1, 5.
- Maillard, B. Additions radicalaires de diols et de leurs dérivés: diesters et acétates cycliques. Thèse de troisième cycle, Université de Bordeaux I, 1971, p 165.
- Martin, B.; Etievant, P. X.; Le Quere, J. L.; Schlich, P. More clues about sensory impact of sotolon in some flor sherry wines. *J. Agric. Food Chem.* **1992**, *40*, 475–478.
- Mathieu. L'oxidation spontanée de l'alcool éthylique. *Bull. Assoc. Chim. Sucrierie Distillerie* **1905**.
- Moureu, Dufraisse. Sur le mécanisme de l'action antioxygène. *Bull. Soc. Chim. Fr.* **1922**, 224.
- Moureu, Dufraisse. Sur le mécanisme de l'action antioxygène. *Bull. Soc. Chim. Fr.* **1924**, 424.
- Muller, C. J.; Kepner, R. E.; Webb, A. D. Identification of 4-ethoxy-4-hydroxybutyric acid γ -lactone [5-ethoxydihydro-2(3H)] as an aroma component of wine from *Vitis vinifera* var. Ruby cabernet. *J. Agric. Food Chem.* **1972**, *20*, 193–195.
- Muller, C. J.; Kepner, R. E.; Webb, A. D. 1,3-Dioxanes and 1,3-dioxolanes as constituents of the acetal fraction of spanish fino sherry. *Am. J. Enol. Vitic.* **1978**, *29* (3), 207–212.
- Nishibori, S.; Bernhard, R. A. Model system for cookies: volatile components formed from the reaction of sugar and β -alanine. *J. Agric. Food Chem.* **1993**, *41*, 2374–2377.
- Nishibori, S.; Kawakiski, S. Formation of 2,3-dihydroxy-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one from fructose and β -alanine under conditions used for baking. *J. Agric. Food Chem.* **1994**, *42*, 1080–1084.
- Peltier, M. L'aldéhyde, sa formation et sa disparition dans la fermentation alcoolique. *Ann. Brass. Distillerie* **1932**, *30*, 79–80.
- Pham, T. T.; Guichard, E.; Schlich, P.; Charpentier, C. Optimal conditions for the formation of sotolon from α -ketobutyric acid in the French "Vin Jaune". *J. Agric. Food Chem.* **1995**, *43*, 2616–2619.
- Popoff, T.; Theander, O. Formation of aromatic compounds from carbohydrates. Part III. Reaction of D-glucose and D-fructose in slightly, aqueous solution. *Acta Chem. Scand.* **1976**, *30* (5), 397–402.
- Ribereau-Gayon, J. Contribution à l'étude des oxidations et réductions dans les vins. *Application à l'étude du Vieillissement et des Cassettes*; 2nd ed.; Delmas: Bordeaux, France, 1933.
- Semichon, L. *Traité des Maladies des Vins*; Masson: Paris, 1905; p 654.
- Shu, C.; Lawrence, B. M. Formation of 4-alkoxy- γ -valerolactones from levulinic acid and alcohols during storage at room temperature. *J. Agric. Food Chem.* **1995**, *43*, 782–784.
- Sulser, H.; Depizzol, J.; Büchi, W. A probable flavoring principle in vegetable-protein hydrolysates. *J. Food Sci.* **1967**, *32*, 611–615.

- Tonsbeek, C. H. T.; Koenders, E. B.; Van Der Zijden, A. S. M.; Losekoot, J. A. Components contributing to beef flavor. Fortified precursors of 4-hydroxy-5-methyl-3(2H)-furanone. *J. Agric. Food Chem.* **1969**, *17*, 397–400.
- Torres, P.; Brugirard, A.; Fanet, J.; Seguin, A. *La Dégustation au Service des Vins Doux Naturels à Appellations d'Origine Contrôlées*; Université des vins du Roussillon, station vitivinicole du Roussillon: France, 1991.
- Tressl, R.; Grünewald, K. G.; Silwar, R.; Bahri, D. Chemical formation of flavour substances. In *Progress in Flavour Research*; Land, D. G., Nursten, H. E., Eds.; Applied Science Publishers: London, 1978; Vol. 17, pp 197–213.
- Trillat, M. A. L'aldéhyde acétique dans les vins. Origine et effets. *Ann. Inst. Pasteur* **1908**, *22*, 704–719, 754–895.
- Vernin, G.; Parkanyi, C. Mechanisms of formation of heterocyclic compounds in Maillard and pyrolysis reactions. In *Chemistry of Heterocyclic Aroma Compounds in Flavours and Aromas*; Vernin, G., Ed.; Ellis Horwood, Chichester, U.K., 1982; Chapter 3, pp 151–207.
- Webb, A. D.; Kepner, R. E.; Maggiora, L. Sherry aroma. VI. Some volatile components of flor sherry of spanish origin. Neutral substances. *Am. J. Enol. Vitic.* **1967**, *18*, 190–199.
- Wildenradt, H. L.; Singleton, V. L. The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Vitic.* **1974**, *25*, 119–126.
- Williams, P. J.; Strauss, C. R. Studies of volatile components in the dichloromethane extracts of australien flor sherries: The identification of the isomeric ethylidene glycerols. *J. Inst. Brew.* **1978**, *84*, 144–147.

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