

## 3-Hydroxy-4,5-dimethyl-2(5H)-furanone Levels in Fortified Madeira Wines: Relationship to Sugar Content

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The maturation of Madeira wines usually involves exposure to relatively high temperatures and humidity levels >70%, which affect the aroma and flavor composition and lead to the formation of the typical and characteristic bouquet of these wines. To estimate the levels of sotolon [3-hydroxy-4,5-dimethyl-2(5H)-furanone] and their behavior over time, 86 aged Madeira wines samples (1–25 years old), with different sugar concentrations, respectively, 90 g L<sup>-1</sup> for Boal, 110 g L<sup>-1</sup> for Malvazia, 25 g L<sup>-1</sup> for Sercial, and 65 g L<sup>-1</sup> for Verdelho varieties, were analyzed. Isolation was performed by liquid–liquid extraction with dichloromethane followed by chromatographic analysis by GC-MS. The reproducibility of the method was found to be 4.9%. The detection and quantification limits were 1.2 and 2.0 μg L<sup>-1</sup>, respectively. The levels of sotolon found ranged from not detected to 2000 μg L<sup>-1</sup> for wines between 1 and 25 years old. It was observed that during aging, the concentration of sotolon increased with time in a linear fashion ( $r = 0.917$ ). The highest concentration of sotolon was found in wines with the highest residual sugar contents, considering the same time of storage. The results show that there is a strong correlation between sotolon and sugar derivatives: furfural, 5-methylfurfural, 5-hydroxymethylfurfural, and 5-ethoxymethylfurfural. These compounds are also well correlated with wine aging. These findings indicate that the kinetics of sotolon formation is closely related with residual sugar contents, suggesting that this molecule may come from a component like sugar.

**KEYWORDS:** Madeira wine flavor; wine aging; aroma; sotolon; sugar contents; furanic derivatives

### INTRODUCTION

Madeira wine, one of the most famous wines known all over the world, can be characterized by typical vinification and aging methods that include fortification, to obtain an ethanol content of ~18–20% (v/v), followed by a baking process known as “estufagem”, during which the wine is submitted to rather high temperatures (45–50 °C) for a long period (90 days). After this treatment, the wine is allowed to undergo a normal maturation process in oak casks (640 L) for a minimum of 3 years. The noble varieties from which Madeira wine is produced are Boal, Malvazia, Sercial, and Verdelho. The chemical characterization of monoterpenols, norisoprenoids, and other volatile components with respect to these varieties was recently studied by Câmara et al. (1, 2).

According to the sugar content, four basic types of Madeira wines can be distinguished: sweet, medium sweet, medium dry,

and dry. They are named after the principal grape variety from which they were obtained. Malvazia is fortified early in order to produce a distinctly sweet wine with a residual sugar content of ~110 g L<sup>-1</sup>. Boal is fortified after approximately half of the sugar has been converted to ethanol. This yields a medium sweet wine (90 g L<sup>-1</sup>). Verdelho ferments more than Boal to produce a medium dry wine (65 g L<sup>-1</sup> of sugar). Sercial is allowed to ferment until a large fraction of the total sugar has been converted to ethanol, giving a dry wine with a 25 g L<sup>-1</sup> sugar content.

It should be noted that the traditional Madeira wine classification based on residual sugar contents ranging from sweet to dry does not correspond to other common designations (3).

The aroma is one of the most important factors in determining wine character and quality. The volatile fraction of a wine can consist of >800 different compounds (4, 5), only several tens of which are recognized as odor-active (5–8). Several papers report the importance of temperature as a key parameter in changes related to the chemical profile and consequently the organoleptic and sensorial character of beverages (9–15).

In addition to estufagem, it is important to note that Madeira wines undergo long periods of aging, 20 years or longer, in

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cellars with temperatures of 30–35 °C, due to climatic conditions, and humidities of 70–75%. Changes in chemical composition and consequently of the sensorial perception of Madeira wines during maturation can be related primarily to the temperature (16) at which the wine is stored and to the period during which the wine is aged in a barrel.

3-Hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) is a very powerful odorant, which contributes to the characteristic sensorial impression of several foods (17–19).

Its presence in wine has been reported for the first time on the “flor sherry” (20). Subsequently, it was found in other types of wines such as the botrytised wines (21, 22), Jura wines (23–27), “vins jaunes”, “vins doux naturelles” (28, 29), Port (30), and Tokay (25, 31).

The correlation between the typical aroma of aged fortified wines and the respective chemical composition was attempted by a few authors, and it was shown that sotolon plays a major role in the sensorial quality of flor sherry wines (26).

More recently sotolon was recognized as the key molecule in the “perceived age” of barrel storage Port wine and consequently in the aroma quality of the product (10). The odor threshold value was estimated at 19  $\mu\text{g L}^{-1}$  in Port wines (10), close to the 15  $\mu\text{g L}^{-1}$  reported for flor sherry (26). These findings provide valuable information for this study, because barrel-aged Port wine bouquet resembles that of old Madeira wines.

Kobayashi et al. (32) reported a strictly chemical formation pathway of sotolon during sugar manufacture, as an aldol condensation between acetaldehyde and  $\alpha$ -ketobutyric acid (derived from threonine) followed by lactonisation. During barrel aging of wines ethanol is continuously converted into acetaldehyde (33), which reacts with the ketoacid to form sotolon.

On the other hand, several authors reported a relationship between the presence of sotolon and sugar derivatives, such as 5-hydroxymethylfurfural (10), 2,3-butanedione, and hydroxyacetaldehyde (34–36). It is important to note that all categories of Madeira wines are very rich in sugar ( $>25 \text{ g L}^{-1}$ ).

The aim of our study was to evaluate the influence of the aging period and sugar contents on the levels of sotolon and to establish a relationship between sugar and furanone concentration, to gather more information concerning the mechanism of sotolon formation in fortified wines.

## EXPERIMENTAL PROCEDURES

**Reagents.** The chemicals, 3-octanol, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, and furanic aldehydes, were obtained from Sigma-Aldrich (a high-purity grade,  $>99.0\%$ ). Dichloromethane was purchased from Lab Scan. Anhydrous sodium sulfate and ethanol were obtained from Merck, Darmstadt, Germany.

**Wine Samples.** The 86 Madeira wine samples used in this study were made from four different varieties corresponding to the following types: Malvazia (sweet), 22 samples; Boal (medium sweet), 26 samples; Verdelho (medium dry), 21 samples; and Sercial (dry), 17 samples; all aged from 1 to 25 years old.

They were produced following standard winemaking procedures used for Madeira wine. Following the typical sugar contents of the grapes varieties used and the residual sweetness admitted for the particular brand, the fermentation process is stopped by the addition of alcohol to obtain an ethanol content of 18–19% v/v. The new wine is then placed in large coated vats, and the temperature is slowly increased, at  $\sim 5 \text{ °C}$  per day, and maintained at 45–50 °C for 3 months. After controlled cooling, the wine is moved to conventional lodges to undergo wood aging and flavor development.

All samples were matured in oak barrels (640 L), submitted to the same oxidative storage conditions, and, after sampling, were stored at  $-28 \text{ °C}$  until analysis.

The wines were supplied by Instituto do Vinho da Madeira (40 samples) and Madeira Wine Co. (46 samples) and correspond to generic wines.

**Sotolon Extraction.** The extraction procedure was based on the method described by Silva Ferreira et al. (10). Fifty milliliters of aged samples of Madeira wines were spiked with 50  $\mu\text{L}$  of 3-octanol in a hydroalcoholic solution (1:1, v/v) at 422  $\text{mg L}^{-1}$  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 (Lab Scan). The two organic phases obtained were blended and dried over anhydrous sodium sulfate (Merck). Two milliliters of this organic extract was concentrated to 0.4 mL under nitrogen stream. Two microliters of the extract was injected into the GC with an MS detector.

**Gas Chromatography–Mass Spectrometry (GC-MS) Conditions.** Several dichloromethane extracts from different aged Madeira wines were analyzed by GC-MS using a Varian STAR 3400Cx series II gas chromatograph, equipped with a 30 m  $\times$  0.25 mm i.d., 0.25  $\mu\text{m}$  film thickness, Stabilwax fused silica capillary column, connected to a Varian Saturn III mass selective detector, according to the method described by Câmara et al. (16). Splitless injections were used. The initial oven temperature was set to 40 °C for 1 min. The temperature was increased in three steps: from 40 to 120 °C, at 1 °C  $\text{min}^{-1}$ ; from 120 to 180 °C, at 1.7 °C  $\text{min}^{-1}$ ; and from 180 to 220 °C, at 25 °C  $\text{min}^{-1}$ . Each step was preceded by a short constant-temperature period of 2, 1, and 10 min, respectively. The injector temperature was 250 °C, and the transfer line was held at 220 °C. The detection was performed by a Saturn III mass spectrometer in the EI mode (ionization energy, 70 eV; source temperature, 180 °C). The acquisition was made in scanning mode (mass range of  $m/z$  30–300; 1.9 spectra  $\text{s}^{-1}$ ).

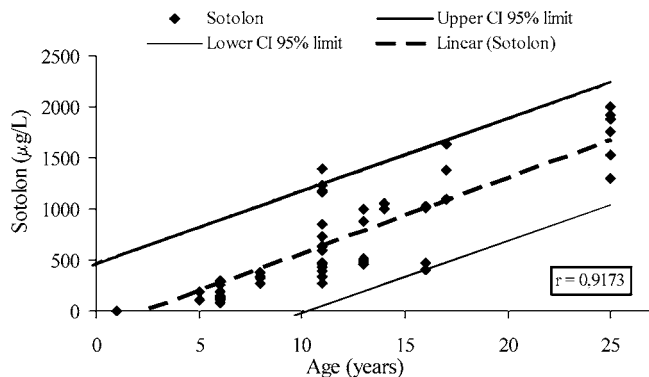
**Validation of Analytical Conditions.** The reproducibility of the extraction method was calculated from 10 analyses of a wine containing 91.0  $\mu\text{g L}^{-1}$  of sotolon. The variation coefficient was found to be 4.9%. The linearity of the method was tested using a young Madeira wine as a matrix; the quantitative analysis of sotolon additions showed that the method was linear for this compound. The concentration range tested was between 4.1 and 810  $\mu\text{g L}^{-1}$ , and the correlation coefficient between levels added and levels assayed was  $r = 0.999$ .

Detection and quantification limits were established in a Madeira wine diluted with a 20% (v/v) aqueous alcoholic solution in order to bring the sotolon peak to a magnitude as near as possible to the background. The detection limit was calculated by adding 3 times the standard deviation of the average of 10 replicates and the quantification limit by adding 10 times the standard deviation to the average value. Thus, detection and quantification limits were found to be 1.2 and 2.0  $\mu\text{g L}^{-1}$ , respectively.

**Sotolon Identification.** The information on sotolon was obtained on the basis of the injection of pure standards. The mass spectrum of the sotolon wine sample, with a retention index of 2209 on a Stabilwax capillary column, presented a fit of 86.7% in comparison to the compound indexed in the NIST92 library. Analysis carried out with electronic impact ionization mass spectrometry (GC-EIMS) showed a basic peak with  $m/z$  83, which corresponds to  $(\text{M}^+ - \text{CO} - \text{OH})^+$  (middle for an unsaturated  $\gamma$ -lactone). Moreover, the retention index (RI) of the compound was 2209 for the Stabilwax column. These values were identical to those obtained for the wine extract and are in agreement with those reported in the literature with columns of similar polarity (10, 19).

## RESULTS AND DISCUSSION

**Sotolon Levels in Madeira Wines versus Time of Storage.** Considering the categories, it was observed that sotolon concentration was clearly dependent on the time of barrel storage, as the high correlation coefficient ( $r = 0.917$ ) demonstrated. These trends are in agreement with the previously reported behavior for Port wines (10). The concentration of 3-hydroxy-4,5-dimethyl-2(5H)-furanone increases with time from 100  $\mu\text{g L}^{-1}$  in wines 6 years old to  $\sim 1000 \mu\text{g L}^{-1}$  in 25-year-old wines. The highest contents were observed for wines



**Figure 1.** Formation of sotolon concentration with aging in Madeira wines ( $\mu\text{g L}^{-1}$ ).

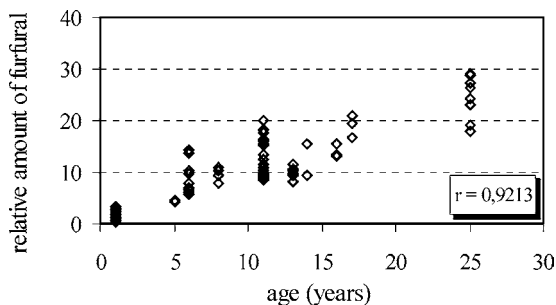
**Table 1.** Values of Furanic Derivatives Determined in Different Types of Madeira Wines, Sensory Threshold in Beer, and Odor Descriptors

wine variety	furanic compounds (relative amount)					
	furfural	5-methyl-furfural	sotolon	HMF <sup>a</sup>	EMF <sup>b</sup>	
Malvazia	minimum	0.5	0.02	nd	5.7	0.0
	maximum	23.3	3.9	3.1	100.3	13.2
	$\bar{x}$ (n = 22) <sup>d</sup>	9.7	1.7	1.2	44.9	3.6
Boal	minimum	0.8	0.02	nd	2.9	0.0
	maximum	24.1	1.9	1.9	74.3	10.9
	$\bar{x}$ (n = 26)	8.6	0.7	0.7	29.1	2.4
Verdelho	minimum	0.3	0.05	nd	0.7	0.0
	maximum	21.0	2.3	1.3	46.6	11.9
	$\bar{x}$ (n = 21)	7.5	0.9	0.5	14.7	1.5
Sercial	minimum	0.2	0.0	nd	1.2	0.0
	maximum	19.0	2.9	0.6	39.1	5.9
	$\bar{x}$ (n = 17)	5.7	0.6	0.3	10.4	0.5

	LOP (mg L <sup>-1</sup> )	odor descriptors
furfural	150	paper, green fruits
5-methylfurfural	20	curry, nut
HMF	100	aldehyde, caramel
EMF		spice, curry, coffee

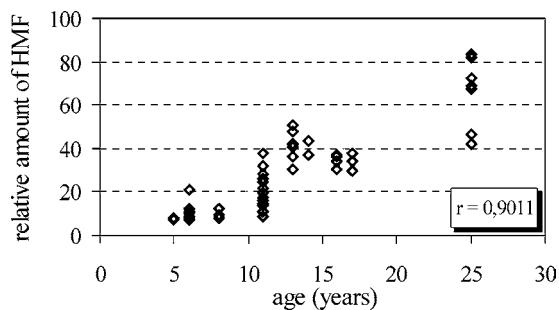
<sup>a</sup> 5-Hydroxymethylfurfural. <sup>b</sup> 5-Ethoxymethylfurfural. <sup>c</sup> Mean value. <sup>d</sup> Number of samples.



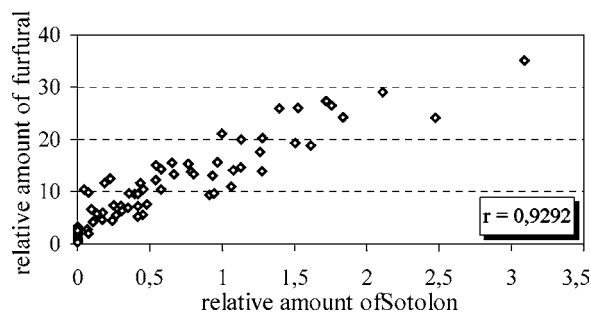
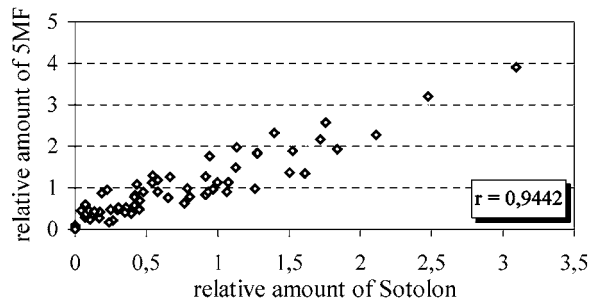
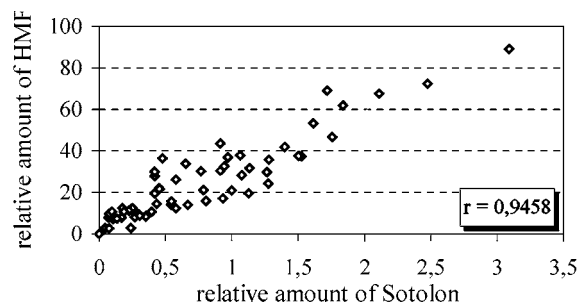
**Figure 2.** Formation of furfural with fortified Madeira wine aging (n = 86).

25 years old,  $>1 \text{ mg L}^{-1}$ . If we consider the wines of each variety, the linear correlation is very pronounced. For the Boal category,  $r = 0.963$ , and for Malvazia, Sercial, and Verdelho categories the correlation coefficients were 0.932, 0.924, and 0.948, respectively. Finally, considering the odor threshold reported for a Port wine, after 25 years in the sweet wine category, sotolon reached an odor aroma value (OAV = concentration/odor threshold) of  $>20$  (Figure 1).

**Sotolon versus Sugar Content.** Here, the apparent relationship between the quantity of sotolon formed during the aging process and the sugar content has to be noted. The average



**Figure 3.** Formation of 5-hydroxymethylfurfural (HMF) with fortified Madeira wine aging (n = 86).

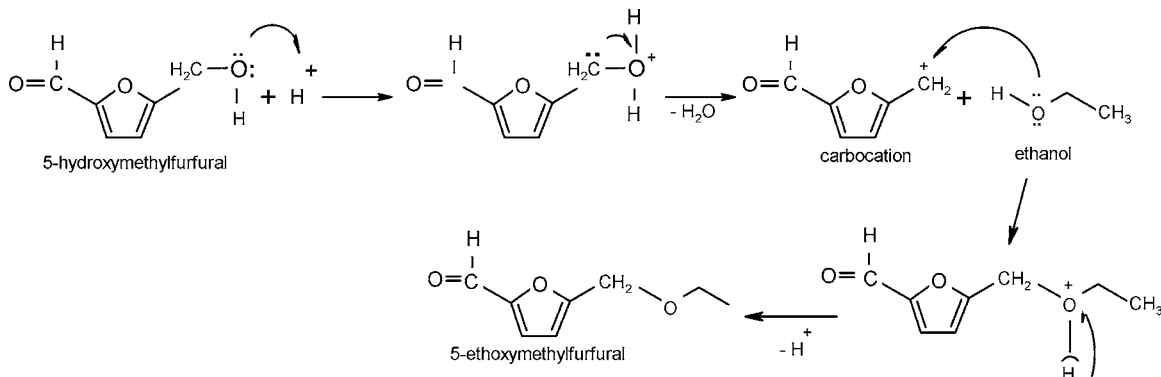


**Figure 4.** Correlation between furanic derivatives (5-hydroxymethylfurfural, HMF; and 5-methylfurfural, 5MF) and relative amount of sotolon.

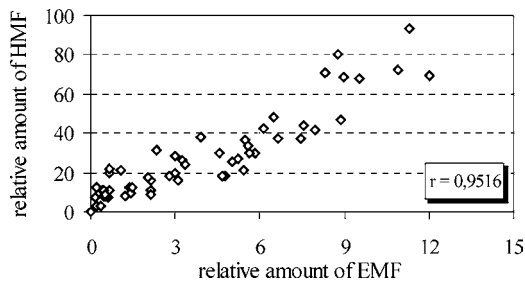
values observed for sotolon rise from dry (Sercial,  $25 \text{ g L}^{-1}$ ) to sweet (Malvazia,  $110 \text{ g L}^{-1}$ ) wines. Considering the same period of storage, that is, 11 years, sweet wines show a higher average value of sotolon ( $825.8 \mu\text{g L}^{-1}$ , n = 22) as opposed to dry wines that have a lower content ( $258.7 \mu\text{g L}^{-1}$ , n = 17). The median values calculated for medium sweet and medium dry wines are  $540.6 \mu\text{g L}^{-1}$  (n = 26) and  $430.3 \mu\text{g L}^{-1}$  (n = 21), respectively.

In fact, during this time frame, the increment in sotolon concentrations in sweet fortified white wines was  $6.7 \pm 1.4$ , whereas it reached only  $1.3 \pm 0.2$  in dry fortified Madeira wines. These results suggest that under similar storage conditions the formation of sotolon is favored by a high sugar content.

The relative rate of formation (RRF) between each of the categories and the one with the lowest sugar content (dry wines) was calculated. This value was obtained by taking the slope of the curve of sotolon concentration and the time of storage as a



**Figure 5.** Possible pathway for synthesis of 5-ethoxymethylfurfural from 5-hydroxymethylfurfural (40).



**Figure 6.** Correlation between 5-ethoxymethylfurfural (EMF) and 5-hydroxymethylfurfural (HMF).

rough estimate of the rate of formation, assuming that the concentration varied linearly with time, for each category. The RRF values were then defined for the three combinations, as the slope ratio between (sweet wine/dry wine), (medium sweet wine/dry wine), and (medium dry wine/dry wine) and were calculated as 4.4, 3.6, and 2.6, respectively. The kinetics of sotolon formation is highly dependent on sugar contents, and these results point toward a sugar-related pathway.

It has been demonstrated that sugar degradation products due to Maillard reaction were the starting material for the formation of the furanone (19, 28, 34–37). Thus, attempts were made to quantify some well-known sugar derivatives (furanic molecules) and relate them to sotolon concentration.

**Furanic Derivatives versus Sotolon Levels.** The average values ( $n = 3$ ) determined for furanic derivatives in Madeira wines are shown in **Table 1** as the threshold of sensory perception and the associated odor descriptors for the furanic compounds. The results show that higher levels of wine sugars ( $110 \text{ g L}^{-1}$ ) correspond to a higher content of 5-hydroxymethylfurfural. On the contrary, the dry wines (sugar content of  $25 \text{ g L}^{-1}$ ) present lower levels of this compound, indicating the formation of furanic derivative compounds arising from the degradation of sugars.

In the time considered (1–25 years), we observed a significant increment of contents of these compounds, with distinction for furfural and 5-hydroxymethylfurfural. The correlation coefficients calculated for furfural, 5-methylfurfural, and 5-hydroxymethylfurfural (HMF), 0.921, 0.906, and 0.916, respectively, demonstrated linear behavior of these compounds over time during aging (**Figures 2 and 3**), which makes these compounds, as well as sotolon, wine age indicators. The same behavior was observed by Silva Ferreira et al. (10) for Porto aged wines.

Besides the high levels in the aged wines, the sensory impact of these compounds for the characteristic aroma of Madeira wines seems to be weak due to the respective high threshold values (38).

At wine pH value, 5-hydroxymethylfurfural gives rise to 5-ethoxymethylfurfural (EMF) (30, 39, 40). This compound can also be formed by condensation between ethanol and 5-hydroxymethylfurfural (**Figure 5**). Depending on the concentration, 5-ethoxymethylfurfural is responsible for several descriptors such as spice and curry notes.

The identification by GC-MS presents a fit of 84.2% with the proposed by NIST92 fragmentation. The retention index in the Stabilwax capillary column is 2128.

The behavior observed for 5-ethoxymethylfurfural in the course of aging process is similar to that of the other furanic derivatives, with apparent linear ( $r = 0.912$ ) increment with time of wine maturation. **Figure 6** shows a strong correlation ( $r = 0.952$ ) between 5-ethoxymethylfurfural and 5-hydroxymethylfurfural.

These compounds arising from sugar degradation are strongly related to the formation of sotolon during aging (**Figure 4**). All of them show a high correlation coefficient with sotolon (HMF versus sotolon,  $r = 0.9458$ ; furfural versus sotolon,  $r = 0.9291$ ; 5-methylfurfural versus sotolon,  $r = 0.9442$ ; and EMF versus sotolon,  $r = 0.9045$ ). These facts illustrate that in Madeira wines sotolon synthesis is, probably, a product of Maillard reaction.

**Conclusions.** The results of this work show that Boal, Malvazia, Sercial, and Verdelho varieties have different profiles of sotolon. Malvazia (a sweet wine), as opposed to Sercial (a dry wine), has a higher total amount of these compounds than other varieties.

The quantification of this compound showed a high dependence between sotolon levels and maturation time, sugar wine contents, and furanic compounds.

Nevertheless, the high correlation coefficient observed between furanic derivatives—furfural, 5-methylfurfural, and 5-hydroxymethylfurfural—and sotolon suggests that the mechanisms related to sugar degradation could be responsible for the observed constant rate of formation.

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