Agriculture and Food

AIMS Agriculture and Food, 3(3): 172–180.
DOI: 10.3934/agrfood.2018.3.172
Received: 02 May 2018
Accepted: 03 July 2018
Published: 06 July 2018

http://www.aimspress.com/journal/agriculture

Research article

Odor detection threshold (ODT) and odor rejection threshold (ORT) determination of sotolon in Madeira wine: A preliminary study

João M. Gaspar[1], Vanda Pereira[1,2]* and José C. Marques[1,2]

[1] Faculty of Exact Sciences and Engineering, University of Madeira, Campus da Penteada, 9020-105 Funchal, Portugal
[2] Institute of Nanostructures, Nanomodelling and Nanofabrication (I3N), University of Aveiro, Aveiro, Portugal

* **Correspondence:** Email: vpereira@uma.pt; Tel: +351291705122.

Abstract: Madeira is a fortified wine, well renowned worldwide. It is during the aging process that its characteristic bouquet is developed, through the formation of specific aromas. Sotolon (3-hydroxy-4,5-dimethyl-2(5*H*)-furanone) is frequently pointed out as one of the molecules responsible for the aroma of the finest Madeiras. The present work serves as a preliminary insight on the sensorial impact of this compound in Madeira wine. The odor detection threshold of sotolon in a sweet-type Madeira was obtained by the 3-Alternative Forced Choice method. The estimated threshold value was obtained by 19 non-trained and non-expert panelists, within the spiked range 4–314 µg/L. An odor threshold of 112 µg/L was obtained using a 3-year-old Madeira with 6.3 ± 0.4 µg/L endogenous sotolon. This result is about 6-fold higher than those previously reported for other fortified wines. A Paired Preference test was chosen to determine the concentration at which the panelists would reject the wine spiked with sotolon. Nineteen panelists assessed a series of spiked concentrations ranging from 253–3464 µg/L. Within this range, it is not possible to define the concentration value from which the aroma of sotolon it is no longer pleasant. Thus, an odor rejection threshold could not be obtained. Indeed, the study also suggests that sotolon does not become unpleasant at higher concentrations.

Keywords: fortified wine; wine key-aromas; sensory analysis; 3-alternative forced choice; paired preference test

1. Introduction

Madeira wine is a well-known fortified wine (17 to 22% alcohol by volume (ABV)), which comes from the small volcanic islands of the Portuguese archipelago of Madeira, situated in the Atlantic Ocean. The production of Madeira can be distinguished from other wines mainly due to its vinification process and unusual aging methods. White grape varieties, like Sercial, Verdelho, Bual and Malvasia (or Malmsey), commonly called as "noble" grape varieties, are usually used to produce high-quality Madeira, in different styles (Sercial produces the driest style while Malvasia produces the sweetest style). However, most of Madeira wine's production is derived from the red-grape Tinta Negra, which can be used to produce the different styles of Madeira: dry, medium-dry, medium-sweet, and sweet. The alcoholic fermentation process is conducted by indigenous yeasts and is ceased by fortification, adding vinous alcohol 96% ABV. In this sense, depending on the moment when fortification is done, different wine styles can be obtained. After vinification, Madeira is oxidatively aged under unique conditions. The wines can, firstly, follow the *estufagem* process, in which wines are artificially heated to about 45 ˚C in stainless steel tanks, for at least 3 months, followed by an aging period in wood casks. If the fortified wine has exceptional characteristics, it can exclusively follow the *canteiro* process, in which it rests, for at least 3 years, in oak casks placed in the producer's warehouses exposed to natural heating. During both aging processes, important changes take place. Non-enzymatic browning reactions occur, since favorable conditions for its occurrence are met. This kind of reactions play an important role in the development of key odorant compounds, characterizing the typical bouquet of Madeira wine [1,2].

Sotolon (3-hydroxy-4,5-dimethyl-2(5*H*)-furanone) is a powerful odorant compound with caramel to curry-like notes, characteristic of older Madeira wines, and it is usually considered a key-aroma compound of these fortified wines [3]. Its positive contribution to the aroma of Madeira wine is also reported in other wines such as Vin Jaunes [4,5], Port wine [6] and French fortified wines [7]. On the other hand, sotolon is considered to give an off-flavor character to prematurely aged dry white wines [8]. The formation pathways in wine are not well elucidated. Yet, it is recognized that sotolon formation in Madeira wine is highly dependent on aging time and sugar content [9]. Despite being identified as a key-aroma of Madeira wine, the odor threshold of sotolon in this fortified wine has not yet been established.

The detection threshold is defined as being the minimum value of a sensory stimulus needed to give rise to a sensation [10]. The difference threshold is usually determined when the control sample already contains the stimulus in study. In fact, the detection threshold can be considered as a kind of difference threshold measurement, in which the control sample doesn't contain the stimulus, or its presence is unknown [11]. In case of threshold determinations in complex matrices, such as beer or wine, the detection and difference thresholds are generally considered the same entity [12,13]. The ability to detect a stimulus is influenced by physiological factors related to the assessors [14]. Factors such as the level of training and expertise of the panel performing the sensory tests will also influence the perception of a stimulus [15]. Depending on the purpose of the sensory test, the type of panel may be chosen accordingly. A common procedure for the odor detection threshold (ODT) or difference threshold determinations involves the so-called "ascending forced choice method of limits" [16]. The 3-Alternative Forced Choice (3-AFC) method is a type of triangular test that consists of an ascending concentration series of presentations, each containing one target sample with the target stimulus and two identical control samples, containing the media without the stimulus.

In this method, the panelist is required to identify the sample with the target stimulus or to discriminate the sample that differs from the others. Even if the panelist can't discriminate, a guess must be made. The American Society for Testing and Materials (ASTM) E679 standard practice describes the use of the 3-AFC method for a rapid and reliable determination of the detection threshold of a stimulus from 50 to 100 3-AFC presentations [14]. This practice prescribes the best estimate threshold (BET) detection method that is based on the correct and/or incorrect response pattern of the panel rather than the conventional threshold, usually determined as the corresponding concentration when 50% of the panelists, above chance, can detect the stimulus. The BET is then obtained by taking the geometric mean of the concentrations at which the panelists' responses change from incorrect to consistently correct.

Considering that sotolon is reported as imparting a pleasant odor to fortified wines and unpleasant to white wines and that an odorant stimulus may exhibit a pleasant scent at lower concentration levels while becoming unpleasant at higher levels it becomes relevant to study the odor rejection threshold (ORT). Prescott et al. [17] proposed the concept of consumer rejection threshold (ORT evaluation by a consumer panel) as a mean to determine the point at which the concentration level of a compound becomes unpleasant. These authors investigated the ORT of 2,4,6-trichloroanisole which is responsible for the cork taint defect in white wines. The ORT determination uses the Paired Preference test which is based on the Paired Comparison test method described in the International Organization for Standardization (ISO) 5495 [18]. It follows a similar practice to the 3-AFC method but only uses two samples: One target sample and one control sample. The sensory analysis is translated into statistical language establishing a null hypothesis (H_0) that a distinction cannot be made between the two samples in order of preference (there is an equal probability of 0.5 that a panelist will randomly select sample A or sample B). Statistically, the preference test is a two-sided test, thus the alternative hypothesis is written as $P_A \neq P_B$ ($P_A > P_B$ or $P_A < P_B$). At a 5% significance level, the null hypothesis is rejected if the number of selections for one sample is at least equal to that expressed in the statistical tables for the Paired Preference test [16,17].

The present work serves as an attempt to estimate the sotolon's ODT in sweet Madeira wine by determining the point from which sotolon's concentration becomes olfactory perceptible. Additionally, it was also intended to evaluate if there is a sotolon concentration level that becomes unpleasant in sweet Madeira wine.

2. Materials and methods

2.1. Panelists

Non-trained and non-expert individuals from the University of Madeira, including students and staff personnel, were asked to participate in the study. Twenty-two assessors (13 females and 9 males) aged between 21 and 62 years old accepted to participate in the study as volunteers, providing an informed-consent statement by email. Participants were also asked to fill a short online screening questionnaire, to characterize the participating panel. Only 16 assessors responded to the questionnaire. According to the responses, the majority (69%) had previously participated in similar sensory tests. Most of the assessors rarely drink fortified wine (69%), while 25% were occasional drinkers (a few times a month) and of these, 25% occasionally drink Madeira wine, while the remaining rarely or never drink it. Additionally, 31% identified themselves as "having some knowledge about wine in general", 31% as "interested in wine", 19% as being "consumers only" and 19% didn't identify themselves in any of the presented categories.

2.2. Wine samples

Two commercial wines were locally bought and used for the sensory tests. A 3-year-old sweet Madeira wine was used for the ODT evaluation, holding low levels of sotolon (6.3 ± 0.4 μg/L), as is required for ODT assessments. A wine with higher aroma complexity was used for the ORT assessment, a 5-year-old sweet Madeira wine holding 174 ± 6 μg/L. This kind of wines are blends and are mainly produced from the Tinta Negra grapes. The sotolon concentration of these base wines (non-spiked wine) was determined by liquid chromatography-mass spectrometry (LC-MS/MS). The sotolon extraction and quantification followed an optimized and validated in-house methodology (unpublished method). The analysis was carried out in the LCMS-8040 (Shimadzu, Japan).

A 100 mg/L stock solution of sotolon (SAFC, St. Louis, MO, USA) in synthetic wine (6 g/L tartaric acid; 18% ethanol; pH 3.50) was used to spike the wine samples in use during the tests. For the ODT determination, a concentration series was established with reference to the previously reported threshold for sotolon in Port wine [6]. To prepare the spiked solutions, the following six increasing concentrations were added to the 3-year-old wine: 4, 14, 34, 74, 154 and 314 μg/L. For the ORT assessment five 2-fold concentration scale-steps, ranging from 253–3464 μg/L, were prepared by spiking the 5-year-old sweet wine with the appropriate amounts of sotolon standard stock solution.

2.3. Sensory test

Forced choice tests were used. The sensory evaluation tests were conducted in a room free of odors at the University of Madeira. Each sensory study (ODT and ORT) was composed of 4 sessions (2 sessions per day for 2 consecutive days) using the same assessors whenever possible. Panelists were also asked not to eat, drink or smoke during the 30 min prior to the testing. As aforementioned, a set of 22 individuals agreed to participate in the sensory tests, however not all were available to contribute for both tests.

2.3.1. ODT

The 3-AFC method described in the ASTM E679 standard practice was used [14]. From the initial set of 22 participants, 19 panelists (11 females and 8 males, aged between 21 to 62 years old) have performed the ODT sensory tests. Most panelists (90%) completed the sensory test at least twice (during two sessions) and a total of 318 3-AFC presentations were obtained. Six sets of three coded samples containing 20 mL of wine were prepared in ISO tasting glasses: two control samples (non-spiked wine) and one sample spiked with sotolon per set. Assessors were asked to sniff each triad of samples, starting with the presentation of the lowest concentration (as conventional for an ascending forced choice method of limits) having to choose the sample that was different from the other two. As the method employed forced choice, each assessor was required to guess when he/she could not discriminate between the samples. The order of presentation of the samples was randomized for each triad and in every other session. Each assessor completed the evaluation of all six concentrations scale-steps of each set and responses were collected in a paper form.

2.3.2. ORT

The ORT analysis was done following the procedure of Prescott et al. [17] using a Paired Preference test based on the ISO 5495 standard practice [18]. From the set of 22 participants, another group of 19 panelists (12 females and 7 males, aged between 21 to 62 years old) have performed the ORT evaluation tests. Replication tests were made to obtain a sufficient amount of evaluations. Proper randomization was applied between replicate sessions. The procedure was the same as for ODT, but this time only five sets of two coded samples were used: one control sample (non-spiked wine) and one spiked sample. Assessors were asked to sniff each pair of samples, starting with the presentation of the lowest concentration, having to choose the sample that was preferred. As the method employed forced choice, each assessor was required to guess when he/she could not discriminate or had no preference for one of the samples. Each assessor completed the evaluation for all five concentrations scale-steps of each set and responses were collected in a paper form.

2.4. Data analysis

2.4.1. ODT

Each individual BET was determined by taking the geometric mean of the highest incorrect concentration and the next correct concentration. For the panelists who incorrectly identified the spiked sample at the highest concentration, the individual BET was determined by taking the geometric mean between the highest concentration tested and the next higher (hypothetical) concentration. Similarly, for the panelists who got a whole run of correct responses, the BET was determined by taking the geometric mean between the lowest and the next lower (hypothetical) concentration. Then, the final individual BETs were calculated as the arithmetic mean of each panelist replicant. The group BET was finally calculated as the geometric mean of all individual BETs.

2.4.2. ORT

The preference data for sotolon stimulus was collected and analyzed, and the proportion of panelists preferring the control sample was plotted against the sotolon concentration. The minimum responses necessary to establish a significative preference for one of the samples at a 5% significance level was 32 out of 48 (or 67% of the total responses) [17].

3. Results and discussion

3.1. ODT

The cumulative proportion of assessors that correctly identified the wine samples containing the sotolon stimulus at each tested concentration scale-step is depicted in Figure 1.

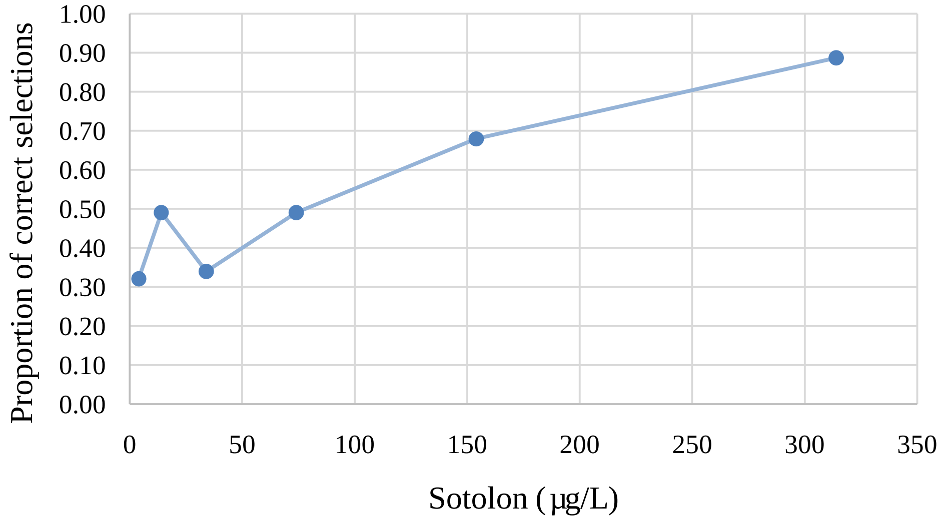

Figure 1. Proportion of assessors that correctly identified the wine sample spiked with sotolon at the different concentration scale-steps evaluated in the study.

Above 154 µg/L added sotolon, more than 68% of the panel chose correctly. These results are a representation of the raw data obtained during the test and include correct responses that may have been obtained by guessing. Using the procedure described by ASTM E697 practice this data can be processed to give the BET for sotolon's stimulus in sweet Madeira wine. Figure 2 represents the distribution of the obtained individual BETs.

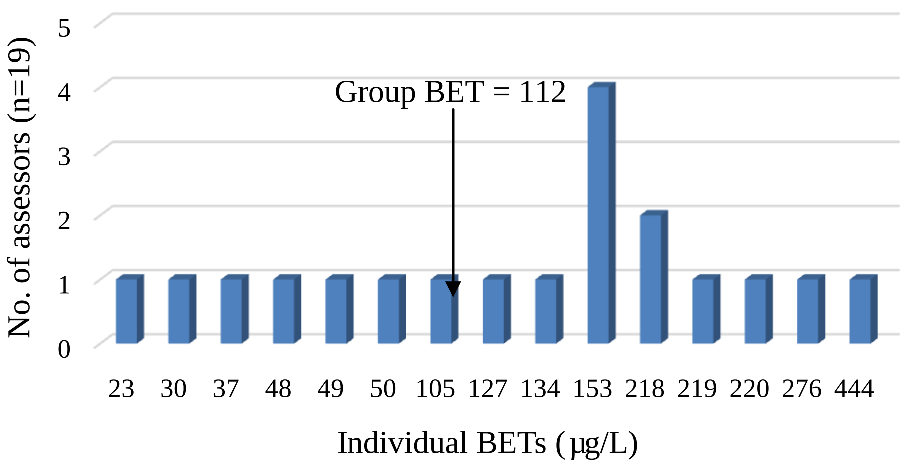

Figure 2. Distribution of the individual best estimate thresholds (BETs) for the detection of sotolon in sweet Madeira wine.

The group BET, calculated by the geometric mean of all the individual BETs, was 112 µg/L added sotolon, with a $\log_{10}$ standard deviation of 0.36. This threshold value was obtained in a sweet-type Madeira containing 6.3 ± 0.4 µg/L of endogenous sotolon. Despite most individual BETs being within 134–219 µg/L, they ranged from 23 to 444 µg/L, which highlights the differences between panelist's sensitivity level. These differences can also result from many other factors besides assessor's sensory abilities, such as fatigue, lack of training and repeated exposure to the stimulus, which can influence the results. The obtained ODT value is about 6-fold higher than the ODT of sotolon obtained by Silva Ferreira et al. [6] for Port wine. Although Port and Madeira are both fortified wines, their production processes impart differences in the bouquet of each wine, which may have implications in sotolon's ODT. Thus, this discrepancy could be due to matrix effects. In fact, several studies have shown that the nature of the wine matrix influences the threshold determinations, affecting the volatility and perception of the stimulus [18–20]. However, it should be kept in mind that caution should be taken when comparing threshold data obtained using different sensory analysis methods [11,21].

3.2. ORT

The Paired Preference test was used to determine the ORT, as previously described, and the results were translated into statistical language. Figure 3 illustrates the proportion of preference responses given by the panelists for each tested concentration. The minimum responses necessary to establish a significative preference for one of the samples at 5% significance level is 32 (67%), which is represented in Figure 3 by the dashed line. During these tests, the maximum preference responses for the non-spiked sample was lower than 46% at any of the tested scale-steps. Thus, this result suggests that samples spiked with sotolon were not rejected by this panel since non-spiked wines were never chosen as preferred by at least 67% of the panel.

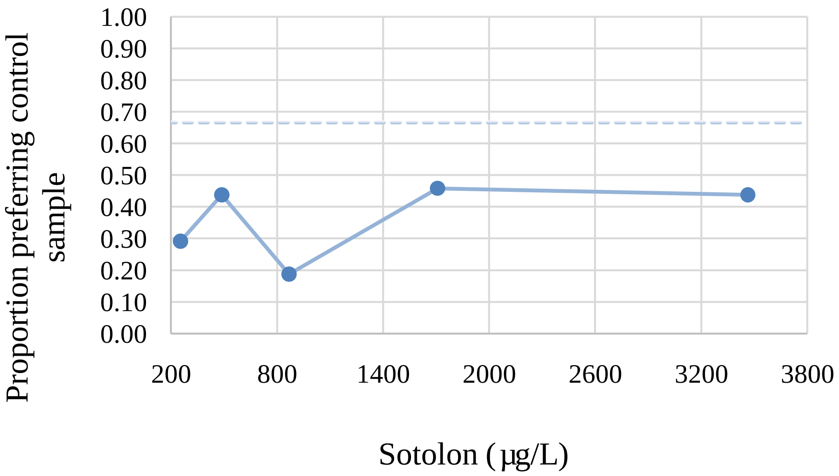

Figure 3. Proportion of preference responses given by the panelists for the non-spiked sample. The dashed line indicates the 5% significance criterion according to the binomial distribution for the Paired Preference tests.

4. Conclusions

The study presents a preliminary study about the ODT and ORT estimates of sotolon in Madeira wines. Indeed, this is the first study reporting threshold data for sotolon in Madeira wine. From 3-AFC evaluations of 19 non-trained panelists, an ODT value of 112 µg/L was obtained for sotolon in sweet Madeira wine, which is quite above the values previously reported for other fortified wines. Furthermore, the panel did not find the spiked wine with sotolon unpleasant at any concentration level within the concentration range 253.4–3464.2 µg/L. Thus, this result suggests that sotolon does not become unpleasant in these wines, even at higher concentrations.

This study can also be used as a base to determine at which concentration level sotolon has an influence on the overall aroma of Madeira wines. Further studies will be developed to estimate the ODT of sotolon in different styles of Madeiras, namely using an expert panel.

Acknowledgments

Vanda Pereira (Post Doc fellowship) is thankful to the *Agência Regional para o Desenvolvimento da Investigação Tecnologia e Inovação* for the financial support through their grants in the scope of the project M1420-09-5369-FSE-000001. The financial support was given by the European Regional Development Fund (ERDF) (+*Conhecimento* program, VALIMED project, MADFDR-01-0224-FEDER-000006). The authors are thankful to the sensory panelists for their kind contribution.

Conflict of interest

All authors declare no conflicts of interest in this paper.

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