OXFORD UNIVERSITY PRESS | Chemical Senses

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Journal:	*Chemical Senses*
Manuscript ID	Draft
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a

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Key Words:	detection thresholds, specific anosmia, psychophysics, olfaction

Individual Differences in Thresholds for Rotundone added to Red Wine

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Running Title: Specific anosmia for rotundone

Key Words: detection thresholds; specific anosmia; psychophysics; olfaction

Abstract

Rotundone is an odor-active compound found in the skin of some grape varietals that contributes the peppery note associated with wines such as Shiraz and Noiret. Previous research suggests there may be a specific anosmia for rotundone, as some individuals are unable to detect the presence of this compound even at high concentrations, despite having an otherwise normal sense of smell. However, subtle methodological differences limit the broader application of these results. Here, we estimate detection thresholds for rotundone added to red wine in a convenience sample of non-expert consumers in central Pennsylvania. We use a well-established standardized psychophysical method, and compare thresholds determined via orthonasal (n=56) and retronasal assessment (n=53). We found approximately 40% of our sample was anosmic to rotundone, and that ortho-and retronasal detection thresholds were nearly identical in a wine matrix. These results confirm a specific anosmia for rotundone within in a North American cohort, and suggest that peppery aroma experienced by sniffing a wine closely mirror the peppery flavor experienced when tasting the same wine. This suggests future research on rotundone perception may be able to rely on orthonasal assessment of samples. We also suggest additional work is warranted to uncover the genetic basis for this anosmia, in order to better evaluate potential regional differences in rotundone perception.

Introduction.

Rotundone is a sesquiterpine with a peppery, spicy aroma. It is responsible, in part, for the characteristic aroma of black pepper. Commercially, it is highly pertinent to the wine industry, as it is also found in some red wines such as Australian Shiraz or Pennsylvania Noiret. Rotundone was first isolated a little over a decade ago (Wood et al., 2008), and subsequent work has shown rotundone is found in the skins of certain grape varietals (Caputi et al., 2011), including Shiraz/Syrah (Wood et al., 2008) and Noiret (Homich, Elias, Vanden Heuvel, & Centinari, 2017). The concentration of rotundone in the skins of these grapes can be altered via viticulture practices (Geffroy et al., 2019, 2014; Homich et al., 2017). Similarly, the amount of rotundone in wines made from these grapes can be controlled via oenology practices, including how long the wines are left to ferment on the skins (Caputi et al., 2011). Noiret grapes are a hybrid varietal that grows well in Pennsylvania, and anecdotally, these grapes are typically used to make sweet, low-rotundone wines for the local market. Conversely, in Australian Shiraz, the peppery aromas provided by rotundone can be highly desirable, as wines with a strong peppery character are able command premium prices in the marketplace.

Prior work on rotundone perception also suggests it may exhibit a *specific anosmia* in some percentage of the population: based on extant data, roughly 1 in 4 or 1 in 5 individuals are unable to perceive this peppery aroma (Geffroy et al., 2018; Wood et al., 2008). A specific anosmia is the inability to smell a single odorant, despite an otherwise normal sense of smell. In humans, the sense of smell relies on approximately 350 different types of olfactory receptors (Zozulya, Echeverri, & Nguyen, 2001), each of which responds to a specific class of molecules (reviewed in Buck & James, 2004), and odors arise from the pattern of activation across these different receptors (see Silva Teixeira, Cerqueira, & Silva Ferreira, 2016 for a review). The specific receptors in an individual's nose, as well as the number of each type of receptor they express, are genetically determined, resulting in individual differences in odor perception across the population (Olender et al., 2012; Trimmer et al., 2017). That is, some individuals may lack a certain receptor type, and be unable to smell molecules of the specific class to which that receptor responds, while having an otherwise normal sense of smell. Several compounds have been identified as having specific anosmias in the population (e.g., Amoore, Venstrom, & Davis, 1968; Lawless, Antinone, Ledford, & Johnston, 1994); for a few of these (e.g., the smoky odor of guaiacol or the floral note of beta-ionone), a specific allele responsible for the anosmia has been identified (Jaeger et al., 2013; Mainland et al., 2014).

Because rotundone was isolated relatively recently and is typically present in only a few wine varieties, studies examining the perception of this compound have been quite limited. In the first study from Australia in 2008, one quarter to one fifth of participants were anosmic to the compound in red wine (9 of 47) and water (12 of 49). Among the responders, the orthonasal detection threshold was estimated to be 8 ng/L (in water) or 16 ng/L (in red wine), whereas anosmic individuals were unable to detect rotundone even at concentrations as high as 4000 ng/L (Wood et al., 2008). This specific anosmia was subsequently confirmed by a French study, which estimated ~31% of participants could not detect rotundone at 200 ng/L (i.e., a concentration well above threshold for responders) using both orthonasal and retronasal (in mouth) assessment (Geffroy et al., 2018).

Given the popularity of Australian Shiraz wines that contain rotundone, we wondered whether there might be a market in Pennsylvania for wines containing moderate to high-rotundone concentrations. Because perception of rotundone has not been previously studied in a North American sample, and because the prior reports used different methods to estimate thresholds, here we wished 1) to determine the detection threshold of rotundone in red wine in a convenience sample of wine consumers in Pennsylvania, 2) to compare orthonasal and retronasal delivery on threshold estimates, and 3) to determine the percentage of our participants who would be anosmic for rotundone. As we were preparing rotundone dilutions for use in this study, our team noted that rotundone appeared to be less intense when sniffed orthonasally than when assessed retronasally by swishing rotundone-spiked wine in the mouth. Given the discrepancy in delivery method between the two prior studies (Geffroy et al., 2018; Wood et al., 2008), we directly compared ortho- and retronasal detection thresholds for rotundone (in red wine) in participants drawn from the same population.

Methods.

Overview.

A total of 109 participants were recruited for a single test session. Upon arrival to the laboratory, they were randomized to one of two conditions in a pairwise fashion: roughly half of participants smelled (sniffed) the wine samples but did not taste them, and the other half were asked to taste the samples by mouth before spitting them out. Hereafter, for convenience and readability, these two conditions will be referred to as the *orthonasal* condition and the *retronasal* condition (with the caveat that the second condition is not solely retronasal in nature, as taste and chemesthestic inputs are also present when tasting via the mouth). Also, we presented rotundone in red wine rather than water for increased ecological relevance; that is, this compound is typically encountered in wine, and prior work shows that threshold estimates differ substantially between water and wine (e.g., Perry & Hayes, 2016). Within a single visit, each participant was given 5 separate triads of samples, for a total of 15 samples; no replicates were obtained. There was no overlap in participants across conditions (i.e., participants only completed orthonasal assessment or retronasal assessment, but not both). They provided informed consent for both the screener to determine eligibility and the study itself, and all procedures were approved by the Penn State University Institutional Review Board. Participants who visited the laboratory received a small cash incentive for their time. Data were collected using Compusense Cloud, Academic Consortium (Guelph, ONT).

Participants

Participants were recruited from an existing database of 1200+ individuals maintained by the Sensory Evaluation Center at Penn State. Study qualifications included the following: not pregnant or breastfeeding, nonsmoker, no food allergies, no history of choking or difficulty swallowing, no known smell or taste defect, no self-reported history of alcohol dependency or religious aversion to consuming alcohol. The orthonasal condition was completed by 56 participants (12 men, 44 women) while the retronasal condition was completed by 53 participants (10 men, 40 women, and 3 not reported). The modal respondent was female who self-reported as Caucasian, and was 25-30 years old (Table 1).

Table 1. Age distribution of sample.

	Sample (n = 109)	Proportion
22-24	12	11.3%
25-30	36	34.0%
31-35	25	23.6%
36-40	17	16.0%
41-45	14	13.2%
46-50	0	0%
51-55	2	1.9%
Not reported	3	–

Stimuli

Based on prior reports (i.e., a detection threshold of 16ng/L in red wine, and 31% of the sample being unable to detect rotundone at a fixed concentration of 200ng/L in water), we selected a concentration range above and below these values. Because we wanted to minimize fatigue, we used 5 concentrations of rotundone: 0.2, 2, 20, 200, and 2000 ng/L. Stock solutions of rotundone in ethanol (95% USP grade ethanol, Koptek, King of Prussia PA) were prepared and then added to 4L jugs of a neutral, fault free red wine (Carlo Rossi Burgundy, Carlo Rossi Vineyards, Modesto, CA) to create a single jug of each rotundone concentration, which were used for all participants in both the orthonasal and retronasal testing groups. Rotundone was kindly provided by Dr. Markus Herderich and the

Australian Wine Research Institute (Glen Osmond, South Australia). Concentrations were as prepared by the research team; no attempt was made to quantify these via instrumental chemical analysis.

Psychophysical Task Completed by the Participants

Detection threshold estimates were determined in accordance with ASTM Method E679-04 ("Standard Practice for Determination of Odor and Taste Thresholds By a Forced-Choice Ascending Concentration Series Method of Limits"). Briefly, participants completed a series of triangle tests, where every triad contained one spiked sample and two blank samples. The triads were presented in a fixed order so that the spiked sample increased in concentration across triads (to minimize adaptation and fatigue), and the order of samples within a triad was randomized. A break of 90-seconds was enforced between sets. Each sample consisted of 20 ml of wine (spike or control) in a standard ISO wine tasting glass. For each set (triad), participants were asked either to sniff (orthonasal) or taste (retronasal) the three samples in the order presented on the tray, and to identify which sample was the most different among the three (i.e., standard triangle test instructions). Participants in the retronasal condition who sampled the wine by mouth were also instructed to expectorate the wine after tasting. After each set, participants were asked to use water to cleanse their palates before the next trial. Participants received their first three triads on a single tray, then exchanged the tray after their third trial to receive their fourth and fifth triads. In total, testing took approximately 20 minutes.

Threshold Definition and Data Analysis

For the triangle test at each concentration, we recorded whether or not the participant got the triangle test correct. We used these data to calculate the best estimate threshold (BET) for each individual. To do so, we used the standard ASTM E679 decision rule, with two minor modifications. Per the standard method, for most individuals, we defined their BET as the geometric mean of the first concentration where the participant got all subsequent levels correct, and the next concentration (level) down. For the two participants who were correct at the lowest concentration given, their threshold was calculated as the geometric mean of the lowest concentration given (0.2 ng/L) and the next hypothetical concentration down (i.e., 0.02 ng/L), again in accordance with the standard method. However, for four participants (of 109), an alternative decision rule was used to define their individual BET. Specifically, these four individuals each got three lower concentrations in a row correct before getting a higher concentration incorrect; because the probability of getting three in a row correct by chance is quite low (3.7%), we reasoned these individuals may be sensitive to rotundone and that the incorrect answers at higher (and nominally easier) concentrations were thus due to adaptation. For these four individuals, their BET was instead calculated as the geometric mean of the first concentration where the participant began the run of three correct, and the next concentration (level) down. Finally for the non-responders (i.e., those who got the triangle test at the highest concentration presented wrong, and did not have a run of three lower concentrations correct), the BET was imputed as the geometric mean of the top concentration (2000 ng/L) tested and the next theoretical concentration that would have been tested (20,000 ng/L); this value was only used for visualization in histograms and was not included in calculated means or any statistical testing. Using the individual BETs for all of the responders, we then calculated a group threshold estimate as a geometric mean. This was done by calculating the arithmetic mean of the logged BETs, and then taking antilog of this value. To formally test for differences in thresholds across the two conditions (orthonasal versus retronasal) in the responders, we used an unpaired t-test on the logs of the individual BETs.

As an alternative method of analysis, we also used regression of the individual responses at concentration to visualize and calculate group thresholds, using the graphical method of Lawless (Lawless, 2010) as modified by Perry and colleagues (Perry, Byrnes, Heymann, & Hayes, 2019). Briefly, individual responses at each concentration were coded as 0 for incorrect and 1 for correct, and a regression line was fit to these points. The resulting line was used to determine the logged concentration where 67% performance was achieved by the group (i.e., halfway between perfect performance of one and chance performance of one third). This value was then antilogged to estimate the group threshold for that condition in ng/L. As discussed elsewhere (Perry et al., 2019), this approach provides a threshold estimate that a) is adjusted for chance, b) does not vary with participant number, and c) does not require specialized software.

Results.

Across all participants, we observed substantial variation in individual thresholds (BETs), as shown in Figure 1. The proportions of anosmic individuals and responders were similar for both routes of odorant delivery: of 56 participants who smelled the samples, 22 were anosmic (i.e., 60.7% were responders), while of 54 participants who sampled the wines by mouth, 23 were anosmic (i.e., 57.4% were responders). After excluding anosmic individuals, the estimated threshold for the responders (i.e., the geometric mean of the individual BETs) was 36.8 ng/L (±10.7 SD) for the orthonasal condition and 73.4 ng/L (±21.2 SD) for the retronasal condition. Critically, these values were not significantly different from each other ($t_{63} = 1.01$; $p = 0.30$, on logged BETs), suggesting that route of delivery does not influence the detection of rotundone.

From the graphical approach (Figure 2), we also see clear evidence of individuals who are able to detect rotundone (top), and of individuals who are anosmic (bottom). After excluding those who are unable to detect the rotundone, it again appears that the route of delivery does not influence the detection of rotundone, as the estimated thresholds for both conditions are very similar (139.9 versus 145.5 ng/L). From the confidence interval of the graphical method, the lower and upper bounds of the orthonasal estimate were 38.9 and 831.8 ng/L. For the retronasal group, the lower and upper bounds of the estimate were 57.5 and 473.1 ng/L.

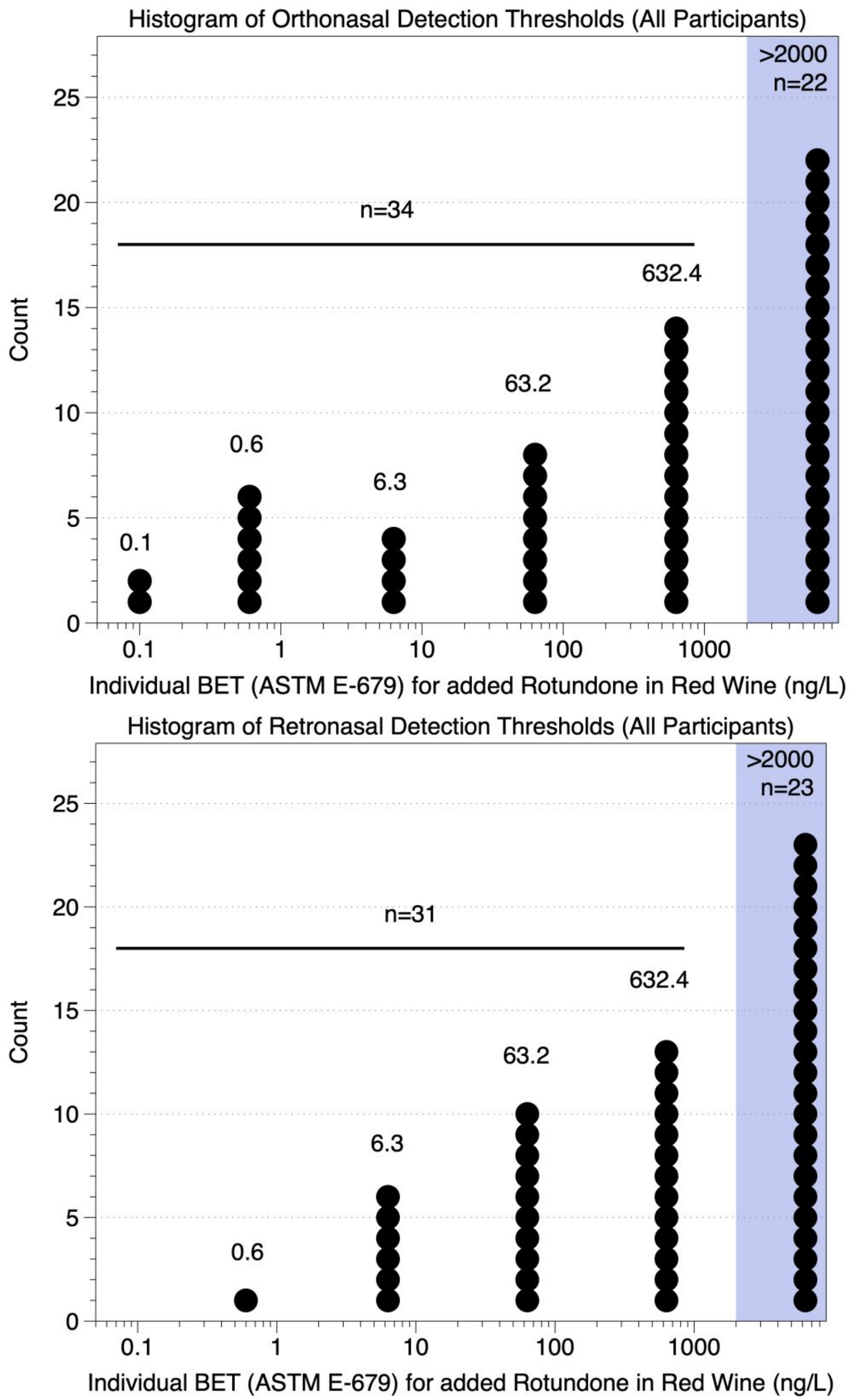
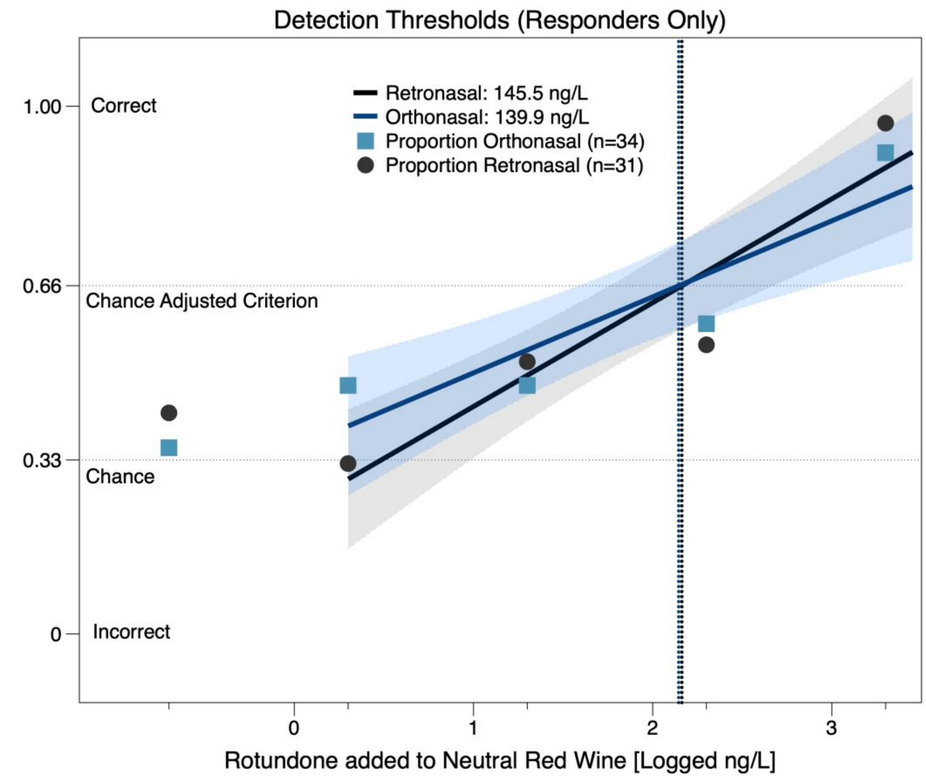


Figure 1: Estimated detection thresholds for rotundone in red wine assessed via the nostrils (orthonasal; top) or via mouth (retronasal; bottom).



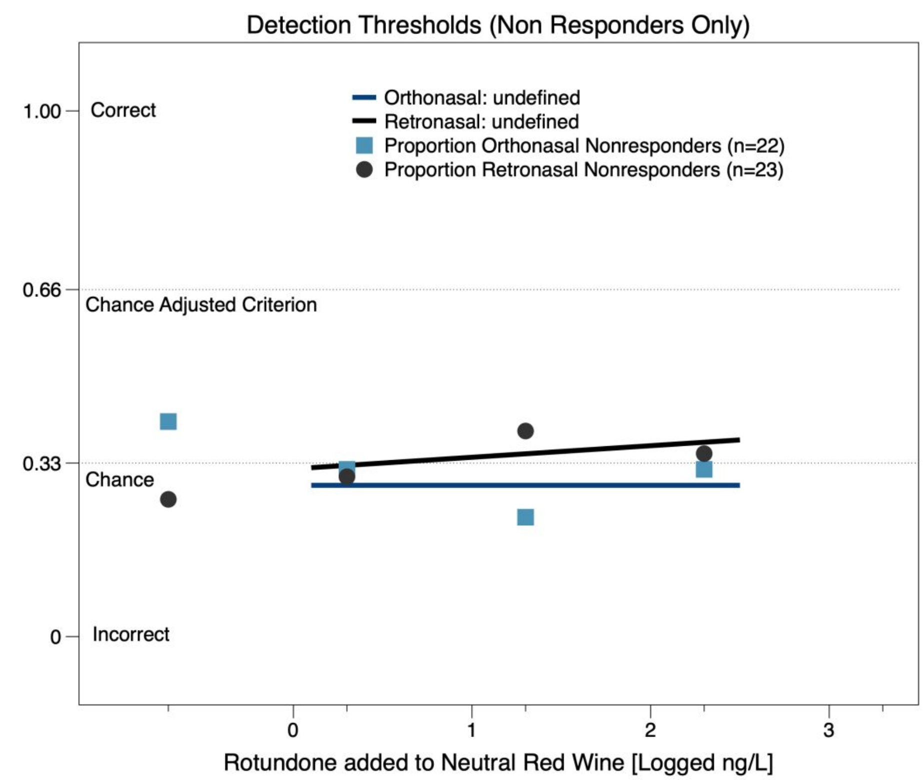


Figure 2: Estimated detection thresholds for rotundone in red wine using the graphical method, for responders (top) and non-responders (bottom)

Discussion.

The main aims of this study were to determine detection thresholds for rotundone in red wine via both ortho- and retronasal assessment, and to use these thresholds to assess the percentage of participants in our sample who display a specific anosmia for rotundone. Using the ASTM method, the orthonasal detection threshold was 36.8 ng/L, while the retronasal detection threshold was 73.4 ng/L , and these values were not significantly different from one another. Using the graphical method, we found that detection thresholds were 139.9 and 145.5 ng/L for ortho-and retronasal methods respectively. Approximately 40% of our participants were anosmic to rotundone at the highest concentration (not counting those who exhibited evidence of adaptation).

Regarding the differences between the two methods for determining group threshold, Lawless previously suggested that the ASTM method might yield lower threshold values than the graphical method, particularly if some participants in the sample exhibited adaptation at higher concentrations, as a handful of our participants appeared to do. We prefer the graphical method for several reasons. First of all, it is less sensitive to issues of adaptation and one-off mistakes (i.e., incorrect answers) from panelists who are momentarily distracted (Lawless, 2010). Secondly, this method does not require hand-coding to determine individual BETs, which is both laborious and potentially subject to coding errors. Particularly for experiments with large numbers of participants and/or those employing many concentration levels, the graphical method has a clear advantage in this respect. Our threshold values using the graphical method are somewhat higher than the previously reported group mean orthonasal threshold of 16ng/L (Wood et al., 2008). Still, from the graphical method, the lower end of our confidence interval for the estimated orthonasal threshold was 38.9 ng/L, which is roughly similar to the values reported previously by Wood and colleagues.

There was a slightly lower percentage of responders in our sample than either of the two previously published studies. This could be due to several factors. It is possible that there are simply regional variations in the distributions of responders and non-responders, as we used a convenience sample drawn from the northeastern United States, while Wood et al. (2008) conducted their study in Australia, and Geffroy et al. (2018) tested participants in France. However, there are also methodological considerations. The participants in Wood and colleagues' study (2008) were employees or students at the Australian Wine Research Institute (AWRI). Presumably, these individuals are more likely than naïve Pennsylvanian consumers to be familiar with peppery wines, given both the popularity of Shiraz in the Australian market and their occupational exposure to wine and its sensory evaluation. Other prior work suggests that exposure to an odor can lower the detection threshold for that odorant (reviewed in Royet, Plailly, Saive, Veyrac, & Delon-Martin, 2013). As such, AWRI employees may have lower thresholds for rotundone due to more familiarity with the compound. Additionally, of course, it is also possible that employment at a wine research institute may be self-selecting for individuals with greater olfactory expertise or interest relative to the typical consumer (see discussion in Hayes & Pickering 2012).

The present study has some limitations that should be mentioned. Here. we only used only a single set of concentrations, and only a single threshold estimate was determined for each participant. Had we used an interleaved series of concentrations across participants, or had participants assessed more concentration levels across multiple days, we may have gotten a slightly better fit for the regression line in our graphical threshold method; similarly, additional test samples at intermediate concentrations may have resulted in smaller confidence intervals. Further, our sample was unbalanced in terms of participant gender; we did not have have any sex or gender specific hypotheses for this compound, so our study was not powered to assess potential differences between men and women. Still, given the potential for sex differences in olfactory sensitivity (e.g., (Doty & Cameron, 2009)), future research should potentially revisit this question. Finally, due to limitations on the project scope, we did not make any effort to confirm the rotundone concentrations via chemical analysis; that said, we have no reason to believe the concentrations delivered deviated from the amounts prepared by research staff. Indeed, given the similarity between our orthonasal threshold estimation and that of Wood et al. (2008), we are relatively confident that our levels were approximately correct.

Conclusions

Our data confirms previous work suggesting that there is a specific anosmia for rotundone, and extends it to a population not previously tested. We also found a slightly greater proportion of non-responders – this could be due to either regional or ethnic differences across populations, or methodological differences between our study and the two prior studies examining the perception of rotundone in red wine (Geffroy et al., 2018; Wood et al., 2008). Further, we found no differences between the detection thresholds for rotundone in red wine using orthonasal and retronasal delivery. This is particularly pertinent to the wine industry, as it suggests that the peppery odor obtained by sniffing a wine may be a good representation of how a consumer can expect that wine to taste in the glass. Additionally, there are clear implications for the experience of non-responders. While it is true that expectation strongly influences olfactory perception (Herz & Von Clef, 2001), unwitting rotundone-anosmic consumers who purchase a peppery wine may be disappointed to find that they are unable to detect a peppery flavor. Educating consumers about the possibility of having a specific anosmia might help to avoid this disappointment, which could be unfairly blamed on the vintner. Additional work is needed to see how disconfirmation of expections may influence consumer satisfaction. Separately, given the differences in proportions of anosmic participants across the three regions tested to date (Australia, France, and Northeastern USA), we suggest additional research is needed to deduce the genetic basis for this specific anosmia, which may allow more accurate assessment of the incidence of rotudone nonresponders around the globe. Such information is both biologically interesting and commercially relevant given the rapid growth of wine consumption in emerging markets like India and China.

Acknowledgments

The authors wish to thank Dr. Helene Hopfer for help with calculations and dilution of the wine samples, Dr. Alyssa Bakke for consultation on the study design, and the staff and students in the Sensory Evaluation Center for their help in executing the test. We also thank our study participants for their time and participation.

Funding

This work was supported by a competitive grant from the Pennsylvania Wine Marketing and Research Board administered via the Pennsylvania Department of Agriculture, and discretionary funds from the Pennsylvania State University. Dr. Hayes receives additional support from the United States Department of Agriculture (USDA) via National Institute of Food and Agriculture (NIFA) and Hatch Act Appropriations [Project PEN04565 and Accession #1002916]. None of these organizations had any role in study conception, design or interpretation, or the decision to publish these data. The findings and conclusions in this publication are those of the authors, and do not represent the views of the U.S. Department of Agriculture, and do not represent any US Government determination, position or policy.

Conflict of interest

JMG and ANB have no potential conflicts to report. JEH has received speaking, travel, and consulting fees from nonprofit organizations, federal agencies, commodity boards, and corporate clients in the food industry. Additionally, the Sensory Evaluation Center at Penn State routinely conducts taste tests for industrial clients to facilitate experiential learning for students.

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Supplemental Table 1. Participant demographics.

	Sample (n = 104*)	Prevalence
How often do you consume wine?		
Never	3	2.88%
A few times a year	15	14.42%
Once a month	13	12.5%
2-3 times a month	35	33.65%
Once a week	19	18.27%
2-3 times a week	17	16.35%
4-6 times a week	1	0.96%
Every day	1	0.96%
How often do you consume beer?		
Never	10	9.61%
A few times a year	14	13.46%
Once a month	10	9.61%
2-3 times a month	30	28.85%
Once a week	21	20.19%
2-3 times a week	15	14.42%
4-6 times a week	4	3.85%
Every day	0	0%
How often do you consume liquor straight?		
Never	13	12.26%
A few times a year	41	39.42%
Once a month	18	17.31%
2-3 times a month	17	16.35%
Once a week	6	5.77%
2-3 times a week	7	6.73%
4-6 times a week	2	1.89%
Every day	0	0%
How often do you consume mixed drinks?		
Never	4	3.85%
A few times a year	38	36.54%
Once a month	21	20.19%
2-3 times a month	26	25%
Once a week	7	6.73%
2-3 times a week	7	6.73%
4-6 times a week	0	0%
Every day	1	0.96%

*5 participants did not provide this information.

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