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Determination of odour detection threshold for α-guaiene in water highlights potential wine aroma contribution, especially for panellists who are anosmic to rotundone

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ABSTRACT

Rotundone is the main aroma compound responsible for peppery notes in wine. This work aimed to estimate orthonasal odour detection thresholds (ODTs) of rotundone and its precursor, α-guaiene, in water. For both compounds, ODTs were measured using a panel of 63 subjects and ten three-alternative forced-choice (3-AFC) tests, with concentrations ranging from 1 to 3814 ng/L and a step factor of 2.5 between each series. Unlike rotundone, which exhibited a bimodal distribution suggestive of a genetically driven specific anosmia or hyposmia, individual thresholds of α-guaiene followed a normal distribution. The absence of cross-anosmia between the two compounds suggests that α-guaiene may be perceived by individuals with reduced sensitivity to rotundone. Group detection threshold was determined for α-guaiene at 120 ng/L, a value slightly higher than that of rotundone (55 ng/L). Although preliminary, these findings highlight a potentially significant aromatic contribution of α-guaiene, opening a new field of investigation to determine its ODT in red wine and to evaluate its concentration range in this latter matrix.

KEYWORDS: rotundone, α-guaiene, individual thresholds, ISO 13301

INTRODUCTION

Red wines, particularly those produced from *Vitis vinifera* L. Syrah and Duras, are often described as peppery, a sensory characteristic primarily linked to high levels of the sesquiterpenoid rotundone (Geffroy *et al.*, 2014; Wood *et al.*, 2008). A significant proportion of the general population, ranging from 20–42 % according to previous studies, has proved to be hyposmic or anosmic to this compound (Gaby *et al.*, 2020; Geffroy *et al.*, 2018; Geffroy *et al.*, 2020; Wood *et al.*, 2008). Interestingly, some individuals who failed to detect rotundone in a formal three-alternative forced-choice (3-AFC) test and for whom the omission of rotundone did not provoke any changes in aroma perception may still report perceiving peppery notes in wine (Geffroy *et al.*, 2020; Geffroy *et al.*, 2024)

One possible explanation for this phenomenon is that the peppery perception in wine by hyposmic or anosmic individuals could originate from oak ageing, which enhances compounds such as furfural, 4-ethylphenol, guaiacol, 4-ethylguaiacol, and eugenol, some of which contribute to wine spiciness and may be mistaken for pepperiness (Pérez-Coello & Díaz-Maroto, 2009). However, this hypothesis is unlikely, as a recent study based on 101 face-to-face interviews with French Syrah producers highlighted that the conceptualisation of peppery notes in red wines did not differ between panellists with high or low sensitivity to rotundone (Geffroy *et al.*, 2024). In contrast, a significant regional influence was observed with experts from the Northern Rhône valley considering this characteristic as an indicator of wines produced from under-ripe grapes, while for those from the Languedoc-Roussillon region, it was related to full-bodied wines made from very ripe grapes, such as those grown in the south of France.

An alternative hypothesis is the involvement of other aroma compounds, particularly sesquiterpenoids such as α-guaiene, a precursor to rotundone (Huang *et al.*, 2014), whose concentrations in grapes have been shown to correlate with rotundone and for which levels up to 20 times higher than those of rotundone have been reported in grapes (Takase *et al.*, 2016). The odour detection threshold (ODT) of α-guaiene, which imparts sweet, woody, balsamic, and peppery notes, remains undetermined (Li *et al.*, 2020).

This work aimed to estimate the orthonasal odour detection threshold (ODT) of α-guaiene in water. The ODT for rotundone was also assessed to investigate the existence of a possible cross-anosmia for this latter compound and α-guaiene.

MATERIALS AND METHODS

1. Panellists and test solutions

The panel consisted of 63 volunteers with an average age of 32 ± 13 years (mean ± SD), comprising 62 % women and 38 % men. Participants were recruited from students and staff of the École d'Ingénieurs de Purpan, as well as wine experts from the Gaillac protected designation of origin. Participants were not remunerated, and ethics approval was not obtained, as it was not required by École d'Ingénieurs de Purpan for such a sensory study. However, all were informed of the General Data Protection Regulation (GDPR) rules and provided written consent.

Rotundone (purity > 99 %) and α-guaiene (purity > 95 %), supplied by Firmenich (Geneva, Switzerland) and Eptes (Vevey, Switzerland), respectively, were used to prepare test solutions at varying concentrations. The solutions were prepared in MilliQ water (pH 7.0 ± 1.8) between 24 and 96 hours before testing and stored at 4 °C in the dark until used. Ten mL of each test solution was placed in a 30 mL brown glass flask with a plastic screw cap supplied by Embelia (Charenton-le-Pont, France).

Three test solutions for each compound were analysed following the method proposed by Mattivi *et al.* (2011), confirming that the targeted concentrations had been achieved.

2. Procedure for ODT measurements

Orthonasal ODTs were measured for rotundone and α-guaiene using ten three-alternative forced-choice (3-AFC) tests and an ascending procedure (from lowest to highest concentration) according to the American Society of Testing and Materials (ASTM) E679 (ASTM, 2004) and ISO 13301 (ISO, 2018) methods.

For both compounds, concentrations ranged from 1 to 3814 ng/L with a step factor of 2.5 between each series. The spiked concentrations were chosen based on preliminary tests conducted with four experienced panellists, and previous ODT values reported for rotundone in water (Wood *et al.*, 2008).

ODTs for both compounds were measured across three separate sessions conducted over a two-week period. The sessions were conducted in Toulouse and Gaillac at 20 °C in neutral rooms with white walls and natural lighting, and the spacing between the panellists ensured that no communication occurred. Samples were blinded using 3-digit codes, served at room temperature. For each series, panellists were asked to smell each flask in the prescribed service order and to identify the spiked sample. All panellists evaluated both sesquiterpenes, with the first compound in each assessment randomised. They were given a 5-minute break between the two molecules.

3. Statistical analysis

Taking into account the probabilistic nature of detection, individual thresholds were estimated using a method derived from the BET method ASTM-E679. For each subject, the threshold was estimated as the geometric mean between the lowest concentration in three consecutive correct responses and the previous concentration (Wise *et al.*, 2008). Another criterion was that the taster could not have more than one wrong answer for the following concentrations (ASTM, 2004; Wise *et al.*, 2008).

Individual thresholds were analysed using the Kruskal–Wallis test to assess differences across the three sessions using XLSTAT software (Addinsoft, Paris, France).

Group thresholds were defined as the concentration at which the probability of detection was 50 %. This statistical value was determined according to an adaptation of the ASTM-E1432 method. The curve representing the chance-corrected probability of detection as a function of log10 concentration was modelled as a sigmoid using SigmaPlot software (non-linear regression by ANOVA transform). The sigmoid equation was used to determine the threshold of the compound under study (concentration corresponding to a detection probability of 0.5) (ISO, 2018).

RESULTS AND DISCUSSION

No statistically significant differences were observed for rotundone or α-guaiene across the three sessions (Kruskal–Wallis tests, p-value > 0.05).

The distribution of individual orthonasal thresholds for rotundone and α-guaiene in water is presented in Figure 1. A clear bimodal distribution is observed for rotundone, with (i) a sensitive group comprising 54 % of the panellists, who can detect rotundone at a concentration of 61.8 ng/L or below, and (ii) a hyposmic group representing 46 % of the panellists, who can only detect rotundone at concentrations strictly exceeding 61.8 ng/L. It is unlikely that the 61.8 ng/L individual threshold concentration represents an overlap between the two populations. This study provides the first evidence of such a clear bimodal distribution for rotundone. In contrast, Wood *et al.* (2008) reported that 24 % of panellists could not detect rotundone even at 4000 ng/L in water. Our results suggest a greater sensitivity in comparison with the previous research conducted in Australia. It cannot be excluded that age differences between the panels might explain the discrepancy observed, as it was recently proposed that younger panellists may exhibit a greater sensitivity towards rotundone (Geffroy *et al.*, 2023). Nevertheless, this hypothesis is unlikely. Although the study by Wood *et al.* (2008) did not mention the average age of the panellists, the panel, like ours, included both staff and students from the institution.

A likely explanation is that rotundone sensitivity varies between the predominantly Caucasian French population and the more ethnically diverse populations of Australia. Indeed, it has been shown for β-ionone, another compound for which specific anosmia/hyposmia has been reported, that a higher frequency of the functional gene can be found in some African populations (Razafindrazaka *et al.*, 2023). A bimodal distribution of individual detection thresholds or a high proportion of subjects with specific anosmia, both associated with genetic variation, has already been observed for other compounds such as β-ionone (Jaeger *et al.*, 2013; Tempere *et al.*, 2012), androstenone (Wysocki & Beauchamp, 1984), or certain musks (Whissell-Buechy & Amoore, 1973). Together with our findings, this supports the notion of a genetic origin for rotundone sensitivity. If confirmed, this would imply that sensitivity to this odorant could not be induced in certain panellists through repeated exposure (Tempere *et al.*, 2012; Wysocki *et al.*, 1989).

To obtain preliminary answers, 10 panellists with individual thresholds strictly above 61.8 ng/L underwent a one-month training, during which they sniffed a rotundone solution daily, while 7 other anosmic/hyposmic panellists remained untrained. The training solution, prepared in the same 30 mL flask, contained 40 µg/L of rotundone in a 15 % *v/v* hydroalcoholic

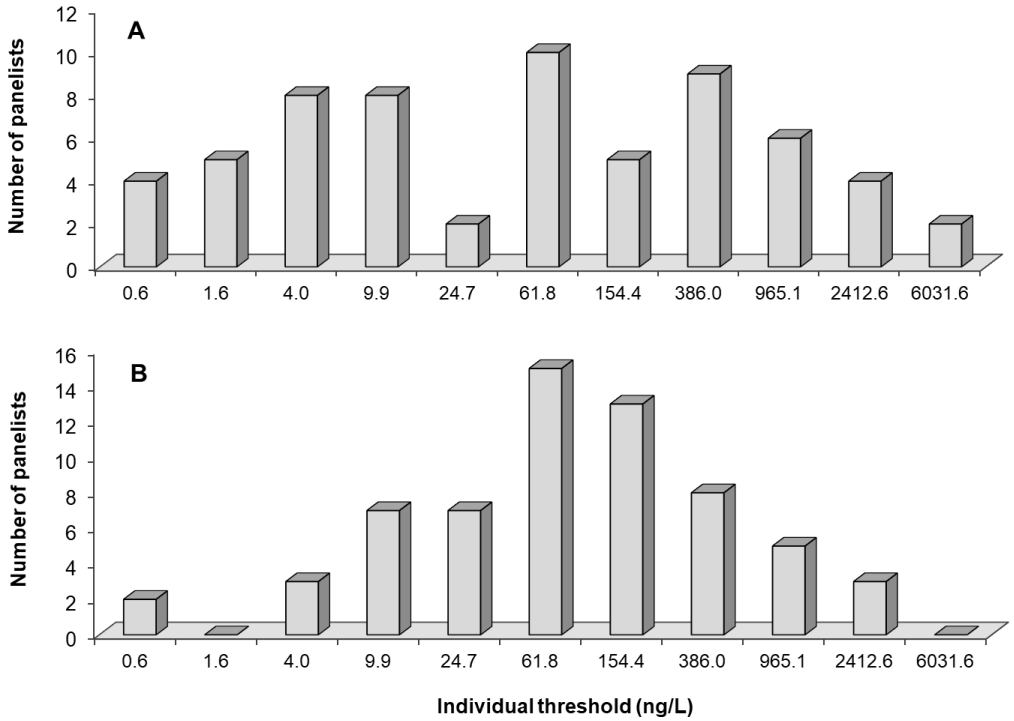


FIGURE 1. Distribution of individual thresholds for (A) rotundone and (B) α-guaiene in water according to the modified ASTM-E679 method (n = 63).

solution. A polypropylene pad from Delahaye Industry (Saint-Aignan-Grandlieu, France) was placed inside the flask to prevent spills. Trained panellists were instructed to sniff the flask for one minute each day over the course of a month. To maintain engagement and ensure consistency, those panellists recorded daily training times, received weekly reminders, and were provided with fresh solutions if intensity decreased. After this period, individual thresholds were reassessed for both trained and untrained panellists under the same conditions as in the initial session. No statistically significant differences were found, suggesting a genetic basis for specific anosmia/hyposmia to rotundone for the trained subjects (Wilcoxon tests, p-value > 0.05). This subgroup exhibited a sensitivity gain (1.7-fold decrease in ODT) in trend compared to the untrained panellists (0.5-fold increase). These findings remain preliminary and should be validated with a larger panel.

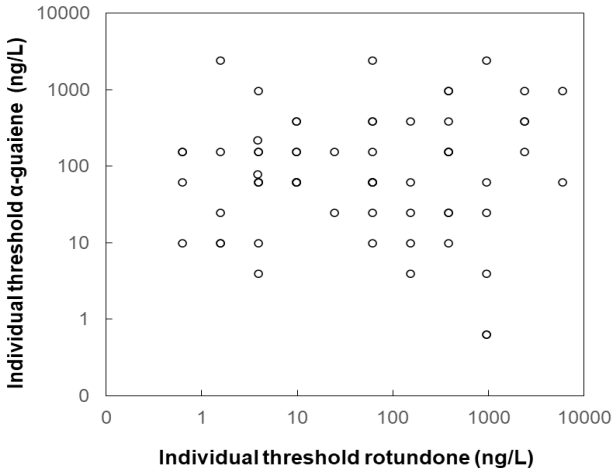


FIGURE 2. Relationship between the individual thresholds determined in water for rotundone and α-guaiene (n = 63).

For α-guaiene, individual thresholds followed a normal distribution (Figure 1B) and showed no correlation with the individual threshold values estimated for rotundone (Figure 2), ruling out the hypothesis of cross-anosmia between the two compounds. This indicates that panellists with reduced sensitivity to rotundone may detect α-guaiene, another sesquiterpene imparting woody or spicy notes. Interestingly, the group threshold for this latter compound was determined at 120 ng/L according to the ISO 13301 method (Figure 3), while for rotundone, values of 55 ng/L were observed.

This provides the first evidence of the potential aromatic impact of α-guaiene with an ODT in water in the ng/L range, alongside other significant grape-derived aroma compounds such as varietal thiols (Tominaga *et al.*, 2000), monoterpenols (Guth, 1997), and methoxypyrazines (Buttery *et al.*, 1969). Although, to our knowledge, this compound has not been reported in wine, its contribution could be significant due to its high concentration in grape exocarp, which can exceed 50,000 ng/kg (Takase *et al.*, 2016). This is particularly likely if its extraction rate from grape to must during fermentation is confirmed to be similar to that of rotundone (≈ 10 %) (Caputi *et al.*, 2011).

CONCLUSION

Our research provided the first estimation of the ODT for α-guaiene in water. Unlike rotundone, which exhibited a clearly bimodal distribution suggesting a genetically driven specific anosmia or hyposmia, α-guaiene followed a normal distribution. The absence of crossed anosmia suggests that this compound could have a sensory impact on panellists with low sensitivity to rotundone. The group detection threshold, estimated at 120 ng/L using the ISO 13301 method, was slightly higher than the one of rotundone (55 ng/L). These findings open new fields for research on α-guaiene, particularly to determine its ODT in red wine and to assess its concentration range in such products.

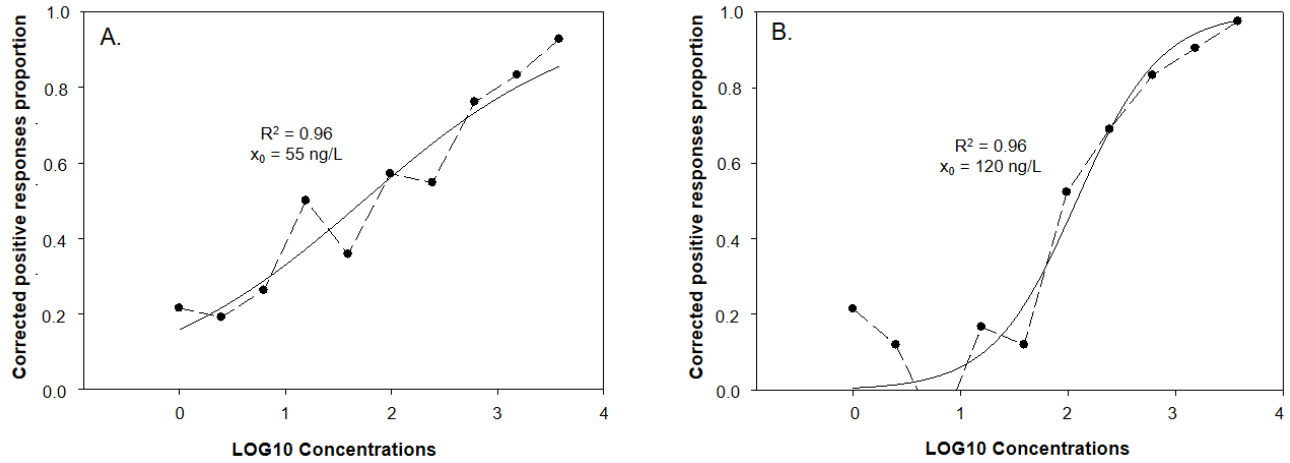


FIGURE 3. Corresponding psychometric functions and absolute detection thresholds (x0) for (A) rotundone and (B) α-guaiene (solid lines = psychometric function, dashed lines = sigmoid curve modelling). The panel thresholds were estimated at 55 and 120 ng/L for rotundone and α-guaiene, respectively (n = 63).

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