



Brief communication

Vanillin modifies affective responses to but not burning sensations from ethanol in mixtures

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ABSTRACT

Vanillin may modulate perception of noxious oral stimuli via TRP receptor interactions; separately, vanillin has also been shown to have top-down influences on flavor perception. Here, we ask whether added vanillin decreases the perceived burn of ethanol either via peripheral or cognitive mechanisms. Participants rated the burn of ethanol with 0, 16, and 160 ppm vanillin. In studies 1 ($n = 102$) and 2 ($n = 82$), participants wore nose clips and rated the burn of 8% and 16% ethanol (study 1) or 32% and 48% ethanol (study 2). In study 3 ($n = 65$), participants were able to breathe freely and rated liking and perceptual qualities of 8% and 32% ethanol at each vanillin level. Vanillin showed no significant effect on ratings of burn or smoothness, but did increase perceived sweetness ($p < .001$) and liking ($p = .004$) in study 3. These data suggest vanillin does not modulate ethanol burn via TRP receptor mediated interactions, but may shift hedonic responses to ethanol via top-down processes.

Aging spirits in wooden vessels has been shown to increase perceived smoothness [4], but limited research has investigated the mechanisms by which aging alters the perception of alcoholic beverages. One recent study suggests carbonyl compounds influence the perceived burn of ethanol via TRPV1 and TRPA1 receptor activation [8]. However, given the breadth of chemical – and thus flavor – changes that result from aging spirits, it is possible that other compounds are also involved in the perception of smoothness. In particular, wood-aging spirits increases the concentration of vanillin [5,19].

Traditionally, the literature on human olfaction has treated vanillin as an odorant lacking a trigeminal sensation (see, e.g., [7]). However, it is somewhat unclear from the extant literature whether this is truly the case. Early work [14] suggests vanillin can indeed be detected trigeminally, and a large body of medical literature demonstrates that vanillin has antinociceptive properties [1,13,15] in animal models, possibly via activation of TRP receptors [12]. Vanillin is a relatively weak agonist for TRPV1, but also modulates TRPV3 and TRPA1 receptors [10], both of which are involved in oral chemesthesis [11]. Recent data suggests vanillin can decrease the perceived burn of capsaicin under some conditions [16,17]. However, vanillin has also been shown to moderate the taste perception of sweet solutions via more top-down, cognitive routes, in which perceptual experiences are driven by cognitive processes such as expectations and context, rather than by sensory input alone. For example, if one asks participants to taste a red-

colored but orange-flavored beverage, top-down processes might lead participants who view the beverage before tasting to experience it as cherry-flavored based on their expectations (cognitive processes) that a red drink will have a cherry flavor, regardless of the actual flavor of the beverage. In contrast, participants who taste the drink while blindfolded are more likely to rely on bottom-up, receptor-dependent sensory processing, and accurately perceive the drink as being orange flavored. In the case of vanillin, implicit associations between vanilla and sweetness seem to have cross-modal influences on flavor perception in a top-down, receptor-independent fashion, as sweet-smelling odors typically do not activate sweet taste receptors [18]. For example, added vanilla (of which vanillin is a major component) has been shown to increase sweetness ratings of milk, both with [20] and without [9] added sucrose. It therefore seems likely vanillin plays some role in the perception of aged alcoholic beverages, but it remains unknown whether this occurs at the receptor level or is more of a cognitive, cross-modal process. Here, we investigated whether vanillin might modulate perceived ethanol burn in humans, thereby contributing to greater perceived smoothness in aged spirits.

To test whether vanillin influences the burn of ethanol, we conducted three experiments aimed at disentangling receptor-level interactions and cognitive influences. In the first two studies, we investigated whether different concentrations of vanillin influenced perceived burn of different concentrations of ethanol. By having

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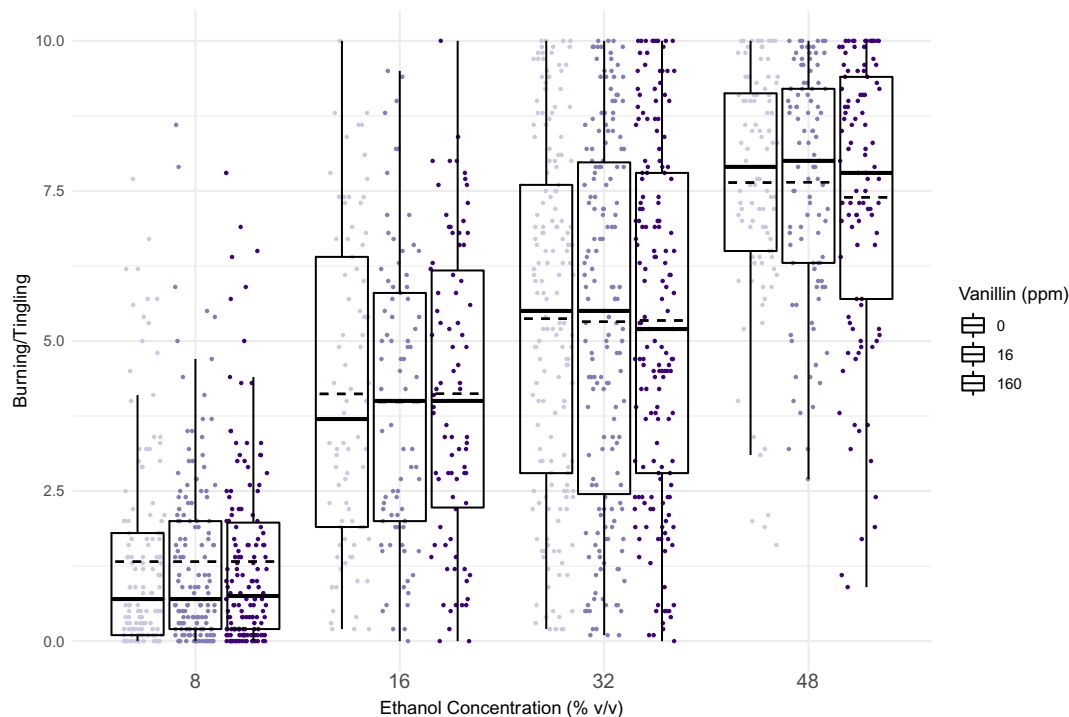


Fig. 1. Burning/tingling sensations on a visual analog scale for Study 1 ($n = 102$) and Study 2 ($n = 82$). The dots show individual ratings from each participant, and group means are indicated by the dotted line; standard box and whisker plots are also provided, where the solid line is the median, and the box is the interquartile range. As would be expected, burning/tingling increased significantly with ethanol concentration (see text for additional details). However, in the absence of retronasal olfaction, mixed models failed to reveal any evidence of significant differences in ethanol burn at different vanillin concentrations.

participants wear nose clips during testing, we were able to focus on peripheral (and presumably receptor-level) interactions between vanillin and ethanol burn. In the third study, we had participants sample stimuli without nose clips; this created a sensory experience more similar to real world consumption, and also allowed us to measure whether the odor of vanillin might have top-down, cross-modal influences on the perception of ethanol.

We recruited participants aged 21–65 years old from an existing database of 1200+ individuals maintained by the Sensory Evaluation Center at Penn State. 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, willing to wear a nose clip for the duration of the test (studies 1 and 2 only), and willing to taste liquor samples. Individuals who participated in an experiment were not permitted to participate in any subsequent experiments to reduce any effects of expectancy or learning across studies. Because we utilize a separate data collection protocol for recruitment and sensory data collection, we are unable to link specific demographic data with our study participants. We do, however, set enrollment quotas to ensure that our final study population is no > 60% female, and consistent with the demographics of the local region, a large majority of our participants self-identify as white.

Participants who met screening criteria signed up for a single laboratory visit. We used a balanced 3 by 2 design with three vanillin concentrations (0, 16, or 160 ppm) and two ethanol concentrations (varied by experiment). We diluted ethanol (95% USP grade ethanol, Koptek, King of Prussia PA) to the desired concentration (8, 16, 32, or 48% v/v, with calculations corrected to account for using 95% ethanol) using filtered water; these concentrations were based on prior work (e.g., Nolden & Hayes 2015) and their relevance to alcoholic beverages. Vanillin (> 97% pure food grade, Sigma Aldrich, St. Louis MO) was then dissolved in the appropriate ethanol:water solution to achieve the desired concentration of 0, 16 mg/L, and 160 mg/L (which is equivalent

to 0, 16, and 160 ppm, as 1 mg per 1000 mL equals 1 ppm). Participants were given spit cups, and were asked to spit out the samples after tasting, as well as the water used to rinse their mouths. In each experiment, participants rated attributes using computerized visual analog scales (details below). All data were collected using Compusense Cloud, Academic Consortium (Guelph ONT).

In Experiment 1, participants ($n = 102$) rated 32% or 48% ethanol with 0, 16, and 160 ppm vanillin (6 samples total). They wore nose clips for the entire experiment in order to prevent smell from influencing perception. We instructed participants to place the entire 15 mL sample in their mouth, swish for 5 s, then expectorate the sample into a separate spit cup. Participants rated *burning/tingling* on an unmarked VAS anchored at either end by “none” and “a lot”; we chose not to use a generalized scale here as all comparisons were within subjects (see discussion in [6]). Clicks on the scale were converted to a value from 0 to 10 in software; these numbers were never seen by participants. Between samples, participants had a 2-min break, during which they rinsed their mouths with water and then drank as much water as needed to rid their mouths of any residual sensations. Methods for Experiment 2 ($n = 82$) were identical to Experiment 1, but the ethanol concentrations were lowered to 8% and 16% ethanol (again, with 0, 16, and 160 mg/mL vanillin, for 6 samples total). Again, participants wore nose clips throughout the entire test session.

In experiment 3, participants ($n = 65$) rated 8% and 32% ethanol (the extremes from Experiments 1 and 2) with 0, 16, and 160 ppm vanillin *without* nose clips. First, participants sniffed the samples orthonasally and used a visual analog scale to rate the intensity of *vanilla* and *alcohol* aromas in the samples. Then, they tasted the samples and rated *burning/tingling*, *smoothness*, *sweetness*, and *overall liking* on separate VASs. Each unnumbered VAS was anchored by “none” on the left and “a lot” on the right, with the exception of liking, which was anchored with “do not like at all” on the left and “like extremely” on the right. An interstimulus interval of 2-min was enforced via software. We analyzed data in SPSS version 25.0 (IBM, Inc.; Armonk NY) using mixed

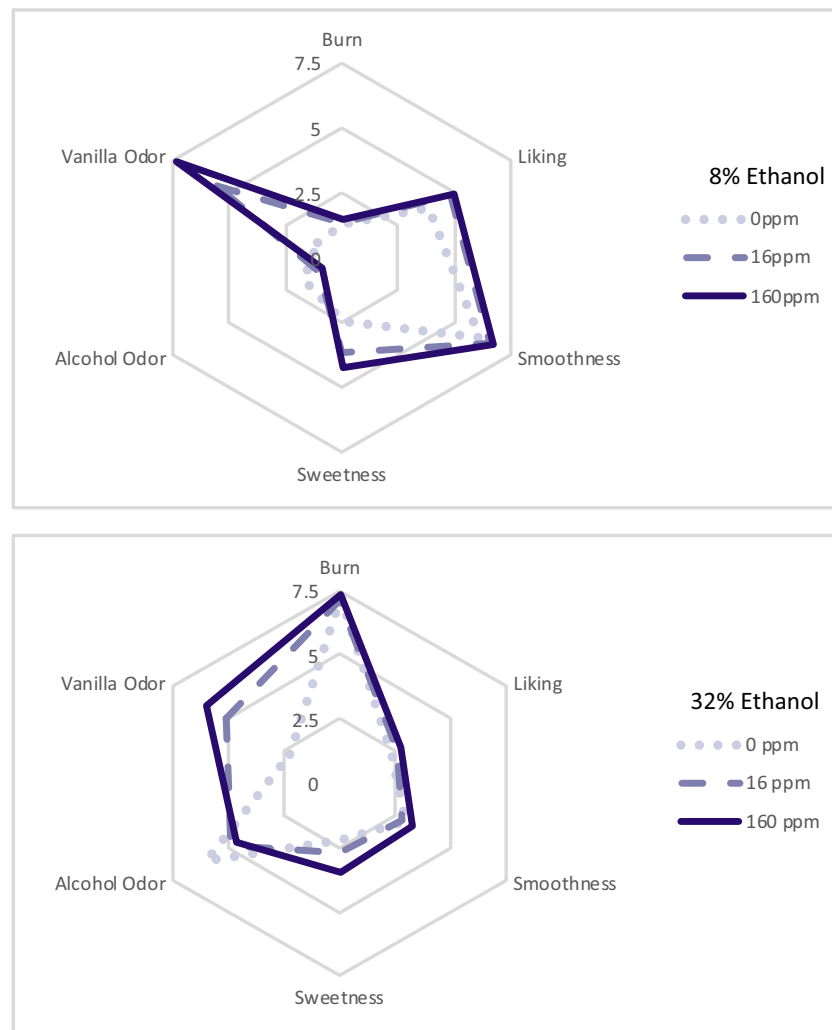


Fig. 2. Summary of results from Study 3 ($n = 65$), which was performed without nose clips. The spider plots in Panels A (8% v/v ethanol) and B (32% v/v ethanol) show the overall influence of added vanillin on the various attributes. When breathing freely, vanillin did not influence burn or smoothness at either ethanol concentration; however, there was a significant increase in sweetness ($p < .001$), liking ($p = .004$), and vanilla odor ($p < .001$), and a significant decrease in alcohol odor ($p < .001$). The data in Panel C correspond to the means shown in Panels A and B; they are exploded to show individual ratings and indicate significant differences across the levels of vanillin for variables of interest (vanilla odor not shown). Separate repeated measures mixed models were tested for each attribute, with ethanol concentration and amount of added vanillin as factors. Within an individual triad, levels of vanillin sharing a letter are not significantly different. Also, as shown in Panel D, there was a significant interaction between vanilla odor and alcohol odor ($p < .001$), such that 32% ethanol had more vanilla odor than 8% at 0 ppm vanillin, while the reverse was true at 160 ppm vanillin (see text for additional details).

models with ethanol concentration, vanillin concentration, and the interaction between ethanol and vanillin concentrations as fixed effects; participants were a random effect.

Across all three experiments, we found no evidence vanillin influenced the perceived burn of ethanol (all p 's > 0.57), as shown in Fig. 1 and Fig. 2. However, in experiment 3, we observed evidence that vanillin modulated several other attributes. Specifically, as shown in panels A-C of Fig. 2, increasing concentrations of vanillin led to increased sweetness ($F_{(2,320)} = 23.911$, $p < .001$), while added vanillin at any concentration increased liking over ethanol with no vanillin ($F_{(2,320)} = 5.68$, $p = .004$). Additionally, we found adding vanillin decreased ratings of alcohol odor ($F_{(2,320)} = 9.581$, $p < .001$) and, as expected, increased ratings of vanilla odor ($F_{(2,320)} = 160.317$, $p < .001$). Interestingly, we found an interaction between ethanol concentration and vanillin concentration for vanilla odor ($F_{(2,320)} = 10.046$, $p < .001$): 32% ethanol with no vanillin was rated as having significantly more vanilla odor than 8% with no vanillin, but at 160 ppm vanillin, 8% ethanol was rated as smelling more strongly of vanilla than 32%. Because ethanol (with no added vanillin) may have a

sweet odor, we speculate that at 32%, this sweetness might be more perceptible than at 8%. Thus, when vanillin is absent, but ethanol concentration is higher, participants may attribute this sweetness to vanilla odor, possibly via dumping. Conversely however, it is also possible that at higher ethanol concentrations, alcohol odor may compete with (i.e., suppressed) the vanilla odor, dampening the perception of 160 ppm vanillin (i.e., odor counteraction; [3]). However, these differences did not lead participants to rate ethanol solutions with added vanillin as being *smoother* ($F_{(2,320)} = 1.34$, $p = .263$).

Experiments 1 and 2 suggest that, at the peripheral level, the concentrations of vanillin used here are unable to modulate TRPV1 activation by ethanol. As our participants wore nose clips throughout the duration of the study, we are confident that our results are based solely on oral sensations (chemesthesis and taste) rather than being influenced by vanilla odor (i.e., olfaction). Experiment 3, in which both ortho- and retronasal air flow were allowed, suggests vanilla odor has some top-down influence on the perception of ethanol, although not for burn or smoothness, which is wholly consistent with the lack of effect in experiments 1 and 2.

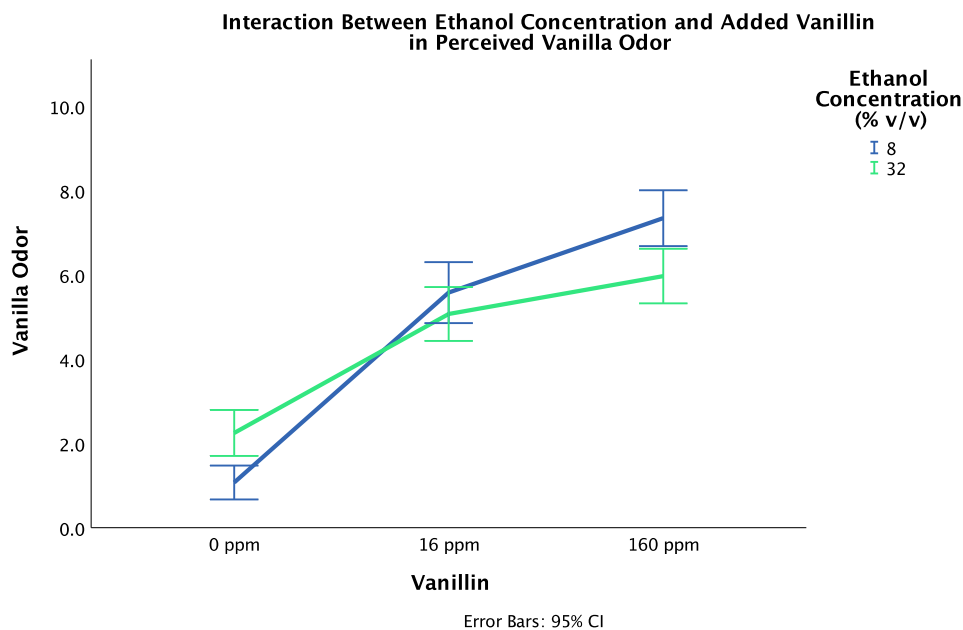
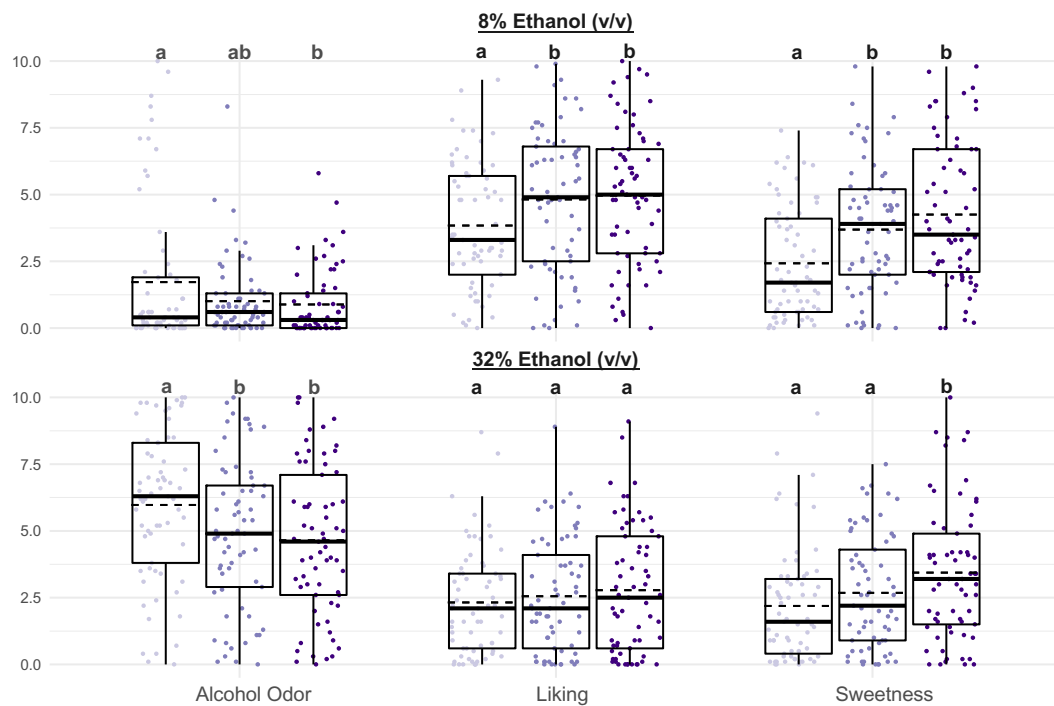


Fig. 2. (continued)

The vanillin concentrations used here were based on levels likely to be present in alcoholic beverages, with further adjustment based on informal pilot testing in our laboratory. While vanillin is typically not found in concentrations higher than 2.5 ppm in oak-aged spirits [19], typical usage levels of vanillin in food and beverage products falls between 26 and 597 ppm, with average use in alcoholic beverages being 30–47 ppm [2]. The concentrations used in our study therefore reflect *ecologically relevant* concentrations that may be present in real beverages (i.e., those likely to be encountered in a real-life consumption context as opposed to just existing in an experimental setting). Conversely, Smutzer et al. [17] employed a 42-fold higher concentration of vanillin in their study – close to 7000 ppm. We note that while the highest concentration used here (160 ppm) does not approach that

concentration, our ethanol samples with 160 ppm vanillin nonetheless had an identifiable vanilla flavor without any perceptible bitterness or trigeminal sensation beyond that of the ethanol alone (based on pilot testing by our team). We chose not to do a lineoleic acid pre-rinse, as was done by Smutzer and colleagues, as we were more interested in whether vanillin might influence burn and smoothness as a component of aged spirits; thus we chose to test the influences of vanillin concurrent with ethanol exposure rather than as a pre-rinse. Accordingly, the lack of an influence of vanillin on burn or smoothness here (in contrast to the reduction in capsaicin burn reported by Smutzer and colleagues) may be attributable to differences between the irritants (capsaicin versus ethanol), or the lower vanillin concentration used, differences in delivery (pre-rinse versus concurrent exposure), or some combination of

these various factors.

Smutzer et al. [17] suggested that vanillin may influence the burn of capsaicin in a receptor-independent fashion. As we did observe some top-down influences of vanillin on perceived alcohol odor, sweetness, and liking, our data are consistent with the idea that vanillin likely has sensory impact via a TRP receptor independent mechanism. However, at least for ethanol, such influences did not ultimately influence perceived burn. Future work is needed to specifically test whether vanillin influences capsaicin burn when applied concurrently rather than as part of a pre-rinse; likewise, it would be interesting to know if the vanillin-linoleic acid pre-rinse employed by Smutzer et al. with capsaicin is capable of reducing ethanol burn. Overall, our data suggest that adding vanillin influences perceived liking and sweetness, and may also diminish the perception of alcohol odor. However, our data also suggest that increased smoothness perceived in aged spirits is not due to their higher vanillin content, but rather is likely due to other flavor or chemical properties, or to additional top-down cognitive influences that do not rely on vanilla flavor or odor.

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Conflict of interest disclosure

JMG and ANB have no conflicts to disclose. JEH has received speaking and consulting fees from nonprofit organizations, federal agencies, and corporate clients in the food and beverage industries. Also, the Sensory Evaluation Center at Penn State routinely conducts taste tests for industry clients to facilitate experiential learning for undergraduate and graduate students. None of these organizations have had any role in study conception, design or interpretation, or the decision to publish these data.

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