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Salivary modulation of organic acid effects on pungency perception in Baijiu

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Abstract: Although ethanol dominates pungency perception in alcoholic beverages, variations among Baijiu samples with equivalent ethanol levels suggest additional modulators are involved. This study investigated the role of salivary physiology in modulating organic acid-induced temporal dynamics of pungency in Baijiu. Salivary α -amylase activity and total protein content were most closely associated with sensory dynamics. Higher amylase levels delayed onset and prolonged decline, whereas elevated protein corresponded to stronger and more persistent intensity. Acetic and hexanoic acids were identified as key modulators with distinct effects, while lactic acid functioned as a background factor with limited acute influence. Although mediation analysis didn't confirm statistical causality, validation demonstrated reproducible acid-specific impacts on salivary physiology. These results provide mechanistic insights into Baijiu pungency and highlight saliva as a critical physiological interface linking composition to sensory perception in complex food matrices.

Keywords: Baijiu, saliva, organic acid, pungency perception, time-intensity analysis, salivary physiology

1. Introduction

Baijiu, a traditional Chinese distilled spirit, is ranked alongside whisky, brandy, gin, vodka, and rum as one of the world's six major distilled beverages (Liu & Sun, 2018). It holds a prominent position not only culturally but also economically in China. In 2023, the total revenue of China's alcohol industry reached 150.7 billion USD, of which Baijiu sales accounted for about 105.6 billion USD, representing nearly 70% of the market share. Given its substantial economic impact, understanding and optimizing Baijiu's sensory qualities is of both scientific and commercial interest. The distinctive flavor of Baijiu is a key determinant of its overall quality and a major driver of consumer purchasing preferences. Historically, sensory evaluation of Baijiu has primarily emphasized aroma compounds, with more than 2,000 volatile aroma compounds, including esters, alcohols, organic acids, and aldehydes, were identified to date (Wang, Jing, Song, et al., 2022; Wang et al., 2024). While aroma has been extensively studied, Baijiu is also characterized by a distinctive oral pungency, often described as a burning or tingling sensation, which plays a decisive role in consumer acceptance, particularly among first-time drinkers. Despite its importance as a fundamental component of Baijiu's sensory profile, research on oral pungency remains surprisingly underexplored, especially with respect to its physiological underpinnings.

The distinctive pungency of Baijiu is closely linked to its high ethanol content, as its alcohol by volume typically ranges from 40-52% (Wang et al., 2025). In the context

of Baijiu, ethanol is well established as the principal contributor to oral pungency, primarily through its stimulatory effects in the oral cavity and activation of trigeminal nerve pathways such as TRPV1 receptors (Danilova & Hellekant, 2002; Green, 1988; Ickes & Cadwallader, 2017). However, ethanol concentration alone cannot fully account for the pronounced differences in pungency observed among Baijiu samples with comparable alcohol levels. Given Baijiu's chemically-rich matrix, it is plausible that additional constituents modulate the perception of ethanol-induced pungency, yielding distinct sensory judgements at similar ethanol concentrations (M. C. Qian et al., 2019). Recent studies have highlighted organic acids, which abundant in Baijiu, as important modulators of oral pungency, acting through mechanisms such as pH-dependent trigeminal activation and synergistic interactions with ethanol (Y. Chen et al., 2025; Ployon et al., 2017). Among these, lactic and acetic acids are frequently regarded within the industry as influential in shaping perceived harshness versus smoothness in mouthfeel (Y. He et al., 2025; Wang, Jing, Song, et al., 2022). Current research on Baijiu mouthfeel faces two major limitations: Methods and mechanism. Methodologically, pungency has often been assessed using static, end-point ratings. This approach is inherently inadequate for a high-strength alcoholic beverage such as Baijiu. Upon entering the oral cavity, high concentrations of ethanol trigger an intense initial stimulation, eliciting a physiological response in which copious saliva is secreted to dilute and mitigate the sensation. This response produces a characteristic time-dependent sensory profile, pungency rises rapidly, then gradually diminishes as

saliva secretion increases, and eventually subsides, often leaving a transient numbness on the tongue, a typical consequence of high ethanol exposure. Consequently, a dynamic sensory evaluation approach is more appropriate (Y. He et al., 2021; MacGarry, n.d.; Pedersen et al., 2002; R. Qian et al., 2024; Wang et al., 2025). Mechanistically, most studies remain at the level of linking specific compounds to corresponding sensory attributes. For example, certain esters have been reported to impart a spicy sensation and contribute uniquely to Baijiu pungency, whereas some organic acids appear to moderate ethanol's impact, rendering the mouthfeel smoother (Gawel et al., 2018; W. He et al., 2023; Y. He et al., 2025). While such compound-attribute associations are valuable, they essentially treat the perception process as a "black box," offering limited insight into the physiological pathways through which these compounds influence perception. To unravel this complexity, it is necessary to identify and investigate the intermediate physiological mediators involved in oral sensory processing. Since Baijiu pungency is primarily perceived within the oral cavity through trigeminal stimulation, saliva serves as a direct and biologically relevant interface for mechanistic investigation.

Baijiu's pungency is mainly perceived in the oral cavity, where interactions between chemical stimuli and saliva shape the sensory experience. Although a throat burn may occur upon swallowing, oral perception represents the dominant site of trigeminal stimulation. Saliva constitutes the first and continuously present biological interface for Baijiu in the oral cavity (J. Chen, 2009; Spence, 2011). It governs

stimulus transport and clearance through dilution and buffering, and modulates chemosensory access via enzymatic and protein-binding interactions(Canon et al., 2018; Criado et al., 2019; Rinaldi et al., 2012). Upon exposure to high-strength ethanol, reflexive salivation is elicited, which dilutes and attenuates the perceived burn. Concomitantly, salivary flow rate, pH, α -amylase activity, and total protein content can shift in a stimulus-dependent manner(J. Chen, 2009; Cretin et al., 2018; Enberg et al., 2001; Fleming et al., 2016). These properties may determine how rapidly, and to what extent, pungent stimuli reach trigeminal endings, thereby shaping the time course of sensation that dynamic methods are designed to capture. At the molecular level, salivary proteins and enzymes can transiently retain, sequester, or transform flavour-active molecules. α -Amylase, which is abundant in stimulated saliva, binds hydrophobic volatiles and can reduce their release, while mucins and other proteins contribute to lubrication and transient binding that alters local concentrations at receptor sites(Canon et al., 2018; Enberg et al., 2001). Ethanol can also modify salivary protein conformation, including that of α -amylase, with downstream effects on aroma and pungency release and mouthfeel(Enberg et al., 2001; Santos et al., 2023). Organic acids and ethanol are perceived together in the oral cavity, and organic acids have the potential to influence intra-oral pH and trigeminal activation thresholds(Y. Chen et al., 2025; Clark et al., 2011). Consequently, they may interact directly or indirectly with saliva's buffering capacity and protein composition to shape pungency perception. Collectively, these observations suggest a

conceptual framework in which saliva may act as a dynamic mediator, transmitting the effects of Baijiu's chemical composition to its sensory perception. Despite this central role, saliva-focused studies have largely addressed basic taste stimuli or simple food systems, and remain scarce for complex, high-ethanol beverages such as Baijiu.

To address this gap, this study investigated whether saliva could function as a physiological mediator linking organic acids in Baijiu to the temporal dynamics of pungency perception. The work first characterized the salivary responses elicited by Baijiu consumption and assessed whether specific salivary indicators are consistently associated with dynamic sensory attributes. It then identified the key organic acids that modulate salivary physiology and explored their potential mechanisms of influence. Building on these associations, mediation analysis was applied to evaluate whether saliva could serve as an intermediate pathway between organic acid composition and sensory perception. Finally, validation experiments in both model solutions and real Baijiu matrices were conducted to confirm acid-specific effects on salivary responses and to establish the robustness of the observed relationships (Figure 1). Through this integrated framework, the study provides new mechanistic insight into how Baijiu's chemical complexity interacts with oral physiology to shape pungency perception.

2. Materials and methods

2.1. Chemicals and Samples

Eleven commercial Baijiu samples (coded S1-S11) were selected for this study, all classified as strong-flavour types with an alcohol content of 52% vol. These samples were sourced in 2023 from leading distilleries located in representative strong- flavour Baijiu production regions, such as Sichuan and Jiangsu provinces, and stored at 4 °C until further analysis. To ensure comparability and minimize variability across samples, all products were produced using traditional solid-state fermentation techniques, including sorghum as the main raw material, medium-temperature *Daqu* as the fermentation starter, and mud pits as the fermentation vessels. Notably, the same set of Baijiu samples was previously subjected to time-intensity (TI) analysis (Table S1) and organic acid (Table S2) profiling in our earlier research. In the present study, these samples were re-used for sensory evaluation and saliva collection to enable an integrated investigation that linked organic acid, salivary responses, and dynamic pungency perception across identical matrices. This alignment ensures full comparability across chemical, physiological, and perceptual dimensions.

In the validation experiments, ethanol (95% purity, food-grade; HuaLin, Yunnan, China) was employed as the base alcohol. To eliminate potential sensory interference, only pure ethanol without added volatiles or flavoring agents was used. It was diluted to the target concentrations using ultrapure water produced by a NW10VF purification system (Heal Force Development Ltd., Hong Kong, China). Lactic acid, acetic acid, and hexanoic acid were obtained from Sigma-Aldrich Co. Ltd. (Shanghai, China) and maintained at -18 °C prior to use to preserve chemical stability.

2.2. Participant recruitment and screening

This study was approved by the Ethics Review Committee of Zhejiang University (Approval No. 2024-2) and conducted in accordance with the Declaration of Helsinki. To ensure that the sensory and salivary data could be directly paired at the individual level, the same group of participants from our previous TI evaluation using the identical Baijiu samples were recruited for the present study. All sixteen participants were healthy, right-handed Chinese adults, including eight females and eight males, aged between 23 and 27 years. Prior to participation, they were all screened to confirm the absence of alcohol intolerance, dependency, allergic sensitivities, or other relevant medical conditions. Each participant received a detailed explanation of the experimental procedures and provided written informed consent. Monetary compensation was offered in recognition of their time and involvement. All sixteen participants successfully completed all experimental procedures, including the validation sessions and physiological measurements. No data were excluded, and all participants were retained for statistical analysis. All training and formal tasting sessions were conducted in a purpose-built sensory evaluation room under controlled conditions (21 ± 1 °C), with participants instructed to abstain from eating, drinking (except water), and using oral hygiene products for at least 1 hour prior to each session to minimize baseline salivary variation.

2.3. Dynamic sensory evaluation and salivary collection

During the evaluation, Baijiu samples (3 mL each) were served in coded single-use plastic cups and presented in randomized order to minimize expectation bias. To prevent sensory fatigue and desensitization, the participants evaluated a maximum of three samples per day, with only one session conducted daily. All evaluations and saliva collections were performed between 09:00 and 11:00 a.m. to control for circadian variation in salivary composition (Spence, 2021). Two types of saliva were collected: unstimulated (baseline) saliva and sample-stimulated saliva. For unstimulated saliva, participants were instructed to gently and passively drool into a sterile centrifuge tube using a disposable plastic straw over a 5-minute period. This sample served as the baseline reference for each individual (Zhang et al., 2022). The procedure for collecting sample-stimulated saliva is schematically illustrated in Figure 1. Prior to each tasting, participants were instructed to rinse their mouths thoroughly with purified water and remain still and calm during a brief delay period to stabilize oral conditions. At the start of the time-intensity (TI) evaluation (defined as 0 seconds), participants introduced the Baijiu sample into the mouth and began tasting. The evaluation proceeded until the participants reported that the pungent sensation had fully subsided, at which point the TI recording was terminated. Immediately thereafter, the entire oral mixture (Baijiu plus saliva) was expectorated into a sterile 15 mL centrifuge tube. Samples were centrifuged immediately at 4 °C (500 g, 30 min) to remove oral debris, and the resulting supernatant was transferred to clean microtubes for biochemical analysis. Between

samples, participants rinsed again with purified water and rested for at least 3 minutes, or until all residual oral sensations had dissipated before proceeding to the next evaluation.

2.4 Determination of salivary biochemical properties

Four key physiological and biochemical parameters of saliva were analyzed in this study: salivary flow rate, pH, α -amylase activity, and total protein content. These indicators were selected to reflect both quantitative and functional responses of the oral environment to Baijiu stimulation. Salivary flow rate was calculated in grams per minute, based on the assumption that 1 g of saliva approximates 1 mL. To account for inter-individual variability and to better capture the stimulatory effect of Baijiu, the flow rate used for analysis was expressed as the net change between the sample-stimulated and unstimulated (baseline) salivary flow rates for each participant. To minimize pH distortion caused by CO₂ loss during centrifugation, salivary pH was measured immediately after sample collection. A digital pH meter (FE20, Mettler-Toledo, Shanghai, China) was used to assess the initial pH within the sealed collection tubes. As with flow rate, individual salivary pH response was expressed as the difference between sample-stimulated and baseline pH. Total protein content in saliva was determined using a commercial bicinchoninic acid (BCA) protein assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), following the manufacturer's instructions. The salivary α -amylase activity was measured using a

corresponding enzymatic assay kit from the same supplier. Results were expressed as enzymatic activity per milliliter of saliva (U/mL).

2.5. Integrated multivariate and mediation analysis

To systematically elucidate the relationships among Baijiu organic acid composition, salivary physiology, and dynamic sensory perception, an integrated analytical framework was applied, comprising multivariate statistical modelling followed by mediation analysis. This approach enabled both the identification of key pairwise associations and the verification of the physiological pathways linking chemical composition to sensory outcomes (Figure 1).

In the first stage, the relationship between salivary responses and dynamic sensory features was examined. Four salivary parameters were modelled against seven TI descriptors using partial least squares regression (PLSR). The parameter I_{\max} was defined as the maximum perceived pungency intensity during the evaluation. Area represented the area under the intensity-time curve, reflecting the cumulative sensory load. Plateau 90% was the duration during which perceived intensity remained at or above 90% of I_{\max} . $TI_{5\%}$ and $TI_{90\%}$ denoted the times to reach 5% and 90% of I_{\max} during the ascending phase, respectively. $TD_{90\%}$ and $TD_{5\%}$ indicated the times for the perceived intensity to decline from I_{\max} to 90% and 5%, respectively, during the descending phase. These parameters collectively characterized the temporal trajectory of oral pungency perception. Baijiu samples were categorized into subgroups

based on their TI profiles, and one-way ANOVA was used to determine whether salivary parameters differed significantly between groups. Correlation analysis was then performed to explore potential mechanistic relationships between specific salivary indicators and TI descriptors.

The second stage focused on identifying key organic acids associated with salivary variation. Hierarchical cluster analysis (HCA) based on the four salivary parameters was used to classify Baijiu samples into response-based clusters. These clusters were used to construct partial least squares discriminant analysis (PLS-DA) models in SIMCA 14.1 (Umetrics, Sweden). Prior to modeling, the data were normalized and mean-centered to eliminate scale differences. Latent variables (components) were selected automatically based on internal seven-fold cross-validation, optimizing for minimal RMSE and maximal Q^2 values. To validate model robustness and prevent overfitting, permutation tests ($n = 200$) were performed, and model quality metrics (R^2 , Q^2) are reported in Supplementary Figures. Variables with VIP scores > 1.0 were considered significant contributors, in line with widely used chemometric standards. Additional PLS-DA models were constructed for each individual salivary parameter to identify compounds selectively associated with specific physiological changes. One-way ANOVA was applied to assess group-wise differences in the concentrations of key VIP compounds, and Pearson correlation analysis was used to further quantify associations between salivary traits and organic acid levels. To correct for multiple

comparisons, false discovery rate (FDR) correction using the Benjamini-Hochberg method was applied to all ANOVA and correlation analyses.

In the final stage, mediation analysis was used to formally test whether salivary physiology acted as a mediator between organic acid composition and dynamic sensory perception. This step was essential for elucidating the physiological pathway by which Baijiu composition influences the perception of pungency. Three complementary mediation testing approaches were used. First, the causal steps procedure of Baron and Kenny was applied, estimating the total effect of the predictor on the outcome (path c), the effect of the predictor on the mediator (path a), the effect of the mediator on the outcome controlling for the predictor (path b), and the direct effect (path c'). Second, the Sobel test was conducted as a parametric significance test of the indirect effect ($a \times b$). Third, and primarily, nonparametric bootstrap resampling (2,000 iterations) using the mediation package in R was implemented to estimate the average causal mediation effect (ACME), the average direct effect (ADE), and the total effect, with 95% confidence intervals. Bootstrap-based results were taken as the main evidence due to their robustness to distributional assumptions, while the other two methods were used to triangulate and interpret the findings. This integrated multilevel analysis allowed us to move beyond simple pairwise correlations, providing a statistically rigorous mapping of the organic acid-saliva-pungency pathway.

2.6. Statistical analysis

All statistical analyses were conducted to examine the relationships among organic acid composition, salivary parameters, and dynamic sensory features. TI data were processed using SensoMaker (Compusense Inc., Canada). PLSR was performed in Microsoft Excel, and PLS-DA in SIMCA 14.1 (Umetrics, Sweden). ANOVA and data visualisation were carried out in R (version 4.3.1), with correlation analyses and matrix plots generated in Python (version 3.13.3). Mediation analysis was performed in R using the mediation package.

3. Results

3.1 Determination of salivary biochemical characteristics

The biochemical characteristics of saliva were evaluated to establish the baseline variability across samples (Table 1). The pH values ranged from 1.80 to 2.61, with most samples distributed around 2.1-2.4, showing limited variation. Salivary flow rate varied between 1.55 and 2.36 mL/min and exhibited only minor differences among samples. In contrast, pronounced fluctuations were observed in α -amylase activity and total protein content. α -Amylase activity ranged from 16,639 to 38,096 U/L, while total protein content varied from 321 to 667 mg/L. These results indicate that enzymatic activity and protein content were more sensitive to sample stimulation than pH and flow rate.

3.2. Associations between salivary parameters and sensory dynamics

To verify whether saliva could serve as a potential mediator between organic acids and sensory perception, this section aimed to establish whether salivary parameters were significantly associated with dynamic pungency attributes. PLSR was applied to explore the overall relationships between salivary physiological parameters and dynamic pungency perception. As a visualization tool, PLSR provided an overview of potential associations that were further examined by subsequent analyses (Figure S1). α -Amylase activity was most strongly aligned with TI_5% and TI_90%, indicating a potential link with the early stage of pungency perception. Total protein content and flow rate were more closely positioned with TI attributes related to I_max and Area. pH showed weak associations with all sensory parameters. TI attributes related to the decay phase, including TD_5%, TD_90% and Plateau_90%, were located farther from the main salivary parameters.

To confirm these potential associations, group comparisons were performed under two complementary schemes to evaluate differences in salivary parameters across sensory-based sample groupings (see Figure 2). In the first scheme, cluster analysis of integrated TI parameters classified the samples into three categories with distinct overall sensory profiles. In the second scheme, individual TI parameters were used to construct sample groupings based on high versus low intensity levels, allowing assessment of parameter-specific differences (Table S3). In the overall TI grouping, α -amylase activity showed significant differences between T1 and T2 and between T2 and T3, while total protein content differed between T1 and T3. When

individual TI attributes were examined, α -amylase and total protein showed significant differences across high, medium, and low categories for TI_5%, with similar trends observed for TI_90% in the high to low comparison. Total protein content also differed significantly for Plateau_90%, I_max and Area, which represent peak intensity and sustained stimulation. Flow rate exhibited significant variation for I_max and Area. For decay-phase attributes including TD_5% and TD_90%, α -amylase was the only parameter showing significant differences. pH did not show significant differences across groups.

Based on the attributes and salivary parameters showing significant differences, correlation analysis was then performed to quantify their relationships. Four salivary parameters and four important TI attributes (TI_5%, TI_90%, I_max and Area) were included (Figure 3). These TI attributes were selected for their importance in real tasting experiences and their observed associations with salivary responses in the previous analyses. Across the dataset, all salivary parameters exhibited positive correlations with early-phase TI attributes. α -Amylase activity exhibited the highest correlation coefficients, with $r = 0.76$ for TI_5% and $r = 0.63$ for TI_90%. Total protein content showed strong positive correlations with I_max ($r = 0.74$) and Area ($r = 0.78$). Flow rate presented a similar pattern, with positive correlations across all four attributes and the strongest associations with I_max ($r = 0.54$) and Area ($r = 0.56$). In contrast, pH displayed weaker and inconsistent correlations with TI attributes. Together, these analyses consistently indicated that α -amylase and total

protein were the main salivary factors associated with sensory dynamics, while pH contributed minimally. In addition, the dynamic sensory attributes TI_5%, TI_90%, I_max, and Area showed stronger and more consistent associations with salivary parameters than decay-related attributes.

3.3. Associations between organic acids and salivary parameters

To identify key organic acids that modulate salivary physiology, multivariate and univariate analyses were conducted in sequential steps. PLSR was first applied using the concentrations of 23 organic acids as predictors and four salivary parameters as responses (Figure S2). The resulting biplot displayed distinct spatial alignments between specific acids and salivary features. For α -amylase activity, acetic acid appeared in the closest proximity, suggesting the strongest association. For total protein and flow rate, hexanoic acid was the nearest, with glycolic acid and butanoic acid also located in the same quadrant. For pH, lactic acid and succinic acid were aligned in the opposite direction to total protein and flow rate, suggesting potential inverse relationships.

To further narrow down candidates, PLS-DA models were constructed under two grouping strategies: (i) comprehensive clustering of all salivary parameters into three global response groups (Figure S3), and (ii) binary classifications of individual parameters based on mean values (Table S4). All models exhibited strong performance and stability (Figures S4-S6), with high R^2Y and Q^2 values and

successful validation by permutation testing ($n = 200$). VIP scores were then calculated to identify acids contributing most to group separation, with those above 1 retained as candidates (Figure 4).

Univariate comparisons were subsequently performed to determine whether VIP organic acids also exhibited significant concentration differences across groups (Figure 4). Under the overall salivary profile grouping, acetic acid levels were significantly higher in S1 than in S2 and S3, whereas hexanoic acid was markedly lower in S3 than in the other groups. In parameter-specific analyses, butanoic acid and hexanoic acid were more concentrated in the low-pH group, while lactic acid was elevated in the low-flow group. In contrast, butanoic acid, glycolic acid, and hexanoic acid were significantly higher in both high-flow and high-protein groups. For α -amylase activity, acetic acid was significantly enriched in the high- α -amylase activity group, while octanoic acid and valeric acid were higher in the low- α -amylase activity group.

To further clarify the quantitative relationships, correlation analysis was performed between four salivary parameters and five important organic acids (Figure 5). Acetic acid showed positive associations with all salivary variables, most strongly with α -amylase activity ($r = 0.52$). Hexanoic acid, butanoic acid, and glycolic acid exhibited similar patterns, with negative correlations to pH and α -amylase, while showing positive correlations with flow rate and total protein content. Lactic acid

displayed a moderate negative correlation with flow rate ($r = -0.44$) and negligible associations with the other parameters.

Across these results, acetic acid and hexanoic acid consistently emerged as the most influential compounds, strongly associated with α -amylase, total protein, and flow rate. Butanoic acid and glycolic acid showed secondary but notable effects, while lactic acid appeared to act as a background factor with weaker but stable associations (Liu & Sun, 2018). Together, these results identified acetic acid, hexanoic acid, and lactic acid as the most representative acids for subsequent mediation testing and validation experiments.

3.4. Mediation analysis of salivary pathways linking organic acids to the perception of pungency

To examine whether salivary parameters mediated the relationship between organic acid composition and pungency perception, mediation models were constructed using four salivary indicators as candidate mediators. Seven temporal parameters of sensory dynamics were included as outcomes. Five organic acids were tested as predictors based on their concentration, statistical significance, and correlations with salivary responses. Each model specified one organic acid as predictor (X), one salivary feature as mediator (M), and one sensory attribute as outcome (Y). Baron-Kenny resampling (Table S5), Sobel tests (Table S6), and the Bootstrap (Table S7) procedure were applied to evaluate mediation effects.

In the bootstrap analysis, only one mediation pathway yielded a significant indirect effect. Including the model with hexanoic acid as predictor, pH as mediator, and TD_5% as outcome, the average causal mediation effect was estimated at -0.0637. The average direct effect was 0.0654, while the total effect was near zero. No other indirect effects reached significance. Sobel tests confirmed the absence of significant mediation, with all *p*-values exceeding 0.05. For the hexanoic acid-pH-TD_5% pathway, the *z*-value was -1.96 with a *p*-value of 0.051, indicating no statistical support for mediation. The Baron and Kenny procedure showed that some paths were significant, such as the negative association between hexanoic acid and pH, and the positive association between acetic acid and α -amylase activity. However, path b, representing the association between salivary mediators and sensory outcomes after controlling for the predictor, was not significant in any model. As a result, no full mediation pathway was established. Taken together, all three approaches consistently indicated that while organic acids influenced salivary physiology, no strong evidence supported saliva as a mediator linking organic acids to sensory perception.

3.5. Validation of Salivary Responses Induced by Key Organic Acids

To validate whether specific organic acids could directly modulate salivary physiology, two sets of experiments were conducted using both simplified model

solutions and real Baijiu matrices. Based on previous multivariate screening, lactic acid, acetic acid, and hexanoic acid were selected for validation.

In the model solution experiment, four groups were prepared with 52% ethanol as the base: ethanol only (E), ethanol with lactic acid (L, 1645.67 mg/L), ethanol with acetic acid (A, 289.22 mg/L), and ethanol with hexanoic acid (H, 748.13 mg/L).

Concentrations were adjusted to match those observed in the high-acid Baijiu sample S6 (Table S2). In the validation using the real Baijiu matrix, three representative samples were analyzed: a low-acid sample (S10), a high-acid sample (S6), and an acid-supplemented version of S10 (S10+OA), which was adjusted with lactic acid (687 mg/L), acetic acid (52 mg/L), and hexanoic acid (93 mg/L), based on the concentration differences between S10 and S6. This adjustment was made to replicate the key organic acid profile of S6 while keeping the ethanol and flavour matrix consistent with S10.

In the model solution experiment (Figure 6A), all three organic acid groups showed significantly lower salivary pH compared with the ethanol-only control ($p < 0.001$), while no significant differences were observed among the acid groups themselves. Flow rate was significantly higher in group H compared with all other groups ($p < 0.001$), whereas groups L, A, and E did not show significant difference. For α -amylase activity, all acid groups differed significantly from E. Group A exhibited the highest activity, while groups L and H were significantly lower than E.

Total protein content was significantly higher in groups A and H compared with E ($p < 0.001$), whereas group L showed a significant decrease ($p < 0.05$).

In the real matrix experiment (Figure 6B), no significant differences were observed amongst S6, S10, and S10+OA for pH or flow rate. However, α -amylase activity was significantly lower in both S10+OA and S6 compared with S10 ($p < 0.001$), with a smaller but significant difference between S6 and S10+OA ($p < 0.05$). Total protein content was significantly higher in S10+OA and S6 as compared with S10 ($p < 0.01$), with no significant difference between S10+OA and S6.

4. Discussion

The present study was designed to test whether saliva functions as a physiological mediator linking organic acids in Baijiu to dynamic changes in sensory perception. Although salivary composition has been implicated in oral sensory responses, systematic integration of chemical composition, salivary physiology, and sensory dynamics has remained limited to date. To address this gap in the literature, a multi-layered framework was applied, incorporating chemometric analyses, mediation modeling, and validation experiments. Associations between salivary parameters and sensory dynamics were first examined, followed by the identification of organic acids that most strongly influenced salivary responses. These results then formed the basis for mediation analysis to evaluate whether saliva acted as an intermediate pathway. Finally, validation experiments in both model solutions and real Baijiu matrices were

conducted to provide direct physiological evidence of acid-specific salivary effects.

Together, this framework established a structured approach to reveal mechanistic pathways underlying the perception of pungency.

Distinct associations were identified between salivary parameters and the TI features of Baijiu-induced pungency, indicating phase-specific contributions of α -amylase activity, total protein, and flow rate. Among these, α -amylase activity emerged as the most consistent predictor. Elevated α -amylase activity levels were associated with delayed onset and slower buildup (TI_5% and TI_90%), as well as prolonged decline (TD_5% and TD_90%), which suggests that α -amylase activity may influence both the initiation and resolution of pungency. This interpretation is consistent with previous reports that α -amylase activity modifies oral viscosity and facilitates the transport of compounds to trigeminal receptors (Pagès-Hélary et al., 2014; Ployon et al., 2017). Total protein showed stronger and more consistent associations with attributes reflecting intensity and persistence, including I_max and Area. Higher total protein levels corresponded to more intense and longer-lasting sensory responses, which may be explained by the known binding capacity of salivary proteins such as PRPs, mucins, and enzymes for hydrophobic irritants, potentially enhancing their oral retention and cumulative perception (Çelebioğlu et al., 2020; Enberg et al., 2001). These results suggest that total protein may function as a physiological amplifier of trigeminal stimulation, potentially contributing to inter-individual variation in sensitivity (Espinase Nandorfy et al., 2025; Fleming et al.,

2016). Flow rate showed positive correlations with intensity-related features, although the associations were weaker than those observed for total protein. This pattern may indicate that flow rate acts primarily as a reflexive marker of stimulus-driven secretion rather than as a direct determinant of sensory dynamics (Chong et al., 2021; Proctor, 2016). By contrast, salivary pH showed weak and inconsistent associations. This may be partly due to the fact that pH was measured in post-exposure saliva-stimulus mixtures, where the inherent acidity of the Baijiu samples may have masked subtle physiological pH responses. Future studies may benefit from including complementary parameters such as salivary buffering capacity or titratable acidity to better capture acid-related physiological responses. Taken together, these results suggest that salivary contributions to pungency are structured and phase-dependent, with α -amylase activity most closely linked to onset and decline, total protein to intensity and persistence, flow rate to secretion magnitude, and pH to minimal effects. Such structured associations support the interpretation that, in the context of trigeminal stimulation rather than basic taste, saliva functions as a physiological correlate of sensory dynamics. This provides a mechanistic basis for evaluating its potential mediating role between organic acids and perception.

Having established phase-specific associations between salivary physiology and sensory dynamics, the next step was to identify the organic acids most strongly associated with salivary variation, thereby providing chemical drivers of physiological response. Among the 23 quantified acids, a small subset consistently showed strong

associations with multiple salivary parameters, suggesting their key role in shaping oral responses.

Hexanoic acid, butanoic acid, and glycolic acid displayed similar response profiles, characterized by increased flow rate and total protein content accompanied by decreases in α -amylase activity and pH. This pattern suggests a coordinated protective mechanism that may involve enhanced mucinous or serous secretion together with enzymatic suppression. Such effects are consistent with prior evidence that amphiphilic or hydrophobic molecules, such as free fatty acids, can stimulate salivary gland secretion and alter saliva composition through trigeminal or epithelial interactions (Brignot & Feron, 2019; Hodson & Linden, 2004). In contrast, acetic acid exhibited a broad stimulatory pattern, showing positive associations with all salivary parameters and particularly strong links with enzymatic activity and protein concentration. These results imply that acetic acid may function as a generalized enhancer of salivary output. Lactic acid presented a distinct case. Despite being the most abundant organic acid across all Baijiu samples, its effects on salivary physiology were minimal. Only weak negative correlations with flow rate were observed, and no significant group differences were detected, suggesting that it may act primarily as a background constituent contributing to baseline buffering or microbial regulation (Y. He et al., 2025; Wang, Jing, Wang, et al., 2022; Wang, Song, et al., 2022). Taken together, these results support a functional classification of organic acids according to their physiological impact. Hexanoic, butanoic, and

glycolic acids may be regarded as activation-type acids that stimulate protein secretion while reducing enzymatic activity. Acetic acid serves as a generalized enhancer, whereas lactic acid appears to play a background role with limited acute modulation. Across these patterns, α -amylase activity and total protein concentration consistently emerged as the most responsive salivary indicators, reinforcing their potential as physiological markers that link chemical composition to oral responses (Canon et al., 2018; J. Chen, 2009; Mosca & Chen, 2017).

On this basis, formal mediation analyses were conducted to evaluate whether the identified organic acids influenced pungency perception through salivary pathways. Despite strong associations between acids and salivary parameters, and between these parameters and sensory dynamics, statistically robust indirect effects were not confirmed. Among the tested models, only one pathway involving hexanoic acid as the predictor, pH as the mediator, and TD_5% as the outcome yielded a significant bootstrap-based indirect effect. Yet the opposing direction of the direct effect resulted in an unstable and negligible total effect. Additional tests, including Sobel statistics and the Baron-Kenny procedure, did not provide statistical confirmation of mediation, as significant predictor-mediator associations were not accompanied by significant mediator-outcome effects. Several factors may account for these outcomes. Critical components of saliva, such as mucins or glycoproteins known to interact with tastants and oral surfaces, may not have been captured by the selected physiological markers. In particular, future studies could benefit from targeted profiling of proline-rich

proteins, mucins, and statherin, which have been implicated in tastant binding, mucosal adhesion, and oral lubrication. These proteins may play roles in shaping temporal attributes such as plateau intensity and decay duration during trigeminal stimulation, offering more mechanistic insight beyond total protein measures (Canon et al., 2018; Çelebioğlu et al., 2020). The timing of saliva collection may also have limited sensitivity to transient stimulus-induced fluctuations that shape dynamic perception. Furthermore, the modest sample size may have constrained the detection of subtle indirect effects. The absence of statistically validated mediation under the present design is therefore more likely to reflect methodological constraints and the inherent complexity of salivary modulation than the absence of biological relevance (Green, 1988).

To further test the inferred roles of specific acids, targeted validation experiments were conducted in both model solutions and real Baijiu matrices. In model systems, the addition of lactic, acetic, or hexanoic acid led to pronounced decreases in salivary pH compared with ethanol alone. These reductions mainly reflected the intrinsic acidity of the solutions rather than physiological modulation, suggesting that pH may not serve as a reliable indicator in this context. More informative responses were observed for flow rate, α -amylase activity, and total protein content. Hexanoic acid uniquely increased flow rate, whereas lactic and acetic acids showed no comparable effect. For α -amylase activity, acetic acid elevated enzymatic levels, while lactic and hexanoic acids reduced them, consistent with previous findings that organic acids can

modulate salivary enzyme activity(Y. Chen et al., 2025; Santos et al., 2023). In terms of total protein, acetic and hexanoic acids induced increases, while lactic acid caused a decrease. These acid-specific trends aligned with the chemometric predictions, providing experimental evidence for their physiological relevance. To assess whether these effects persisted in more complex systems, validation was also conducted in authentic Baijiu matrices. Comparison of a low-acid sample with both a high-acid sample and an acid-supplemented counterpart revealed consistent decreases in α -amylase activity and increases in total protein, whereas pH and flow rate remained stable. Notably, the acid-supplemented sample closely approximated the high-acid sample, demonstrating that targeted adjustment of organic acid composition within a real beverage matrix can reproducibly elicit predictable salivary changes.

Collectively, these validation results reinforced the chemometric findings and highlighted mechanistic roles for salivary α -amylase and total protein as sensitive indicators of organic acid influence. Acetic and hexanoic acids were confirmed as primary modulators, while lactic acid functioned as a background constituent. Although mediation analysis did not statistically confirm saliva as a causal intermediary, the convergence of multivariate screening and experimental validation underscores its potential as a physiological pathway linking organic acid composition to sensory perception. Together, these findings establish a coherent framework that connects chemical composition, salivary physiology, and sensory outcomes, offering mechanistic insight into pungency modulation in alcoholic beverages.

5. Conclusion

This study investigated whether saliva could serve as a physiological pathway linking organic acids in Baijiu to dynamic pungency perception. The results demonstrated that α -amylase activity and total protein content were the salivary parameters most closely associated with sensory dynamics. Higher α -amylase activity delayed the onset and prolonged the decline of pungency, whereas elevated protein levels corresponded to stronger and more persistent intensity. Among the organic acids, acetic acid broadly stimulated enzymatic activity and protein secretion, hexanoic acid enhanced protein while suppressing α -amylase, and lactic acid exerted little acute effect, consistent with its background role. These acid-specific effects were reproducible in both model solutions and real Baijiu matrices, establishing α -amylase and total protein as reliable physiological markers of organic acid influence. Mediation analysis did not statistically confirm saliva as a causal intermediary, which may reflect limited biomarker coverage, fixed-time saliva sampling, and modest sample size. These constraints likely reduced sensitivity to transient physiological changes that directly modulate sensory responses. Additionally, our current modeling strategy did not explicitly incorporate subject-level random effects or repeated trial-wise data, which may have further limited the detection of indirect effects. Despite this, the consistent convergence between chemometric analyses and validation experiments provides converging evidence that salivary physiology plays an

important role in modulating oral perception. Future studies incorporating higher-resolution salivary profiling, time-resolved sampling, and mixed-effects modeling frameworks may help more precisely delineate causal pathways between chemical stimuli and dynamic perceptual responses. Moreover, the potential synergistic or antagonistic interactions among organic acids warrant further investigation, ideally through full-factorial experimental designs that can systematically evaluate combination effects on salivary and sensory responses.

Overall, this work provides mechanistic insight into how organic acids shape salivary physiology in distinct and reproducible ways, contributing to the biochemical basis of pungency perception. These findings advance understanding of saliva's role in sensory science and provide a physiological basis for exploring and modulating flavor perception in complex food and beverage systems.

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AUTHOR CONTRIBUTIONS

Guangnan Wang: Conceptualization, Writing - Original Draft; **Charles Spence:** Supervision, Writing - Review & Editing; **Zhuoshun Dai:** Investigation, Methodology; **Fuping Zheng:** Conceptualization, Writing - Review & Editing; **Xingqian Ye:**

Conceptualization, Writing - Review & Editing; **Baoguo Sun:** Resources, Supervision;

Huan Cheng: Resources, Supervision

NOTES

The authors announce that they have no conflicts of interest concerning the publication of this manuscript.

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Table 1 Salivary biochemical properties in response to stimulation by different Baijiu samples.

| | pH ^a | Flow rate ^a | Amylase activity | Total protein |
|-----|-----------------|------------------------|-------------------|---------------|
| S1 | 1.80±0.43 | 2.36±0.70 | 37489.18±12312.52 | 622.02±231.33 |
| S2 | 2.31±0.62 | 2.31±0.86 | 38095.58±8387.84 | 666.77±164.06 |
| S3 | 1.99±0.50 | 2.24±0.94 | 26515.08±9022.92 | 365.83±128.39 |
| S4 | 2.34±1.00 | 1.99±0.52 | 27986.12±8393.92 | 320.65±85.33 |
| S5 | 2.12±0.63 | 2.29±0.89 | 16638.51±779.59 | 557.65±272.13 |
| S6 | 2.19±1.03 | 2.01±0.53 | 17638.09±4365.26 | 442.87±188.57 |
| S7 | 2.61±1.20 | 2.07±0.84 | 21563.21±7310.48 | 435.43±131.53 |
| S8 | 2.40±0.88 | 1.55±0.53 | 21336.67±8768.77 | 354.35±165.56 |
| S9 | 2.33±0.61 | 1.83±0.50 | 22191.86±8028.37 | 392.40±129.80 |
| S10 | 2.16±0.46 | 2.07±0.82 | 21168.21±9722.66 | 344.62±94.70 |
| S11 | 2.17±0.85 | 1.70±0.68 | 18605.33±6326.82 | 336.42±110.85 |

^apH values and flow rate represent the difference relative to each participant's non-stimulated baseline.

Figure Captions

Figure 1: Schematic of the experimental workflow.

Figure 2: Comparison of salivary parameters across groups defined by time-intensity sensory attributes. Statistical significance was evaluated by one-way ANOVA followed by Benjamini-Hochberg FDR correction. Asterisks indicate significant differences.

Figure 3: Correlation analysis between time-intensity attributes and salivary parameters in Baijiu samples.

Figure 4: Comparison of VIP organic acid concentrations between salivary response-based groups. Statistical significance was evaluated by one-way ANOVA followed by Benjamini-Hochberg FDR correction. Asterisks indicate significant differences.

Figure 5: Correlation analysis between key organic acids and salivary physiological parameters.

Figure 6: Validation of the effects of organic acids on salivary parameters using model solutions (A) and real Baijiu samples (B). In Panel A: E, ethanol-only control; L, lactic acid model; A, acetic acid model; H, hexanoic acid model. In Panel B: S10, low-acid Baijiu sample; S10+OA, low-acid sample supplemented with key organic acids; S6, high-acid Baijiu sample. Statistical significance was evaluated by one-way ANOVA followed by Benjamini-Hochberg FDR correction. Asterisks indicate significant differences.

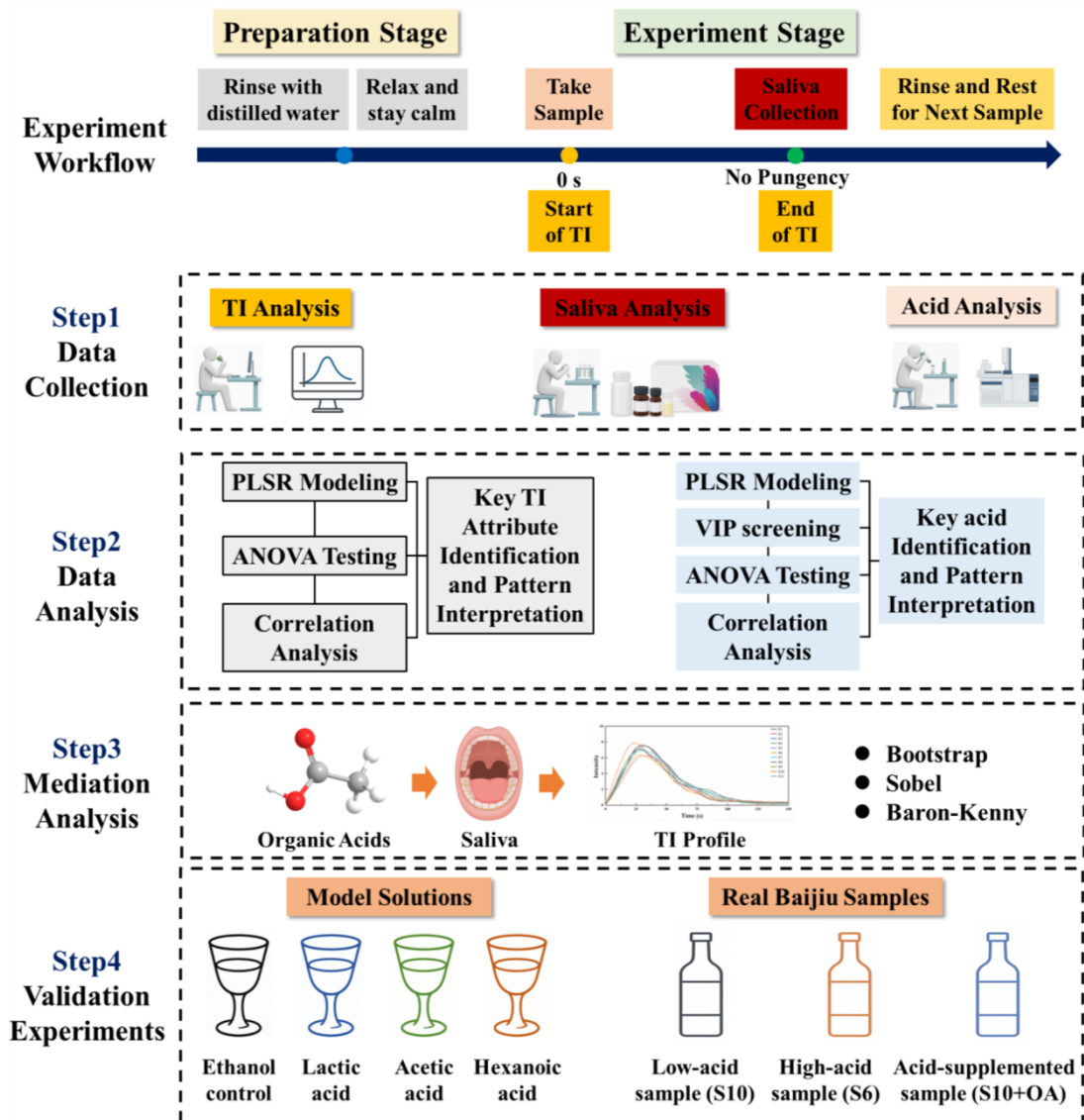


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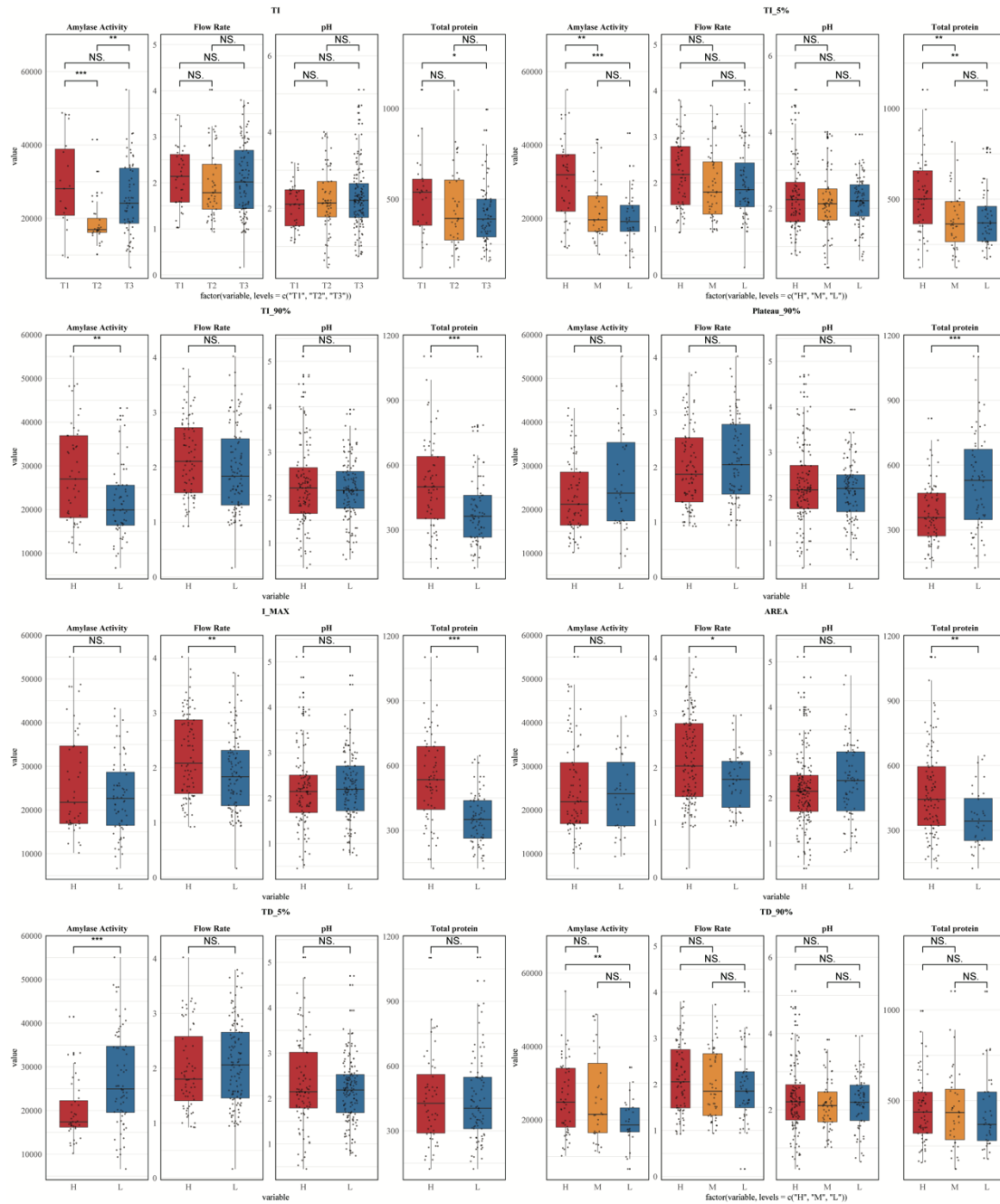


Figure 2: Comparison of salivary parameters across groups defined by time-intensity sensory attributes. Statistical significance was evaluated by one-way ANOVA followed by Benjamini-Hochberg FDR correction. Asterisks indicate significant differences.

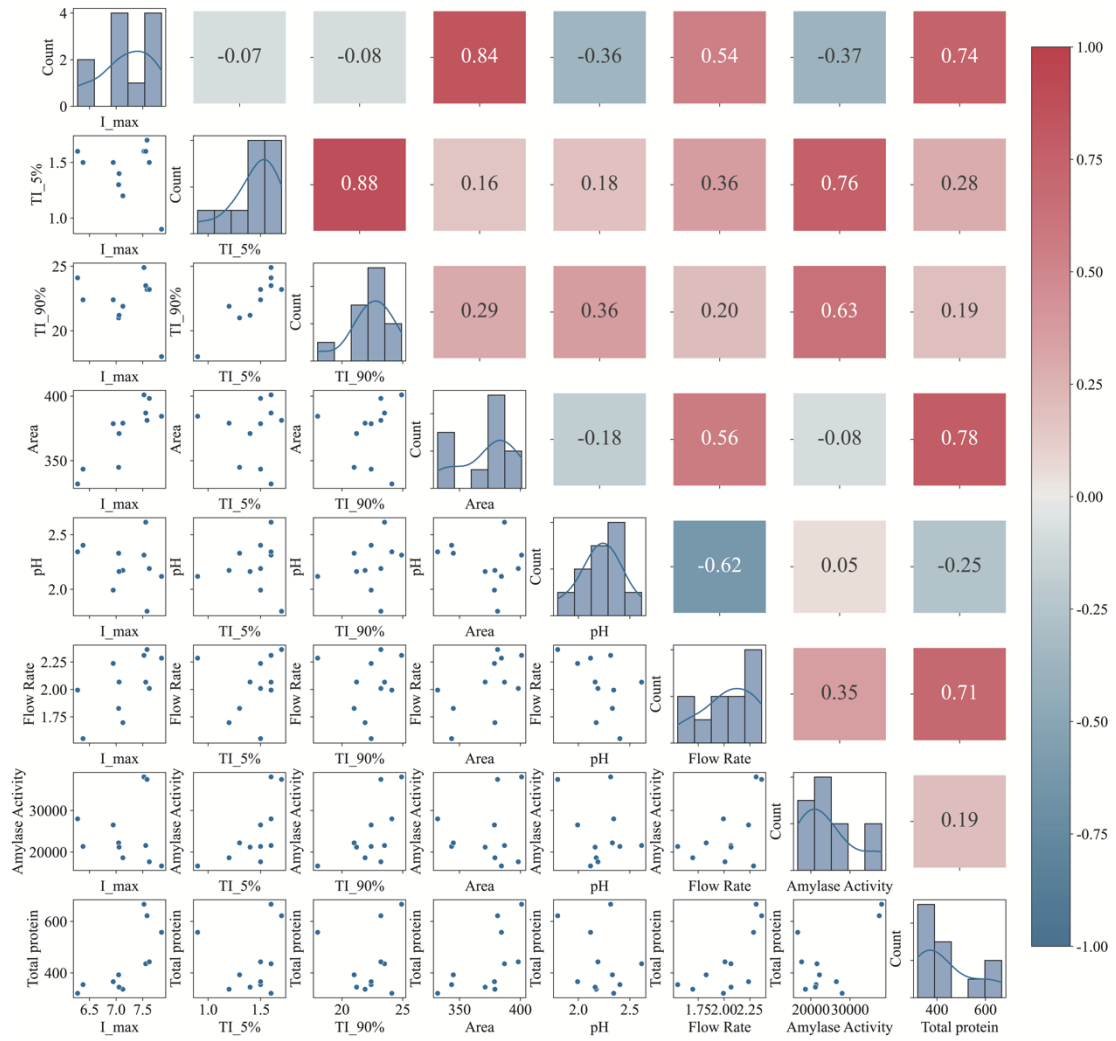


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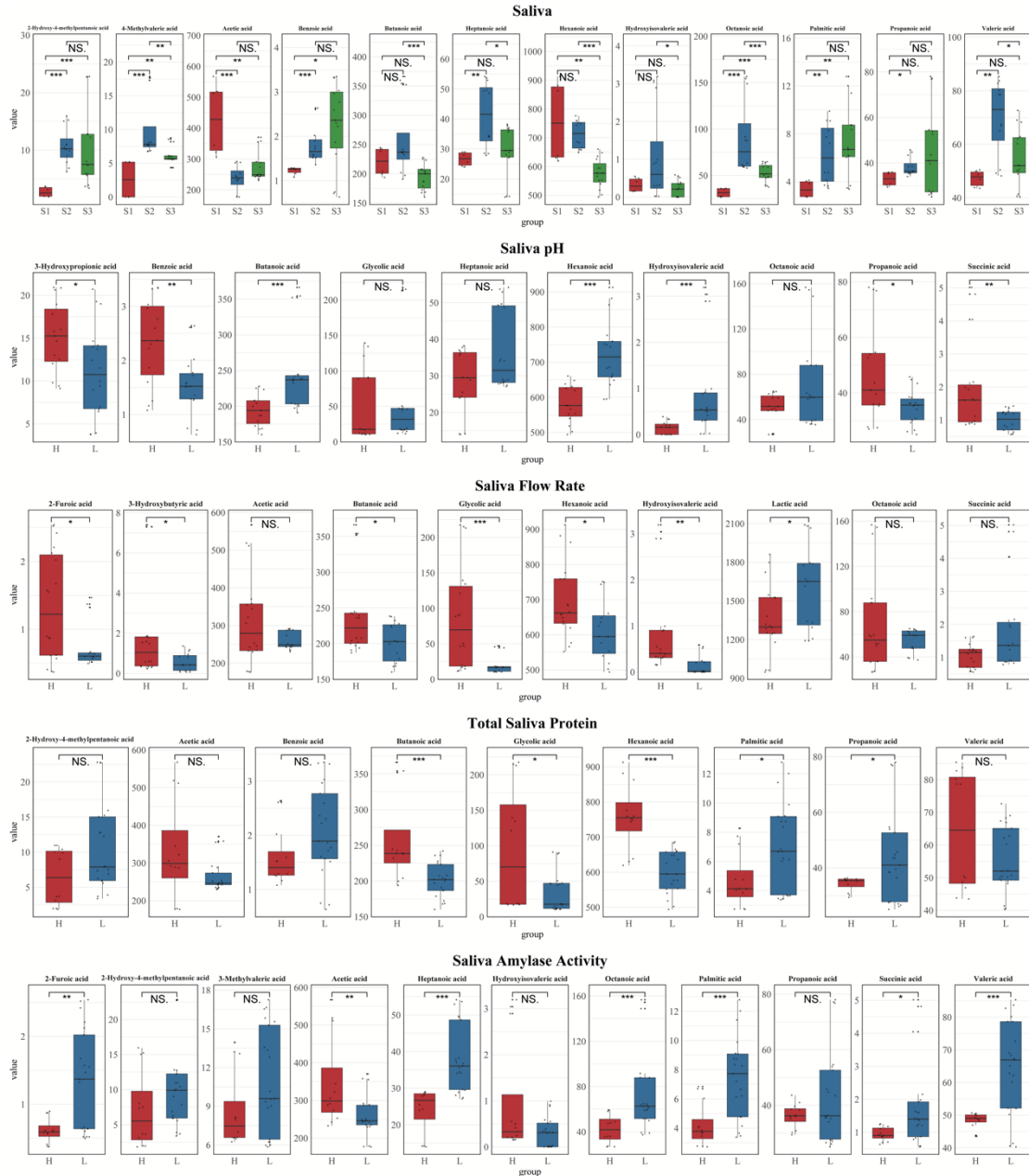


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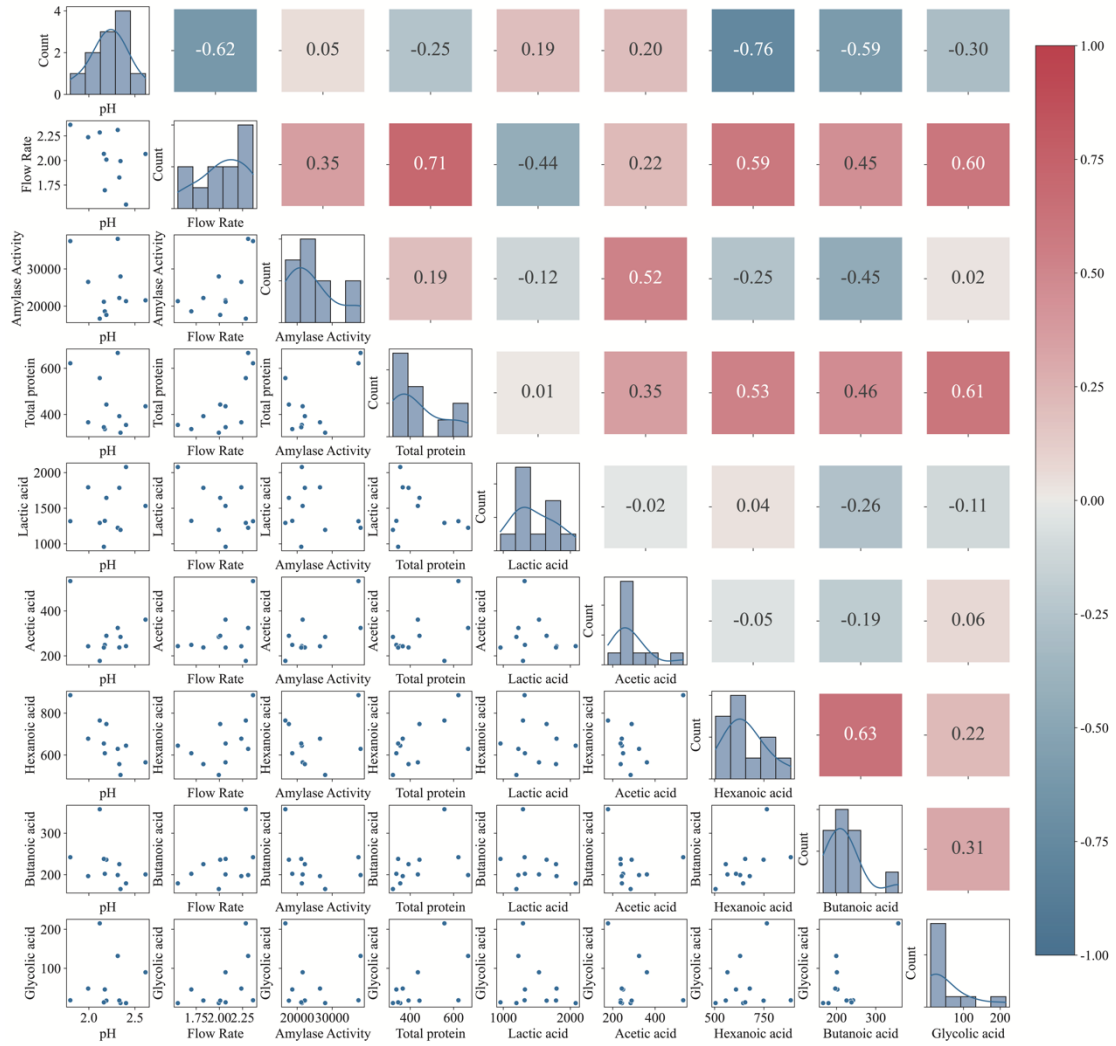


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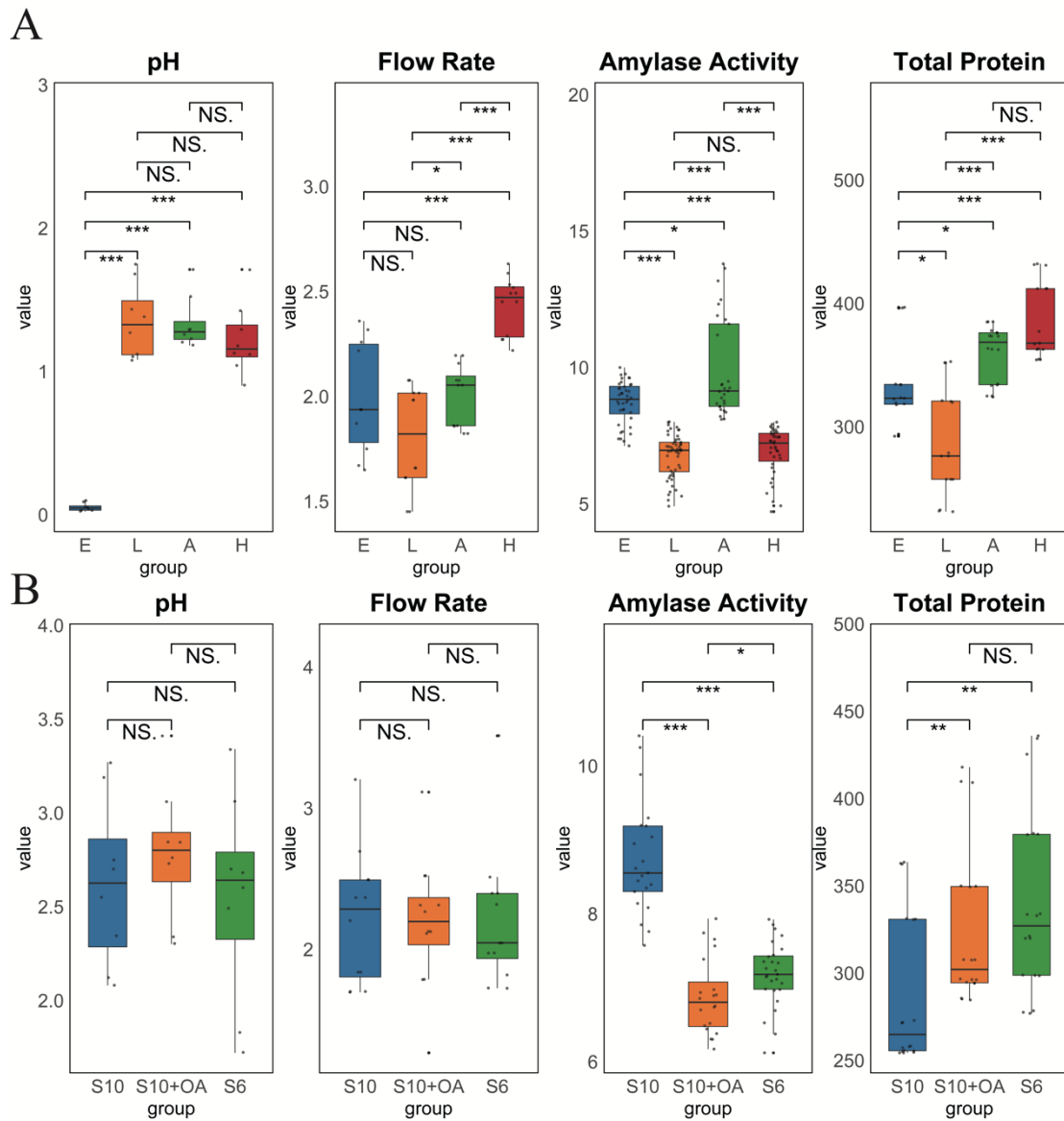
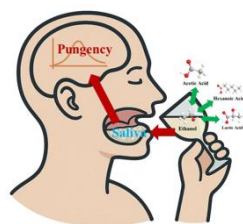
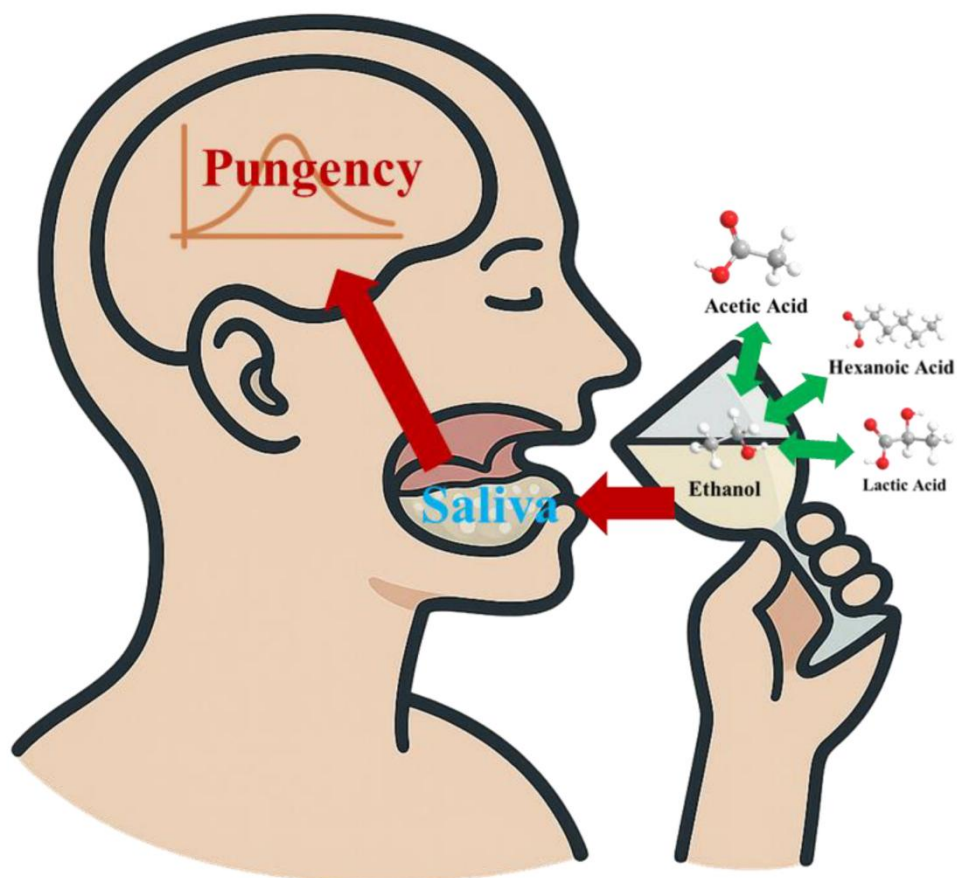


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“TOC graphic”

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1. Salivary α -amylase and protein shape pungency dynamics and respond to acids.
2. Acetic and hexanoic acids modulate saliva; lactic acid plays a minor, stable role.
3. Saliva may modulate the sensory impact of Baijiu's organic acid composition.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: