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Sensory study of potential kokumi compounds in wine

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Abstract. Kokumi is a complex flavour sensation enhancing mouthfeel and continuity in various foods, but its role in wine remains unexplored. This study investigates the putative kokumi sensory effects of Glycyl-l-Valine (Gly-Val), a dipeptide identified in sparkling wines with other oligopeptides. Trained panellists evaluated the impact of Gly-Val in model wine (MW), white wine (WW), and sparkling base wine (SBW) using triangle tests (T), three alternative forced choice test (3-AFC) and descriptive analysis with relative reference scaling (DA with RR scaling). In T the odd sample with Gly-Val was correctly identified only at the highest concentration (120 mg/L) in MW, while at lower concentrations in WW (15-60 mg/L) and SBW (30-90 mg/L). Detection thresholds of Gly-Val, estimated by 3-AFC, ranged from 15 to 60 mg/L in WW, while no threshold was determined in MW. These results show that Gly-Val is more discernible in complex wine matrices compared to model wine. No significant variations ($\alpha \geq 0.05$) were found in the DA with RR scaling, but descriptors trends suggest that Gly-Val may enhance smoothness, mouthfeel and kokumi at lower concentrations. These findings suggest that the kokumi dimension in wine needs further exploration, with Gly-Val identified as a potential kokumi-active compound requiring more sensory research.

1. Introduction

Kokumi is a complex flavour sensation characterised by thickness, mouthfulness and continuity, and perceived as enhanced palatability. In other words, under the influence of kokumi substances, foods/beverages tastes become more flavourful with increased intensity, spread, continuity, richness, harmony, and punchiness which are the six related characteristics corresponding to the kokumi sensory concept [1]. Moreover, kokumi active molecules can enhance the properties of umami substances, to which they have been closely associated [1]. Kokumi active peptides are distributed in many foodstuffs and relevant in fermented ones due to yeasts derived oligopeptides. Kokumi peptides identified from yeast extract, that showed different sensory power tested by sensomic approach, are: five leucyl dipeptides, γ-glutamyl dipeptides and the well-known kokumi-active glutathione (GSH) [2, 3] that is a tripeptide (γ-Glu-Cys-Gly) also present in grape and wine. Recent studies on the free amino acid content of fermented beverages [4] have suggested that beverages having long contact with yeast may contain higher amounts of free glutamate and therefore have a greater capacity to impart umami than beverages with little or no contact with yeast. Wine is the fermented beverage for the which the sensory features represent one of the main quality characteristic driving appreciation and purchase. Among the most worldwide renowned wines, sparkling produced with Champenoise method, involve a second fermentation of the base wine in the bottle followed by an aging period in contact with yeast lees.

Despite these premises, the presence and the sensory impact of kokumi substances in wine has been almost not explored. It has been stated that kokumi for wine is customarily expressed as "body" [5], but no evidence is reported and the kokumi sensory dimension in wine is currently unknown. This gap of knowledge is here addressed by reporting results of a first exploratory sensory study testing the in-mouth impact of Gly-Val, a new putative kokumi oligopeptide detected in wine in the frame of an ongoing chemical study that quantitatively characterized the oligopeptide pattern of classic sparkling wines by UHPLC-ESI-MS/MS (data not showed).

2. Materials and methods

2.1. Materials

Food-grade tartaric acid, sucrose, caffeine, sodium chloride, and tannic acid, monosodium glutamate (MSG), glutathione (GSH) and ethanol (EtOH) were used for

sensory analysis. L-Leucyl-l-Alanine (Leu-Ala) and Glycyl-l-Valine (Gly-Val) were synthesised (purity>98%; Chemspace LLC, USA) and confirmed as safe (ToxibPred, http://crdd.osdd.net/raghava/toxinpred/).

Three different matrices, a model wine (MW: 12% v/v EtOH, 6.30 TA, 3.0 pH), a white wine (WW: 10.5% v/v EtOH, 6.10 TA, 3.2 pH), and a sparkling base wine (SBW: 12% v/v EtOH, 6.37 TA, 3.0 pH), were used for sensory trials.

2.2. Panel

Panellists (22–53 years old; 15 females, 17 males, wine experts) were recruited among students and researchers at the Department of Agricultural Sciences, Division of Vine and Wine Sciences (University of Naples Federico II). Procedures were conducted in accordance with the ethical standards and last amendments of the 1964 Helsinki declaration and approved by the Ethics Committee for Non-Biomedical Human Research (CERSUB) of University of Naples Federico II (PG/2024/0037120). Prior to the experiments, tasters were required to sign an informed consent form disclosing the type of research, voluntary participation and agreement to taste and spit reference solutions and wines. All data were collected anonymously.

2.3. Procedure

In all sessions, 40 mL for each sample were served in covered clear ISO glasses [6] labelled with three-digit code. Samples were served at room temperature (21±1°C) and evaluated in individual booths [7]. Panellists were instructed to rinse their mouth with bottled still water between each sample and/or triad of samples/sample.

2.3.1. Training sessions

During the initial sessions, using still bottled water (Sorgesana, Italy), and in accordance with concentrations reported by the OIV [8], panellists evaluated reference solutions corresponding to sour (tartaric acid: 0.5 and 1 g/L), sweet (sucrose: 6 and 14 g/L), bitter (caffeine: 1.5 and 2 g/L), salty (NaCl: 2 and 5 g/L), umami (MSG: 0.3 and 0.9 g/L), and astringency (tannic acid: 0.75 and 1.25 g/L) sensations and ranked the acidity intensity of tartaric acid solutions at 0, 0.25, 0.5, 0.75, and 1 g/L.

Ten specific sessions were dedicated to train the panel on the concept and complex sensations of kokumi. First, GSH, a kokumi-active compound, was added to still bottled water and white wine both alone and in combination with other taste agents, in concentrations reported in literature [2, 9-11]: i) GSH at 1.54 g/L, ii) GSH (0.2 g/L) + MSG at two concentrations (0.9 g/L and 3.5 g/L), iii) tartaric acid + sucrose + MSG (0.5 g/L, 6 g/L, and 0.3 g/L, respectively) with and without GSH at 0.2 g/L. Then, GSH was added in white wine at three concentrations reported in wine (low: 10 mg/L, medium: 20 mg/L, high: 30 mg/L) [12] to further familiarise panellists with the kokumi concept and to recognise and describe the kokumi sensations. Moreover, to help developing a consensus vocabulary for kokumi sensations, Leu-Ala, a dipeptide reported to exhibit kokumi properties [2], was also employed. GSH (30 mg/L) and Leu-Ala at its threshold (708 mg/L= 3.1 mM [2]) were added to water, model wine (12% v/v EtOH, 6.3 g/L titratable acidity, with pH adjusted to 3.0 using NaOH 1.0 M), and sparkling base wine adjusted to two pH levels (3.0 with H_2SO_4 1.0 M and 3.8 with NaOH 1.0 M). A consensus vocabulary was developed based on descriptors and conceptual references associated with the kokumi concept, and included kokumi and its flavour characteristics: smoothness, harmony, mouthfulness, drying, and long-lasting flavour [1, 5]. A focus group was conducted for the familiarisation with this vocabulary using the dipeptide under investigation, Gly-Val, at 9 different concentrations (ranging from 3.75 to 180 mg/L) along with a control white wine (pH 3.2) without Gly-Val.

Finally, further training sessions focused on sensory test procedures and scales, including triangle tests (T), 3-alternative forced choice trials (3-AFC) and descriptive analysis with relative to reference scaling (DA with RR scaling) [13], utilising a 15-cm line scale with 3 anchor points, low (=1.5), medium (=7.5= reference= white wine), and high (=13.5) intensities [14].

2.3.2. Analysis sessions

T test [15]: trained panellists conducted triangle tests to determine whether Gly-Val could be orally discriminable in the three different wine matrices (MW, WW, and SBW). Six peptide solutions were prepared for each matrix, starting with an initial Gly-Val concentration of 120 mg/L. The samples were gradually diluted 1:1 (v/v) from 120 mg/L to 7.5 mg/L, with an additional sample prepared at 90 mg/L.

3-AFC test [16]: a group of 10 selected subjects participated in 3-AFC trials to determine the oral detection threshold (DT) of Gly-Val at concentrations of 7.5, 15, 30, 60, and 90 mg/L. the objective was to identify the concentration at which participants could detect that the matrix (MW or WW) contained an additional component, distinguishing the odd sample from the other two in a set of three two consecutive replicates as described by Haryono et al. [17].

DA with RR scaling [13]: the 10 selected subjects tested the potential kokumi sensory effect of Gly-Val in white wine using DA with RR scaling. The study assessed the in-mouth modulating effects of added Gly-Val at 15, 30 and 60 mg/L in white wine, with a fourth sample being a control white wine without added Gly-Val (0 mg/L). Panellists were provided with a reference wine (white wine without Gly-Val) to taste before evaluating each sample. During the evaluation, they assessed sensations such as sweetness, saltiness, sourness, bitterness, as well as kokumi-related descriptors developed during training (smoothness, harmony, mouthfulness, drying, and long-lasting flavour). The evaluations were conducted in 2 repetitions, with the intensity of the attributes measured using a 15-cm scale. Panellists were informed that the

mean anchor point (7.5) corresponded to the reference (white wine not spiked with Gly-Val).

2.4. Statistical analysis

The significance of the triangle test was determined according to ISO 4120:2021(E) [15].

Analysis of variance (ANOVA; Tukey and HSD multiple comparison, α=0.05) were performed on data from DA with RR scaling ANOVA was run on the differences with Gly-Val concentration as a fixed effect and the panellist as a random effect.

The XLSTAT (2020.5) software (Addinsoft 2020) was used.

3. Results

Gly-Val has been identified among a pool of 11 putative kokumi peptides and the L-glutamic acid, that were all found in a representative set (34) of classic sparkling wines at total oligopeptides concentration ranging from 9 to 33 mg/L. This pool also included already known kokumi-active peptides such as L-glutathione and Leu-Ala [1, 2]. Although Gly-Val has not been previously reported in the literature as a kokumi substance, it has been chosen as representative of the pool of investigated peptides and therefore tested within the detected total concentration range. This preliminary study aimed to investigate and possibly deny the possibility that Gly-Val could act as a kokumi compound in white wine.

Table 1 shows the results of the T tests performed on the three wine matrices added with Gly-Val.

Table 1. Results of the triangular tests (T) performed on the three wine matrices with different amounts of added Gly-Val.

Added Gly-Val (mg/L)	MW (32 subjects)	WW (26 subjects)	SBW (16 subjects)
7.5	ns	ns	ns
15	ns	***	***
30	ns	***	**
60	ns	***	*
90	ns	ns	***
120	**	nd	ns

ns: not significant; nd: not determined; significance *, **, ***: α≥0.05, 0.01, 0.001.

Results show that the significance (at least α=0.05) of the T test varied depending on the matrix and Gly-Val concentration. In MW, panellists were able to correctly identify the odd sample only at the highest concentration of 120 mg/L Gly-Val. In both real wines, the test was significant at lower concentrations, specifically 15-60 mg/L Gly-Val in WW and 30-90 mg/L Gly-Val in SBW. This suggests that the odd samples spiked with Gly-Val were more discriminable in more complex matrices of real wines compared to the model wine. The odd sample was correctly identified by 57% of panellists for MW, compared to 73% in WW and 75% in SBW. Moreover, the ability to distinguish the added dipeptide at different concentrations in WW and SBW might be related to their compositional differences. The smoother features of WW with lower ethanol content (10.5% v/v), total acidity (6.10 g/L) and higher pH (3.2) compared SBW (12% v/v, 6.37 g/L, 3.0, respectively) seemed to favour Gly-Val perception. The findings from the 3-AFC test support this observation. Indeed, after two testing sessions, the detection threshold (DT) for Gly-Val ranged in real white wine was found from 15 to 60 mg/L, while no DT range could be established for the model wine at the investigated concentrations (Figure 1). In WW, the highest number of replicated correct answers (7/10) was at 15 mg/L Gly-Val, followed by a decreasing trend at higher concentrations-6/10 at 30 mg/L and 5/10 at 60 mg/L Gly-Val. This behaviour highlights nonlinear sensory activity at increasing levels of the peptide.

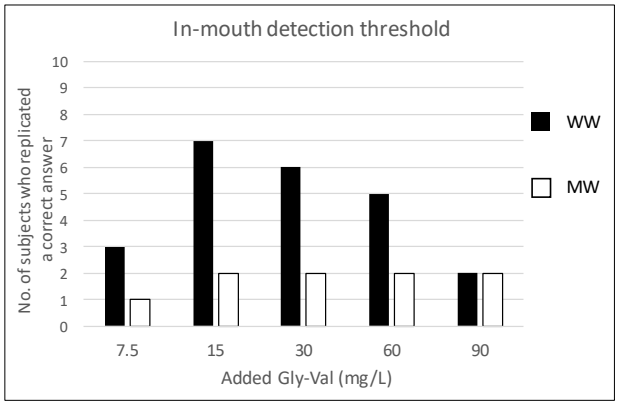

Figure 1. 3-AFC test results: number of subjects that correctly identified the sample added with Gly-Val at 7.5, 15, 30, 60 and 90 mg/L, in two consecutive repetitions.

Based on these results, the potential kokumi sensory effect of Gly-Val in WW was further evaluated using DA with RR scaling. The modulating effects of Gly-Val at 15, 30 and 60 mg/L in WW were assessed. Although the ANOVA test did not highlight any significant variation for the in-mouth features, some aspects seem of interest (Figure 2).

Results referring to the "Reference" samples show that the panel was repeatable in the evaluation of the 5 descriptors. The trend of the kokumi descriptor shows that panellists were consistent in their perception of kokumi, with intensity trends mirroring the in-mouth detection threshold at the same concentrations (Figure 1, black histograms). At 15 mg/L, Gly-Val tended to slightly enhance smoothness, mouthfulness and kokumi. This latter is stable at the higher Gly-Val concentrations (30 and 60 mg/L), while the smoothness, mouthfulness tended to diminish contrary to acidity and bitterness that tended to be perceived as more intense.

The absence of significant differences might be partially attributed to the limited sensitivity of DA with RR scaling compared to traditional DA [14]. However, this method proved useful in terms of repeatability, likely because the reference comparison was helpful in evaluating a complex and acquired sensation such as kokumi. These preliminary results open to future sensory studies.

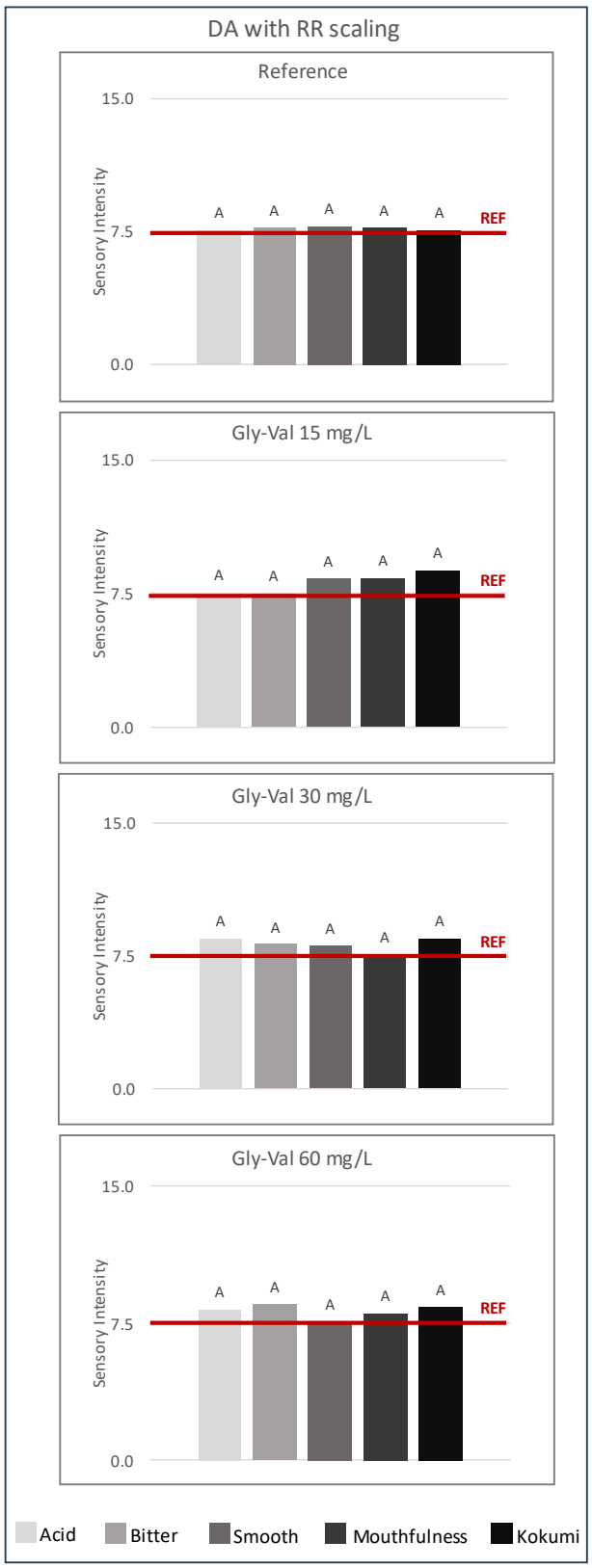

Figure 2. DA with RR scaling results: mean intensity of sensory descriptors measured at different Gly-Val concentrations. Letters refer to significant differences (α≥0.05) between samples within each descriptor. The horizontal light-blue line represents the reference (REF: added Gly-Val 0 mg/L) set at 7.5.

4. Conclusions

The preliminary results here reported support the hypothesis that the kokumi effect exists in white wine and therefore the kokumi dimension need to be explored in wine. The addition of the dipeptide Gly-Val in quantities like the sum of the kokumi oligopeptides found in sparkling wines allowed a trained panel to recognize the spiked wines. Data showed that Gly-Val can be a potential kokumi-active dipeptide in complex matrices at concentrations ranging from 15 to 60 mg/L. Indeed, a kokumi compound is intended as a molecule able to modulate in-mouth sensations due to other sensory active molecules present in the matrix [1]. As a result, Gly-Val could be significantly detected in real white wines, allowing for the estimation of its detection threshold range. However, this was not achievable in model wine, indicating that Gly-Val may only exhibit sensory activity in the presence of other compounds found in real wine but absent in the simplified model wine. According to these first results, the kokumi activity of Gly-Val cannot be directly explicated in wine where it has been detected in traces, but it should be investigated in combination with the other oligopeptides detected in wine, as well as in other food matrices.

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