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# Effect of a *kokumi* peptide, $\gamma$ -glutamyl-valyl-glycine, on the sensory characteristics of chicken consommé

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**Abstract**

**Background:** Recent studies have demonstrated that *kokumi* substances such as glutathione are perceived through the calcium-sensing receptor (CaSR). Screening by a CaSR assay and sensory evaluation have shown that  $\gamma$ -glutamyl-valyl-glycine ( $\gamma$ -Glu-Val-Gly) is a potent *kokumi* peptide. In the present study, the sensory characteristics of chicken consommé with added  $\gamma$ -Glu-Val-Gly were investigated using descriptive analysis.

**Results:** Chicken consommé containing  $\gamma$ -Glu-Val-Gly had significantly stronger “umami” and “mouthfulness” (mouth-filling sensation) characteristics than the control sample at a 99% confidence level and significantly stronger “mouth-coating” characteristic than controls at a 95% confidence level.

**Conclusions:** These data suggest that a *kokumi* peptide,  $\gamma$ -Glu-Val-Gly, can enhance umami, mouthfulness, and mouth coating, implying that the application of this peptide could contribute to improving the flavor of chicken consommé.

**Keywords:** Chicken consommé, Kokumi,  $\gamma$ -Glutamyl-valyl-glycine,  $\gamma$ -Glu-Val-Gly, Sensory evaluation, Descriptive analysis

**Background**

Taste and aroma are important factors in determining the flavor of foods. Sweet, salty, sour, bitter, and umami comprise the five basic tastes with each taste being recognized by specific receptors and associated with particular transduction pathways. However, foods have sensory attributes that cannot be explained by aroma and the five basic tastes alone: texture, continuity, complexity, and mouthfulness. Ueda et al. investigated the flavoring effects of a diluted extract of garlic that enhanced continuity, mouthfulness, and thickness when added to an umami solution and attempted to isolate and identify the key compounds responsible for this effect [1]. Their study indicated that sulfur-containing compounds such as S-allyl-cysteine sulfoxide (alliin), S-methyl-cysteine sulfoxide,  $\gamma$ -glutamyl-allyl-cysteine, and glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH) led to this flavoring effect. These compounds have only a minimal flavor in water, but if added to an umami solution or other types of food, they can substantially

enhance the thickness, continuity, and mouthfulness of the food to which they have been added [2]. They proposed that substances with these properties should be referred to as *kokumi* substances.

Recently, it was reported that *kokumi* substances such as GSH are perceived through the calcium-sensing receptor (CaSR) in humans [3]. These studies confirmed that GSH can activate human CaSR, as can several  $\gamma$ -glutamyl peptides, including  $\gamma$ -Glu-Ala,  $\gamma$ -Glu-Val,  $\gamma$ -Glu-Cys,  $\gamma$ -Glu- $\alpha$ -aminobutyryl-Gly (ophthalmic acid), and  $\gamma$ -Glu-Val-Gly. Furthermore, these compounds have been shown to possess the characteristics of *kokumi* substances, which modify the five basic tastes (especially sweet, salty, and umami) when added to basic taste solutions or food, even though they have no taste themselves at the concentrations tested [1,2,4-8]. The CaSR activity of these  $\gamma$ -glutamyl peptides has also been shown to be positively correlated with the sensory activity of *kokumi* substances, suggesting they are perceived through the CaSR in humans. Among these,  $\gamma$ -Glu-Val-Gly has been reported to be a potent *kokumi* peptide with a sensory activity 12.8-fold times greater than that of GSH [3]. Additionally, it has been reported that  $\gamma$ -Glu-Val-Gly

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was present in several foods such as scallops [9], fermented fish sauces [10], soy sauces [11], and fermented shrimp pastes [12]. Ohsu et al. also reported that adding 0.01%  $\gamma$ -Glu-Val-Gly to 3.3% sucrose solution, 0.9% NaCl solution, and 0.5% monosodium glutamate (MSG) solution significantly enhanced sweetness, saltiness, and umami, respectively [3]. They also reported that adding 0.002%  $\gamma$ -Glu-Val-Gly to chicken consommé prepared from commercial chicken consommé powder significantly enhanced thickness, continuity, and mouthfulness. In that report, sensory evaluation was undertaken with sensory attributes with reference to a method reported previously [1,2]. The sensory attributes used in these previous research works such as thickness, mouthfulness, and continuity were originally extracted using the sensory evaluation which compared the sensory profiles of various foods, mainly soups, with and without MSG [13]. Therefore, to clarify the sensory characteristics of food with added  $\gamma$ -Glu-Val-Gly, a more detailed study comparing the sensory attributes of food with and without this peptide has been needed.

In the present study, we aim to characterize the sensory properties of food with added  $\gamma$ -Glu-Val-Gly, through performing a descriptive analysis of chicken consommé containing the peptide.

## Results and discussion

### Sensory attributes for chicken consommé

During the project-specific orientation session, the panelists developed 17 attributes shown in Table 1. Regarding the attributes related to chicken flavor, since many words related to the chicken flavor were proposed during the project-specific orientation session, three attributes “total chicken/meaty flavour”, “bones/marrow flavour”, and “roasted flavour” were added to the list. Total chicken/meaty flavor was defined as the flavor intensity reminiscent of cooked chicken meat; bones/marrow flavor was defined as the character associated with chicken bones, particularly the marrow of chicken bones; and roasted flavor was defined as the total flavor intensity that is reminiscent of roasted chicken and/or vegetables. Additionally, because the coating sensation was well recognized when the panelists evaluated the chicken consommé with  $\gamma$ -Glu-Val-Gly during the project-specific panel orientation session, the attributes “mouth-coating” and “tongue-coating” were added to the list. “Mouth-coating” was defined as the degree to which there is a leftover residue, a slick, powdery, or fatty coating or film on the mouth that is difficult to clear. “Tongue-coating” was defined as the degree to which there is a leftover residue, a slick, powdery, or fatty coating or film on the tongue that is difficult to clear. Overall, the panelists defined the 17 sensory attributes for chicken consommé listed in Table 1: nine taste and flavor attributes (total flavor, total chicken/meaty flavor, chicken flavor, bones/marrow, roasted flavor,

total vegetable flavor, richness, salty, and umami), seven texture/mouthfeel attributes (viscosity, mouthfulness, mouth coating, tongue coating, salivating, total trigeminal, and swelling perception of soft tissue), and one aftertaste (total aftertaste). The definitions of these sensory attributes and the references are shown in Table 2.

### Sensory characteristics of chicken consommé with added $\gamma$ -Glu-Val-Gly

The sensory characteristics of chicken consommé with or without  $\gamma$ -Glu-Val-Gly are shown in Table 2 and Figure 1. The addition of  $\gamma$ -Glu-Val-Gly at 5 ppm significantly enhanced the intensity of umami and mouthfulness at a 99% confidence level. Furthermore, the addition of this peptide significantly enhanced the intensity of mouth coating at a 95% confidence level. Adding this peptide at 5 ppm did not significantly change the intensity of the other attributes. A recent study has suggested that *kokumi* peptides such as GSH and  $\gamma$ -Glu-Val-Gly enhance the intensity of umami if they are added to 0.5% MSG solution [3], an observation consistent with the present study. Additionally, in the descriptive analysis, umami has been defined not only as the “taste of MSG” but also as “the mouth-filling sensation of compounds such as glutamates that is savoury, brothy, meaty, rich, full, and complex, which is common to many foods such as soy sauce, stocks, ripened cheese, shellfish, mushrooms, ripened tomatoes, cashews, and asparagus”. Therefore, it appears that the enhancement of umami in chicken consommé includes the enhancement of sensations such as richness and complexity. The present results also suggest that  $\gamma$ -Glu-Val-Gly also enhanced mouthfulness. A previous study demonstrated that adding  $\gamma$ -Glu-Val-Gly at 20 ppm to chicken soup significantly enhanced mouthfulness which is consistent with the present study [3]. Regarding other  $\gamma$ -glutamyl peptides, it has been reported that several *kokumi*  $\gamma$ -glutamyl peptides enhanced mouthfulness in food systems. Ueda et al. reported that the addition of GSH ( $\gamma$ -Glu-Cys-Gly) enhanced the intensity of mouthfulness in model beef meat extract [2]. In addition, Ohsu et al. also reported that the addition of GSH enhanced the intensity of mouthfulness in chicken soup [3]. Furthermore, it has been reported that  $\gamma$ -glutamyl peptides such as  $\gamma$ -Glu-Val,  $\gamma$ -Glu-Leu, and  $\gamma$ -Glu-Cys- $\beta$ -Ala found as *kokumi*-active peptides in edible beans enhanced mouthfulness when they were added to chicken broth [5]. In addition, it has been reported that  $\gamma$ -Glu-Glu,  $\gamma$ -Glu-Gly,  $\gamma$ -Glu-His,  $\gamma$ -Glu-Gln,  $\gamma$ -Glu-Met, and  $\gamma$ -Glu-Leu were the key components which impart long-lasting mouthfulness of matured Gouda cheese. From these observations, it is demonstrated that many *kokumi*  $\gamma$ -glutamyl peptides enhance the intensity of mouthfulness.

**Table 1 Definition and reference samples for the descriptive attributes of chicken consommé**

Sensory attributes	Definitions	Reference samples and intensity
Total flavor	The total intensity of all of the flavors of the sample including basic tastes	Kitchen Basics chicken broth (6)
Total chicken/meaty flavor	The flavor intensity reminiscent of cooked chicken meat	Kitchen Basics chicken broth (5)
Chicken flavor	The flavor intensity reminiscent of cooked chicken	Kitchen Basics chicken broth (5)
Bones/marrow flavor	The character associated with chicken bones, particularly the marrow of chicken bones	NR
Roasted flavor	The total flavor intensity that is reminiscent of roasted chicken and/or vegetables	Swanson's chicken broth (6)
Total vegetable flavor	The total flavor intensity of vegetables such as carrots, green vegetables, and herbs in the broth	Kitchen Basics chicken broth (5)
Richness	The degree to which the flavor characters of the sample are harmonized, balanced, and blend well together as opposed to being spiky or striking out	NR
Salty	One of the basic taste, common to sodium chloride	0.2% sodium chloride in water (2) 0.5% sodium chloride in water (5) 0.2% sodium chloride in water (2) 0.5% sodium chloride in water (5)
Umami	One of the basic taste, common to MSG. The taste and mouth-filling sensation of compounds such as glutamates that is savory, brothy, meaty, rich, full, and complex, common to many foods such as soy sauce, stocks, ripened cheese (especially parmesan), shellfish (crab, lobster, scallops, clams), mushrooms (especially porcini), ripe tomatoes, cashews, and asparagus	Kitchen Basics chicken broth (2) 0.5% MSG in Kitchen Basics chicken broth (3.5) Kitchen Basics chicken broth (2) 0.5% MSG in Kitchen Basics chicken broth (3.5)
Viscosity	The degree to which the samples are viscous in the mouth from thin to thick	Water (1) Heavy whipping cream (6)
Mouthfulness	The perception that the sample fills the whole mouth is blooming, or growing, a full-bodied sensation when the sample is held in the mouth	Kitchen Basics chicken broth (1.5) 0.5% MSG in Kitchen Basics chicken broth (3) Kitchen Basics chicken broth (1.5) 0.5% MSG in Kitchen Basics chicken broth (3)
Mouth coating	The degree to which there is a leftover residue, a slick, powdery, or fatty coating or film in the mouth that is difficult to clear	0.5% MSG in water (4) Half and Half (5) 0.5% MSG in water (4) Half and Half (5)
Tongue coating	The degree to which there is a leftover residue, a slick, powdery, or fatty coating or film on the tongue that is difficult to clear	0.5% MSG in water (3)
Total trigeminal	The intensity of the total sensation, including numbing, burning, tingling, or irritation, impaired on the soft tissues of the oral cavity, particularly the tongue	Wintergreen breathsaver (NS) 0.5% MSG in water (5) Wintergreen breathsaver (NS) 0.5% MSG in water (5)
Salivating	The degree to which the sample caused a perceived increase in salivation	NR
Swelling of cheeks and lips	The feeling of swelling of the soft tissue in the oral cavity, specifically the cheeks and lips, reminiscent of the perception of swelling produced by anesthetic treatments at a dental office, but without a distinct numbing effect	0.5% MSG in water (4)
Total aftertaste	The total aftertaste intensity after 5 s of all flavor notes within the sample	NR

NR no reference, NS not scored.

**Table 2 Sensory characteristics of chicken consommé with added  $\gamma$ -Glu-Val-Gly**

Sensory attributes	Control consommé	Consommé with $\gamma$ -Glu-Val-Gly	Changed value	95% confidence interval	99% confidence interval	Significance
Total flavor	6.13 $\pm$ 0.72	6.31 $\pm$ 0.68	0.18 $\pm$ 0.60	0.28	0.36	N.S.
Total chicken/meaty flavor	5.26 $\pm$ 0.61	5.41 $\pm$ 0.59	0.14 $\pm$ 0.59	0.27	0.36	N.S.
Chicken flavor	4.82 $\pm$ 0.55	4.88 $\pm$ 0.78	0.06 $\pm$ 0.69	0.32	0.42	N.S.
Bones/marrow flavor	2.42 $\pm$ 0.85	2.63 $\pm$ 0.98	0.21 $\pm$ 1.14	0.53	0.69	N.S.
Roasted flavor	3.19 $\pm$ 1.03	3.12 $\pm$ 1.03	-0.07 $\pm$ 0.82	0.38	0.50	N.S.
Total vegetable flavor	3.56 $\pm$ 0.75	3.78 $\pm$ 0.81	0.22 $\pm$ 0.64	0.30	0.39	N.S.
Richness	4.01 $\pm$ 0.81	4.27 $\pm$ 0.94	0.27 $\pm$ 0.79	0.36	0.48	N.S.
Salty	2.73 $\pm$ 0.48	2.87 $\pm$ 0.73	0.13 $\pm$ 0.57	0.26	0.35	N.S.
Umami	2.84 $\pm$ 0.65	3.28 $\pm$ 0.67	0.43 $\pm$ 0.66	0.30	0.40	**
Viscosity	2.06 $\pm$ 0.65	2.22 $\pm$ 0.59	0.16 $\pm$ 0.40	0.18	0.24	N.S.
Mouthfulness	2.47 $\pm$ 0.70	2.92 $\pm$ 0.73	0.45 $\pm$ 0.69	0.32	0.42	**
Mouth coating	2.67 $\pm$ 0.66	2.94 $\pm$ 0.65	0.27 $\pm$ 0.56	0.26	0.34	*
Tongue coating	2.56 $\pm$ 0.82	2.72 $\pm$ 0.82	0.17 $\pm$ 0.68	0.32	0.42	N.S.
Salivating	2.42 $\pm$ 0.85	2.58 $\pm$ 0.83	0.16 $\pm$ 1.06	0.49	0.64	N.S.
Total trigeminal	2.76 $\pm$ 0.87	2.98 $\pm$ 0.73	0.23 $\pm$ 0.84	0.39	0.51	N.S.
Swelling perception of soft tissue	2.78 $\pm$ 0.79	2.87 $\pm$ 0.68	0.09 $\pm$ 0.76	1.48	0.46	N.S.
Total aftertaste	4.46 $\pm$ 0.60	4.54 $\pm$ 0.66	0.08 $\pm$ 0.64	0.29	0.38	N.S.

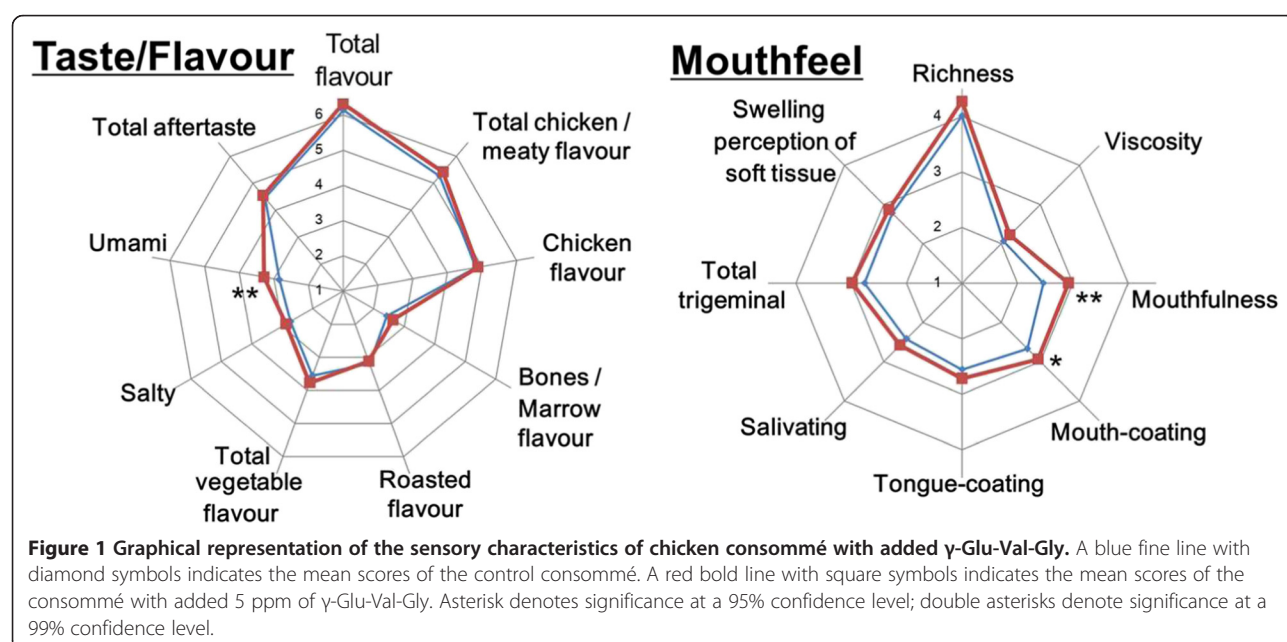
Data was shown as means  $\pm$  standard errors.

N.S. not significant.

\*Significant at a 95% confidence level, \*\*significant at a 99% confidence level.

Interestingly, the present study has revealed that the addition of  $\gamma$ -Glu-Val-Gly at 5 ppm significantly enhanced the intensity of mouth coating. It has been generally known that mouth-coating sensation is evoked by the addition of hydrocolloids such as xanthan gum and locust bean gum, carrageenan [14,15], and fat-containing food materials such as dairy fat emulsion [15]. However,

several studies have reported that low-molecular-weight compounds enhanced the intensity of mouth coating. Dawid and Hofmann reported that 1,2-dithiolan-4-carboxylic acid 6-D-glucopyranoside ester exhibited a buttery mouth-coating sensation [16]. Additionally, the same research group demonstrated that polyphenolic compounds such as vanillin, vanillin-related compounds, americanin



A, and 4',6'-dihydroxy-3',5'-dimethoxy-[1,1'-biphenyl]-3-carboxaldehyde from cured vanilla beans exhibited a velvety mouth-coating sensation [17]. Furthermore, it has been reported that the flavon-3-ol glycosides such as kaempferol glycosides, quercetin glycosides, myricetin glycosides, and apigenin glycoside from black tea induced a mouth-coating sensation [18]. Despite these observations, there have been no reports of a peptide which exhibited the mouth-coating sensation. Therefore, this is the first report which has demonstrated the mouth-coating effect of peptides. Although the viscosity of consommé did not change significantly by adding 5 ppm of  $\gamma$ -Glu-Val-Gly (data not shown), an enhancement of the mouth-coating sensation was observed. The mechanism of this enhancement is interesting and should be clarified by further investigations.

In the present study, the addition of  $\gamma$ -glutamyl-valyl-glycine enhanced the intensity of umami, mouthfulness, and mouth coating. On the other hands, it has been reported that MSG, representative umami compound, also enhances the intensity of mouthfulness and the sensation related to mouth coating [13]. Previously, it has been demonstrated that  $\gamma$ -glutamyl-valyl-glycine enhanced the umami intensity when it was added to 0.3% MSG solution [3]. In addition, as shown in Table 3, the analysis of the chemical components revealed that chicken consommé contained glutamic acid (51.1 mg/dl) and IMP (21.3 mg/dl), and these concentrations of umami components were sufficient to evoke the umami sensation [13]. Therefore, it was considered that the enhancement of mouthfulness and mouth coating by  $\gamma$ -glutamyl-valyl-glycine was possibly caused by the enhancement of the function of the umami components. Regarding difference between the function of *kokumi* compounds and umami compounds, it was considered that the unique character of the *kokumi* compounds is that *kokumi* compounds have no taste themselves. Therefore, it can be assumed that *kokumi* compounds can enhance sensations like mouthfulness and continuity in sweet foods. In our recent study, it was observed that  $\gamma$ -glutamyl-valyl-glycine enhanced aftertaste, oiliness, and mouthfulness in reduced-fat peanut butter [19]. This result suggest that *kokumi* compounds can be used both in savory foods and sweet foods, while umami compounds can be used mainly in savory foods because of the characteristic umami taste. Further detailed studies are necessary to clarify the mechanism of the enhancement of the mouthfulness and mouth-coating sensation by  $\gamma$ -glutamyl-valyl-glycine.

The addition of  $\gamma$ -Glu-Val-Gly significantly enhanced the intensity of umami, mouthfulness, and mouth coating in chicken consommé. The results suggest that adding  $\gamma$ -Glu-Val-Gly can improve the flavor and mouthfeel of chicken consommé. To confirm this possibility, consumer preferences for chicken consommé with added

**Table 3 Contents of free amino acids and 5'-nucleotide in chicken consommé**

Component	Content (mg/dl)
Amino acids	
Taurine	74.9
Aspartic acid	17.5
Threonine	27.5
Serine	20.3
Glutamic acid	51.1
Glycine	15.8
Alanine	23.6
Valine	10.2
Methionine	4.7
Isoleucine	7.0
Leucine	12.4
Tyrosine	9.9
Phenylalanine	8.0
Lysine	17.0
Histidine	6.6
Arginine	23.7
Hydroxyproline	2.5
Proline	10.0
5'-Nucleotide	
5'-IMP	21.3
5'-GMP	N.D.

N.D. not detected.

$\gamma$ -Glu-Val-Gly are now being investigated in our laboratory.

## Conclusions

In the present study, the sensory characteristics of chicken consommé with 5.0 ppm added  $\gamma$ -Glu-Val-Gly were investigated using descriptive analysis. Chicken consommé containing  $\gamma$ -Glu-Val-Gly had significantly stronger "umami" and "mouthfulness" (mouth-filling sensation) characteristics than the control sample at a 99% confidence level and significantly stronger "mouth-coating" characteristic than the control at a 95% confidence level. These data indicated that a *kokumi* peptide,  $\gamma$ -Glu-Val-Gly, can enhance umami, mouthfulness, and mouth coating in chicken consommé. From these results, it was suggested that the addition of  $\gamma$ -Glu-Val-Gly can improve the flavor and mouthfeel of chicken consommé.

## Methods

### Preparation of $\gamma$ -Glu-Val-Gly

The  $\gamma$ -Glu-Val-Gly used in the present study was of food additive grade (FEMA-GRAS No. 4709; JECFA food



flavoring No. 2123) obtained from Ajinomoto Co., Inc. (Tokyo, Japan) and was prepared by a chemical synthetic method reported previously [3].

#### Preparation of chicken consommé

The raw materials for chicken consommé are shown in Table 4. Minced chicken breast meat, minced chicken leg meat, and egg white were mixed. Then, minced chicken wing meat was added and mixed. The raw materials (except bouillon and water) were mixed in a 60-l aluminum pot. Bouillon (Kisco Co. Ltd., Tokyo, Japan) diluted with the same volume of water was added and boiled at between 90°C and 95°C for 30 min. After removing the meat, precipitate, and fat, the resulting chicken consommé was freeze-dried (freezing temperature, -24°C; vacuum <13 Pa; sample temperature, <20°C) using a Freeze Drier (RL-50 MB, Kyowa Vacuum Engineering Co. Ltd., Tokyo, Japan). For sensory evaluation, 5.6 g of the freeze-dried chicken consommé powder and 0.2 g of sodium chloride were dissolved in 100 ml of distilled water and heated to 60°C and presented to the panelists. Approximately 90 ml of consommé was served in foam cups coded with three-digit random numbers.

#### Selection of the panel

Eighteen female panelists participated in the sensory evaluation. The age of the panelists was  $54.0 \pm 8.8$  (mean  $\pm$  standard deviation) years old. They all live in the San Francisco Bay Area, CA, USA. The screening of the panelists was conducted in three phases: phone screening of applicants, on-site acuity testing, and face-to-face interviews with advanced acuity testing.

**Table 4 Raw materials for the chicken consommé**

Materials	Weight (g)
Chicken breast meat (minced)	6,818.2
Chicken leg meat (minced)	6,818.2
Chicken wing meat (minced)	6,818.2
Egg white	1,500.0
Fried onion	1,687.5
Carrot	562.5
Celery	375.0
Tomato	1,406.3
Tomato paste	150.0
Parsley	18.8
Black pepper	5.6
Bouillon (Kisco Co., Inc.)	15,000.0
Water	15,000.0

#### Training of the panel

##### General panel training

All of the panelists were broadly trained in sensory descriptive analysis to evaluate aromas, flavors, textures, and appearance across a wide range of consumer products. This training was conducted for approximately 3 days per week for 3 months, during which the panelists expanded their food sensory vocabularies, learned to use a 15-point scale to rate attribute intensities, and evaluated a wide variety of foods. For example, the sweetness intensity scale was anchored with several concentrations of sucrose in water and the intensity of "sweet aromatic" was anchored with several concentrations of vanilla in milk. The panelists-in-training refined their skills by participating in practice tests using many different types of products. After each test, they were given detailed feedback while retesting the products to help them improve their performance. After this training was complete, the panelists were registered as members of the Descriptive Panel of The National Food Laboratory and began to participate in the descriptive analysis of various kinds of foods.

##### Ongoing panelist feedback

Feedback was routinely provided during panel sessions to maintain and refine the evaluating ability of the panelists. Several times a month, the panelists were given face-to-face performance feedback to help them maintain their calibration. A panel leader tasted the products with the panelists as they reviewed their scores to highlight potential areas for improvement. Feedback was given both on discrimination among products and consistency between replications.

##### Project-specific orientation sessions

The objectives of the orientation training sessions were to understand the effect of  $\gamma$ -Glu-Val-Gly on chicken consommé to generate the list of sensory attributes for the evaluation sessions. This 2-h training session was conducted on the day before the sensory evaluation for the present study. During the session, panelists evaluated samples of chicken consommé with and without  $\gamma$ -Glu-Val-Gly to understand the effect of  $\gamma$ -Glu-Val-Gly. A panel leader led the group in discussion on the differences and similarities between the samples. They developed a list of sensory attributes that described the products' sensory characteristics, focusing on attributes believed to be influenced by  $\gamma$ -Glu-Val-Gly. Each sample was tested at least twice during this orientation session. During this training session, the panelists also developed new attributes such as "total chicken/meaty flavour", "bones/marrow flavour", "roasted flavour", "richness", "tongue-coating", and "salivating". Overall, the panelists defined the 17 sensory attributes listed in Table 2. The panelists practiced rating the samples

on the list so that they were prepared to begin data collection.

#### **Project-specific panelist feedback**

Between each of the six data collection replications, panelists were given feedback about the samples they had evaluated. A panel leader led the group in brief discussions on the differences and similarities between the samples. Panelists were instructed to taste samples (with and without 5 ppm  $\gamma$ -Glu-Val-Gly) for training purposes during the discussions. After each feedback discussion, the panelists took a 10-min break before data collection for the next replication.

#### **Procedure for sensory evaluation**

For the evaluation of chicken consommé, panelists held the product in the mouth for 10 s, expectorated, and then rated flavor, texture/mouthfeel, and aftertaste attributes. They then completed the rating for each attribute (samples with and without 5 ppm  $\gamma$ -Glu-Val-Gly) on a 15-point line scale. The sample serving order was balanced, with each sample being presented approximately an equal number of times in each position for each test. Two days of data collection were completed, each consisted of three replications. Feedback to the panelists was provided after each replication except the final replication. In total, six evaluations were conducted. In the present report, to investigate the effect of  $\gamma$ -Glu-Val-Gly on chicken consommé by an experimental protocol after a single feedback session, we report the result of the second replication of sensory evaluation data, which followed the first panelist feedback session on the first day of data collection. Human sensory analyses were conducted following the spirit of the Helsinki Declaration, and informed consent was obtained from all panelists. The experimental protocol was approved by the ethics board of the Institute of Food Sciences and Technologies, Ajinomoto.

#### **Analyses of free amino acids and 5'-nucleotides in chicken consommé**

Free amino acids were determined using a Model L-8800 amino acid analyzer (Hitachi Corp., Tokyo, Japan) with a lithium citrate buffer (PF-series for nonhydrolyzed amino acid analysis; Mitsubishi Chemical, Tokyo, Japan). The contents of 5'-nucleotides were determined by HPLC equipped with a Hitachi #3013 column with detection at 254 nm.

#### **Statistical analyses**

Statistical analyses were conducted using JMP version 9.0 (SAS Institute Inc., Cary, NC, USA). The data were collected as the means  $\pm$  standard deviation. Data were assessed by the paired *t* test. The data was considered to be significant when the confidence level was more than 95%.

#### **Abbreviations**

$\gamma$ -Glu-Val-Gly:  $\gamma$ -glutamyl-valyl-glycine; GSH: glutathione; CaSR: calcium-sensing receptor; FEMA: Flavour and Extract Manufacturers Association; JECFA: The Joint FAO/WHO Expert Committee; MSG: monosodium glutamate; IMP: inosine monophosphate.

#### **Competing interests**

The authors declare that they have no competing interests.

#### **Authors' contributions**

MK, NM, and TK conceived the idea of this study. TM, MK, and NM designed the detail of experiments. TM and HK conducted the sample preparation and sensory evaluation. TM and MK conducted the analysis of sensory data and wrote the manuscript. All authors read and approved the final manuscript.

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