

Distribution of a *kokumi* peptide, γ -Glu-Val-Gly, in various fermented foods and the possibility of its contribution to the sensory quality of fermented foods

Miyamura N¹, Kuroda M¹, Mizukoshi T², Kato Y², Yamazaki J², Miyano H² and Eto Y²

¹Institute of Food Research & Technologies, Ajinomoto Co., Inc., Kanagawa 210-8681, Japan

²Institute for Innovation, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan

*Corresponding author: Kuroda M, Institute of Food Research & Technologies, Ajinomoto Co., Inc., 1-1 Suzuki-cho, Kawasaki-ku, Kawasaki, Kanagawa 210-8681, Japan, E-mail: motonaka_kuroda@ajinomoto.com

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Abstract

A potent *kokumi* peptide, γ -glutamyl-valyl-glycine (γ -Glu-Val-Gly), was identified and quantified in various fermented foods using high performance liquid chromatography-tandem mass spectrometry (LC/MS/MS). This peptide was present in fermented seasoning such as fish sauces, soy sauces, and fermented shrimp pastes. Among the brewed alcoholic beverages, γ -Glu-Val-Gly was detected in beer. In addition, the content of γ -Glu-Val-Gly positively correlated with the quality grade of dark-colored soy sauce (correlation coefficient $\rho=0.810$, $p<0.05$). These results suggest that γ -Glu-Val-Gly is widely distributed in fermented foods and that the content of this peptide can be an indicator of sensory quality of fermented foods.

Keywords: γ -Glu-Val-Gly; γ -Glutamyl-Valyl-Glycine; Kokumi; Fermented food; Fish sauce; Soy sauce; Shrimp paste; Beer; LC/MS/MS

Commercial fish sauces; soy sauces; fermented shrimp pastes; and beer were purchased from the local market.

Introduction

Recent studies have indicated that *kokumi* substances such as glutathione are perceived through the calcium-sensing receptor (CaSR) in humans [1,2]. These studies have confirmed that glutathione can activate human CaSR; and that several γ -glutamyl-peptides can also activate the CaSR; and these peptides possess the characteristics of *kokumi* substances; which modify the five basic tastes; especially sweet; salty and umami; when they are added to basic taste solutions or food; even though these substances have no taste themselves at the concentrations tested [1,3-8]. Among these *kokumi* peptides; γ -Glu-Val-Gly has been reported to be a potent *kokumi* peptide (1). In our studies; the distribution of γ -Glu-Val-Gly in various foods was investigated. Because the contents of γ -Glu-Val-Gly in foods were very low; a new method for the determination and quantification of this peptide using LC/MS/MS followed by derivatization with 6-aminoquinoyl-N-hydroxysuccinimidyl-carbamate (AQC) reagent was developed [9-11] by modifying the previously reported method [12]. In the present study; the presence and quantities of γ -Glu-Val-Gly in various fermented foods is examined. In addition; possibility of the contribution of γ -Glu-Val-Gly to the sensory quality of fermented foods is also discussed.

Materials and Methods

Materials

γ -Glu-Val-Gly used in the present study was prepared by chemical synthesis as reported previously [1]. Uniformly ¹⁵N-labeled L-arginine (Arg-UN) and L-proline-d7 (Pro-d7) were obtained from Isotec (Tokyo; Japan). An AccQ Fluor reagent kit was purchased from Waters (Milford; MA; USA). Other reagents are of analytical grade.

Sample Preparation and Derivatization Procedure

Samples were filtered through a 0.45- μ m syringe filter (25-mm GD/X disposable filter; Whatman PLC; Maidstone; UK). The filtrates were then passed through an Amicon Ultra Centrifugal Filter (regenerated cellulose 10,000 MWCO; Millipore; USA) at 7,500 g and 4°C for 15 min. Derivatization with AQC was conducted using the AccQ Fluor reagent kit (Millipore) according to the manufacturer's protocol.

Identification and Quantification of AQC-derivatized γ -Glu-Val-Gly

Identification and quantification was conducted as reported previously [10,11,13,14]. Separation of AQC-derivatized γ -Glu-Val-Gly was conducted by HPLC using octadecyl silica column. The peak of AQC-derivatized γ -Glu-Val-Gly was identified by the multiple reactions monitoring (MRM) method with six MRM transition channels. The combination of precursor/product ions (Q1/Q3) and the collision energies (CE (V)) were 474.2/171.2 (51V); 474.2/145.3 (30V); 474.2/300.3 (30V); 474.2/229.4 (20V); 474.2/304.0 (20V) and 474.2/72.1 (50V). AQC-derivatized γ -Glu-Val-Gly in the samples was quantified by the internal standard method using the most sensitive channel (474.2/171.2; Q1/Q3). Internal standards (Arg-UN and Pro-d7) were monitored in the MRM transition channels at 349.0/171.1 (Q1/Q3) and 293.0/171.1 (Q1/Q3); respectively. The typical mass spectrum of AQC-derivatized γ -Glu-Val-Gly is indicated in Figure 1.

Statistical Analysis

Correlation between the γ -Glu-Val-Gly content and quality grade of soy sauce was analyzed by a Spearman's rank correlation test was performed using STAT-VIEW version 5.0 software.

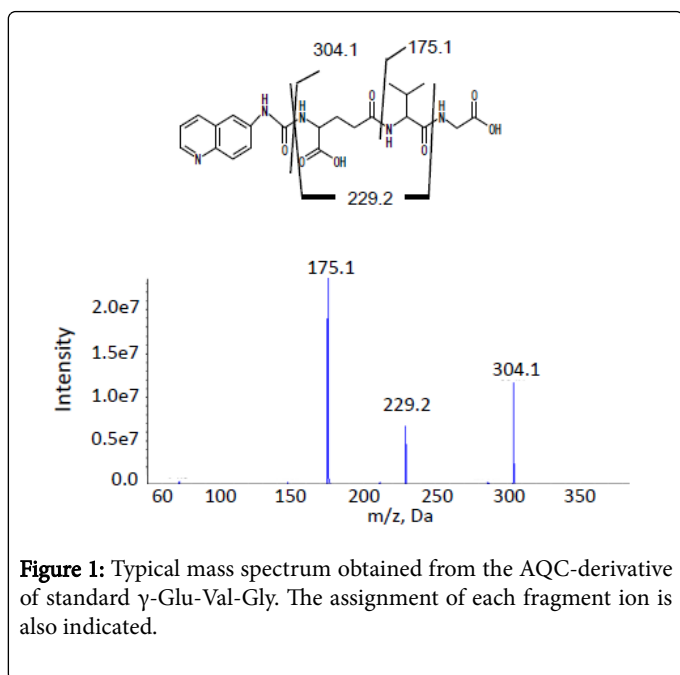


Figure 1: Typical mass spectrum obtained from the AQC-derivative of standard γ -Glu-Val-Gly. The assignment of each fragment ion is also indicated.

Results and Discussion

Distribution of γ -Glu-Val-Gly in Various Fermented Foods

The analytical results are summarized in Table 1. The content of this peptide in 17 brands of commercial fish sauce from Southeast Asia; East Asia and Europe was quantified. The average content in fish sauces was 4.4 mg/L. The contents in Vietnamese fish sauce (*Nuoc Mum*) ranged from 10.4 mg/L to 12.6 mg/L and average was 11.6 mg/L; while those in Thai fish sauce (*Nampra*) ranged from 1.2 to 3.1 mg/L and the average content was 2.3 mg/L. Among the two Chinese fish sauce (Yu-lu), one contained γ -Glu-Val-Gly at 1.1 mg/L. In addition, two of the four Japanese fish sauce contained γ -Glu-Val-Gly at 2.8 and 0.5 mg/L (average content: 0.8 mg/L, n=4). Furthermore; Italian fish sauce (*Garum*) contained γ -Glu-Val-Gly at 0.4 mg/L. These results suggest that γ -Glu-Val-Gly is contained in various fish sauces. γ -Glu-Val-Gly was distributed in commercial Japanese soy sauces at concentrations ranging from 1.5 to 6.1 mg/L [11]; and the average content was 4.0 mg/L. The contents of dark-colored soy sauce ranged from 3.1 to 6.1 mg/L (n=6), while those in light-colored soy sauce ranged from 3.4 to 3.7 mg/L (n=2). The content of the white soy sauce sample was 1.5 mg/L (n=1). These results suggest that γ -Glu-Val-Gly is distributed in soy sauces. As shown in Table 1; commercial fermented shrimp paste condiments from Southeast Asia (n=3) contained this peptide at concentrations ranging from 0.9 to 5.2 mg/kg [13]. Next, the contents of γ -Glu-Val-Gly in various brews including wine; rice wine (sake); and beer were analyzed. The analyses indicated that γ -Glu-Val-Gly was present in all of the beer samples (n=8) at concentrations ranging from 0.08 to 0.18 mg/L [14]. In top-fermented beer (n=2); he content of this peptide was 0.09 mg/L and 0.11 mg/L (average content = 0.11 mg/L) and the bottom-fermented beer (n=6) contained this peptide in the range between 0.08 and 0.15 mg/L (average content=0.12 mg/L, n=6). However; the peptide was not detected in any of the wine or rice wine samples [14]. These results indicate that γ -Glu-Val-Gly is widely distributed in fermented foods such as fish sauces; soy sauces; fermented shrimp paste, and beer.

Samples	Sample Size (n)	Contents of γ -Glu-Val-Gly (mg/L)		
		Average	Minimum	Maximum
Fish Sauces				
Total	17	4.4	<LOQ	12.6
Nampra (Thailand)	5	2.3	1.2	2.7
Nuoc Mum (Vietnam)	5	11.6	10.4	12.6
Yu-lu (China)	2	0.6	<LOQ	1.1
Japanese fish sauce	4	0.8	<LOQ	2.8
Garum (Italy)	1	0.4	0.4	0.4
Soy sauces (Japan)				
Total	9	4.0	1.5	5.3
Dark-colored soy sauce	6	4.6	3.1	5.3
Light-colored soy sauce	2	3.6	3.4	3.7
White-colored soy sauce	1	1.5	1.5	1.5
Fermented shrimp paste				
Total	3	2.4	0.9	5.2
Terasi (Indonesia)	1.0	5.2	5.2	5.2
Bagoong (Philippines)	1	1.0	1.0	1.0
Xiajiang (China)	1	0.9	0.9	0.9
Beer				
Total	8	0.12	0.08	0.15
Top-fermented beer	2	0.11	0.09	0.11
Bottom-fermented beer	6	0.12	0.08	0.15
LOQ: Limit of quantification				

Table 1: The content of γ -Glu-Val-Gly in various Fermented Food

In addition, it was revealed that the contents of γ -Glu-Val-Gly in fermented seasonings such as fish sauce; soy sauce; and fermented shrimp paste were higher than those of brew such as beer. The variation of the contents would be because of the difference in the raw materials; fermentation condition and so on. However; further investigation should be performed to clarify the reasons behind the variation in γ -Glu-Val-Gly contents.

Correlation between γ -Glu-Val-Gly Content and Quality Grade

To clarify the contribution of γ -Glu-Val-Gly to the sensory quality of various fermented foods; the correlation between the content of the peptide and index of the quality of soy sauce [11,15] was investigated. As shown in Table 2, Japanese dark-colored soy sauces (*koikuchishoyu*) are divided into three classes according to the total nitrogen content; special; higher and standard [16]. In addition; the soy sauces in the special class are divided into three grades; ultrasuper, super and ordinary. All of the six dark-colored soy sauces tested in this study are

of special class; and they consist of one ultrasuper grade; three super grades; and two ordinary grades.

Type of soy sauce	Class	Grade	Contents	
			Total Nitrogen	Dry matter of extract**
Dark-colored	Standard		>1.20	
	Upper		>1.35	>14
	Special	Ordinary	>1.50	>16
		Super	>1.65	>16
		Ultrasuper	>1.80	>16

*:Established as Japanese Agricultural Standard (JAS) **: Dry matter except for NaCl

Table 2: Standard for Japanese soy sauces*.

The γ -Glu-Val-Gly content in the ultrasuper grade was highest (6.1 mg/L); followed by the super grade (average content = 4.7 mg/L); and the ordinary grade (average content = 3.7 mg/L). The Spearman's rank correlation test indicated that the content of γ -Glu-Val-Gly was positively correlated with the quality grade of commercial soy sauce (correlation coefficient $\rho=0.810$; $p<0.05$) (Figure 2).

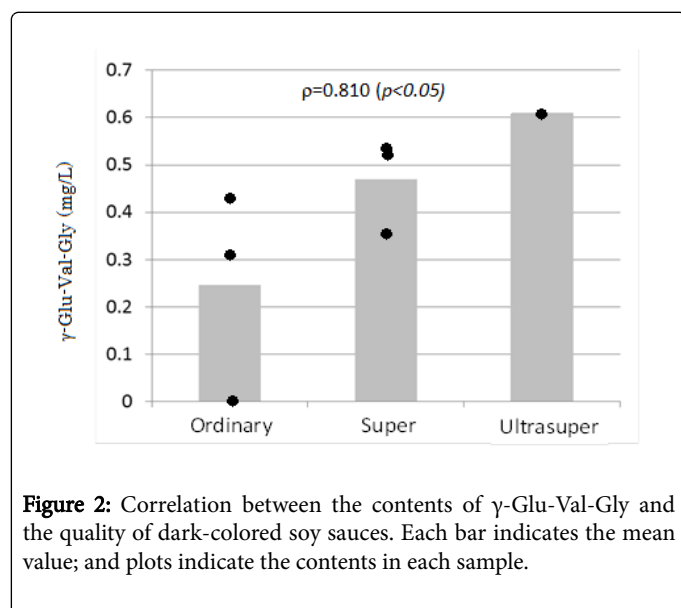


Figure 2: Correlation between the contents of γ -Glu-Val-Gly and the quality of dark-colored soy sauces. Each bar indicates the mean value; and plots indicate the contents in each sample.

These results suggest that γ -Glu-Val-Gly contributed to the sensory quality of fermented foods.

In our laboratory; the contents of γ -Glu-Val-Gly in other fermented foods and the contribution of this peptide to the quality of foods is now in progress.

Conclusion

The content of a *kokumi* peptide, γ -Glu-Val-Gly; in various foods was analyzed by LC/MS/MS followed by derivatization with AQC. The

quantitative analysis indicated that γ -Glu-Val-Gly is distributed in various fermented foods, such as fish sauce, soy sauce, fermented shrimp paste and beer. Furthermore; the content of this peptide has been positively correlated with the quality grade of soy sauces; suggesting that γ -Glu-Val-Gly contributes to the sensory quality of fermented foods.

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