Presence of γ-glutamyl and β-aspartyl isopeptides, diketopiperazines, pyroglutamyl peptides, in addition to normal peptides in fish and soy sauces: Structures, contents and their bioavailability

Running title: Bioavailability of modified peptides in fish and soy sauces

Yudi Rahmadian[a,b], Satoshi Miyauchi[a], Sri Wijanarti[a,c], Tomoko Asai[a] and Kenji Sato[a]*

[a] *Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University, Kitashirakawa Oiwakecho, Kyoto, Japan*

[b] *Department of Animal Products Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia*

[c] *Department of Bioresources Technology and Veterinary, Vocational College, Universitas Gadjah Mada, Sleman, D.I. Yogyakarta, Indonesia*

* Corresponding author: sato.kenji.7x@kyoto-u.ac.jp

ORCID

Kenji Sato: 0000-0002-4891-3066

Yudi Rahmadian: 0000-0002-6864-2283

Abstract

This study identified peptides in fish and soy sauces and elucidated their bioavailability in rats. Over 96 peptides including γ-glutamyl, pyroglutamyl, β-aspartyl peptides, and diketopiperazines were detected. The content of these peptides varied greatly between the products tested. After the administration of Vietnamese fish sauce which had the highest peptide content among samples tested, most normal peptides did not significantly increase in the blood; whereas γ-glutamyl and pyroglutamyl peptides significantly increased in the small intestine and some hydrophobic γ-glutamyl isopeptides and pyroglutamyl-proline significantly increased in the blood. Diketopiperazines and β-aspartyl isopeptides significantly increased in the small intestine and the blood. These findings highlight the presence of modified peptides in fish and soy sauces, which are commonly consumed in daily dishes in East Asia. Only modified peptides such as diketopiperazines, β-aspartyl isopeptides and hydrophobic γ-glutamyl isopeptides survived gastrointestinal digestion, entering blood circulation, suggesting their potential biological activities.

Keywords: Fermented food, Fish sauce, Soy sauce, Peptide, Isopeptide, Diketopiperazine, Bioavailability.

1. Introduction

Salt has been used since ancient times to preserve food products. During salting, endogenous and microbial enzymes can cause changes in ingredients, which can alter the texture, taste, and flavor, and sometimes produce paste and liquid products. This process is known as fermentation. In East Asia, salted and fermented fish, meat, vegetables, beans, and cereals are used as seasonings owing to their strong *umami* taste. Fish, soy sauce, and soy paste are the prevalent seasonings in this area. Fermentation is also used to produce non-salted products, such as yogurt and fresh cheese. These products are usually prepared with a shorter fermentation period than East Asian fermented seasoning. The consumption of East Asian fermented seasonings in this area has decreased in recent decades (Okouchi et al., 2019), possibly because of high salt concentrations. On the other hand, it has been reported that fermented seasonings, especially Japanese-style soy paste *miso* may show beneficial effects on human health, such as lowering blood cholesterol, immune-enhancing, antidiabetic, anti-obesity, anticancer, antimicrobial, and risk-lowering activities against atherosclerosis, osteoporosis, stomach illnesses, and lactose intolerance symptoms (Chatterjee et al., 2018; Endres, 2001; Ito, 2020; Kokubo et al., 2007, 2013; Kondo et al., 2019; Mano et al., 2018; Marco et al., 2017; Nozue et al., 2017; Minamiyama et al., 2003).

These fermented seasoning products produce strong *umami* and *kokumi* sensations and enhance the flavor of daily dishes. Monosodium glutamate in these products is mainly responsible for the *umami* taste. In addition, some peptides such as pyroglutamyl-glutamine (pGlu-Gln) (Kaneko et al., 2011) and γ-glutamyl-valyl-glycine (γ-Glu-Val-Gly) (Kuroda et al., 2020) have been identified to enhance *umami* taste and *kokumi* sensation, respectively. However, the compounds in these products responsible for their health-promoting activities are poorly understood. Recently, some modified peptides such as pyroglutamyl peptides, diketopiperazines (cyclic dipeptides), and β-aspartyl isopeptides have been identified in *miso*, a Japanese-style salted and fermented soy paste (Nagao et al., 2024). These modified peptides show high bioavailability, especially diketopiperazines and β-aspartyl isopeptides, while normal peptides do not. Diketopiperazines are cyclic dipeptides formed from linear peptides (Otsuka et al., 2021) that have been reported to exhibit antioxidant (Zhong et al., 2018), anti-inflammatory (Zhang et al., 2019), antifungal, and antimicrobial activities (Kwak et al., 2018). Pyroglutamyl peptides are generated from a glutamine residue at the amino terminus of peptides by non-enzymatic intramolecular cyclization in a time- and temperature-dependent manner (Suzuki et al., 1999). Oral administration of pyroglutamyl-leucine (pGlu-Leu) in a wheat gluten enzymatic hydrolysate has been reported to have hepatoprotective activity by oral administration (Sato et al., 2013), to ameliorate colitis caused by dextran sulfate sodium in mice by oral administration at 0.1 mg/kg body weight (Wada et al., 2013). Recently, β-

aspartyl peptides have shown anti-fatigue activity in a mouse model (Nakagawasai et al., 2021) and inhibitory activity against angiotensin converting enzyme (Nagao et al., 2024). These modified peptides may be responsible for the bioactivities of fermented foods. However, the presence of these modified peptides in other types of salted and fermented seasonings such as soy and fish sauces is poorly understood. *Miso* is fermented and aged under anaerobic conditions and not sterilized by heat treatment. However, fish and soy sauces are fermented and aged under more aerobic conditions with occasional stirring, and soy sauces are usually heated to terminate fermentation, which can generate new products.

This study aimed to comprehensively identify short-chain peptides in fish and soy sauces and elucidate their bioavailability in rats after oral administration, which can provide fundamental information for understanding the biological activities of East Asian fermented seasonings.

2. Materials and methods

2.1. Reagents

Phosphate-buffered saline (pH 7.4, 10 × PBS), L-pyroglutamic acid, and acetonitrile (HPLC grade) were obtained from Nacalai Tesque (Kyoto, Japan). The amino acid mixture standard solution (Type H) was obtained from Fujifilm Wako Pure Chemical (Osaka, Japan). 9-Fluorenylmethoxycarbonyl (Fmoc) amino acid derivatives including Fmoc-L-Asp α-*t*-butyl-ester, Fmoc-D-Asp α-*t*-butyl-ester, Fmoc-L-Asp β-*t*-butyl-ester, Fmoc-D-Asp β-*t*-butyl-ester, Fmoc-L-Glu γ-*t*-butyl-ester and Fmoc amino acid-bound p-alkoxybenzyl alcohol (Alko) resin, and Fmoc proline-bound 2-chlorotrityl chloride (Barlos) resin were purchased from the Watanabe Chemical (Hiroshima, Japan). Cyclo-Ala-Pro was purchased from the Peptide Institute (Osaka, Japan). 6-Aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AccQ) was purchased from Toronto Research Chemicals (Toronto, ON, Canada).

2.2. Fermented foods

Vietnamese fish sauce (VFS), Thai fish sauce (TFS), Japanese fish sauce (JFS), Japanese soy sauces (two *Koikuchi*-type; JSS-K1 and JSS-K2, and *Tamari*-type; JSS-T) were commercially obtained from local markets in Kyoto, Japan. These products are well-known brands, but they are not representative of the country's products. As references of lightly

fermented foods, Camembert-type cheese produced in Japan and German-style dry salami sausage produced in Japan were also obtained from online markets. All samples were kept at -30 °C until use.

2.3. Identification of peptides in samples without derivatization

Fish and soy sauces were diluted four times with distilled water. For extraction of peptides in the cheese and salami, samples (500 mg) were homogenized with 500 μL of distilled water in a BioMusher II (Nippi, Tokyo Japan). The homogenate was mixed with 500 μL of ethanol and then centrifuged at 12,000 × *g* for 10 min at 4 °C. The supernatant was collected and kept at -30 °C. Aliquots of the extracts were further diluted 10 times with distilled water and then diluents were filtered through a Cosmonice filter (4 mm i.d., 0.45 μm, Nacalai Tesque). Aliquots of the filtrate (10 μL) were directly analyzed using a liquid chromatography-electrospray ionization tandem mass spectrometer (LC-MS/MS; LCMS 8040, Shimadzu, Kyoto, Japan) equipped with an Inertsil ODS-3 column (2.1 mm i.d. × 250 mm, GL Science, Tokyo, Japan). The column was kept at 40 °C. The mobile phases used were 0.1% formic acid (solvent A) and 0.1% formic acid containing 80% acetonitrile (solvent B). The peptides were resolved using a binary linear gradient at a flow rate of 0.2 mL/min. The gradient program was set up as follows: 0–2 min, 0% B; 2–30 min, 0–30% B; 30–40 min, 30–100%

B; 40–45 min, 100% B; 45–45.1 min, 100–0% B; 45.1–55 min, 0% B. Detection was carried out by total ion scan and precursor ion scan targeting immonium ion of pyroglutamyl residue (mass to charge ratio, *m/z* 84.1) at collision energy at -35 V in positive mode. Scan ranges were set to *m/z* 100–200, 200–225, 225–250, 250–275, 275–300, 300–350, 350–400, 400–500, 500–600, and 600–1000. The observed precursor ions of peptides were further analyzed by the product ion scan mode at collision energies of -15, -25, and -35 V to estimate the peptide sequence. The peptide sequences were estimated based on the *m/z* of the precursor and product ions, immonium ions, amino terminal series ions (a, b, and c product ions), and carboxy terminal series ions (x, y, and z product ions) defined by Medzihradszky and Chalkley (2015).

2.4. Identification of peptides in samples with AccQ derivatization

Aliquots (20 μL) of the diluents were added with 20 μL of 0.3% (w/v) AccQ-acetonitrile solution and 60 μL of 50 mM sodium borate buffer (pH 8.8). The reaction was carried out for 10 min at 50 °C. The reactant was mixed with 50 μL of 5 mM sodium phosphate buffer (pH 7.5) and clarified via passing through the Cosmonice filter. The filtrate (20 μL) was injected to the LC-MS/MS. Separation was carried out using same solvents by a binary gradient as 0–3 min, 0% B; 3–20 min, 0–30% B; 20–30 min, 30–100% B; 30–35 min, 100% B; 35–35.1 min, 100–0% B; 35.1–45 min, 0% B. Detection was carried out by total ion scan and

precursor ion scan targeting the AccQ-derived product ion (b1 ion, *m/z* 171.1) at the same collision energy and scan ranges as mentioned before. The observed precursor ions of the peptides were further analyzed in the product ion scan mode.

2.5. Peptide synthesis

The Fmoc strategy was used to synthesize peptides using a PSSM-8 peptide synthesizer (Shimadzu) according to the manufacturer's protocol, with slight modifications. Pyroglutamyl peptides were synthesized using the same protocol, except that L-pyroglutamic acid was used instead of the amino-terminal Fmoc amino acid. L-α-, L-β-, D-α-, and D-β-aspartyl peptides were synthesized using Fmoc-L-Asp-β-*t*-butyl-ester, Fmoc-L-Asp-α-*t*-butyl-ester, Fmoc-D-Asp-β-*t*-butyl-ester, and Fmoc-D-Asp-α-*t*-butyl-ester, respectively (Ejima et al., 2018). Diketopiperazines were synthesized as described by Nagao et al. (2024). L-γ-glutamyl peptides were synthesized by using Fmoc-L-Glu-α-*t*-butyl-ester. The synthesized peptides were purified using a Cosmosil 5C18-MS-II column (10 mm i.d. × 250 mm; Nacalai Tesque). The mobile phases were the same as those described previously. The gradient program was set up as follows: 0–20 min, 0–50% B; 20–30 min, 50–100% B; 30–35 min, 100% B; 35–35.1 min, 100–0% B; 35.1–45 min, 0% B at a flow rate of 2 mL/min. The column was kept at 40 °C. Absorbance at 214 and 254 nm was used to monitor peptide elution. The purity of the isolated peptides was determined by LC-MS/MS. The content of

the purified peptides was quantified by amino acid analysis after HCl hydrolysis (Bidlingmeyer et al., 1984; Sato et al., 1992).

2.6. Quantification of peptides in fish and soy sauces

Aliquots of the sample filtrate (10 μL) was directly analyzed by the LC-MS/MS in the multi reaction monitoring (MRM) mode. An Inertsil ODS-3 column was used to resolve the peptides in the reversed phase mode. Synthetic peptides were used to optimize MRM conditions using LabSolutions LCMS Ver. 5.5 (Shimadzu) and were used as external standards for peptide quantification. The LC-MS/MS elution conditions were the same as those described above.

2.7. Animal experiments

Animal experiments were performed at the Louis Pasteur Center for Medical Research. Guidelines for animal studies from the National Institutes of Health (NIH) were followed for animal experiments. The Animal Care Committee of the Louis Pasteur Centre for Medical Research approved all experimental procedures (No. approval: 20223). Seven-week-old male Wistar rats (210–230 g) were obtained from Japan SLC (Shizuoka, Japan) and acclimatized to the environmental conditions for one week. The rats were kept in a 24-26 °C room, and 40-60% humidity under a 12-h light-dark cycle. The rats were fed a certified rodent diet (MF;

Oriental Yeast, Tokyo, Japan) and had free access to water and food during the acclimatization period. Before the oral administration of the sample, all rats were fasted for 16 h and divided into two groups. The first group was administered 8% salt water (vehicle group, n=3) at 222 μL/200 g body weight using a sonde, and the second group received two × dilutions of VFS (VFS group, n=3). Under isoflurane anesthesia, blood was collected from the portal and abdominal veins using a heparinized syringe, 60 min after administration. The plasma was prepared by centrifugation at 800 × *g* for 10 min. The inner contents of the small intestine were flushed with 10 mL of PBS. The washed small intestine was cut into two parts (upper and lower) and used as the anterior and posterior parts, respectively. All samples were stored at -80 °C for further analyses.

2.8. Quantification of peptides in rats

Aliquots of plasma were mixed with three volumes of ethanol in 1.5 mL tubes. The precipitated proteins were removed by centrifugation at 12,000 × *g* for 10 min. The supernatant was used as the ethanol-soluble fraction of the blood plasma. The suspension of the inner contents of small intestine was mixed with three volumes of ethanol. Aliquots of the center of anterior and posterior parts of small intestine (100 mg) were homogenized with 100 μL PBS in the BioMasher II. The homogenate was then mixed with three volumes of ethanol. The ethanol-soluble fractions were prepared by centrifugation as described above. Aliquots

of the ethanol-soluble fractions (100 μL) were dried under vacuum and kept at -80 °C until use. The residue was dissolved in 100 μL distilled water and then clarified by passing through the Cosmonice filter. The peptides in the filtrates were quantified by LC-MS/MS in the MRM mode under the same conditions mentioned above.

2.9. Statistical analysis

Data are shown as mean ± standard deviation (n = 3). Differences in the peptide content in the rat body between the vehicle and VFS groups were evaluated by Student's *t* test using SPSS 22 (SPSS, Chicago, IL, USA). $P<0.05$ was considered statistically significant.

3. Results

3.1. Identification of peptides

Figure 1A shows MS chromatograms of AccQ-derivatives of amino compounds in VFS. Mass to charge ratios (*m/z*) of the main compounds are presented on each peak. These precursor ions were further analyzed by product ion scan mode. Based on the *m/z* of precursor ion and retention time, amino acids were identified and are indicated by one letter abbreviations on each peak. In addition to amino acids, monoamines, polyamines, and total of 43 normal linear peptides were identified in the VFS based on *m/z* of precursor and product

ions as summarized in Table 1. Estimated peptide structure is also presented on each peak for example GP (Gly-Pro) in the Figures 1A and 1B. In addition to the peptides consisting of leucine and isoleucine, some glutamyl and aspartyl peptides generated multiple peaks with same *m/z* as indicated by arrows in Figure 1B. However, resolution of these peaks was not so good. To resolve these peaks, compounds in the water diluent of VFS were directly subjected to LC-MS analysis without the derivatization. MS chromatograms of the non-derivatives in the VFS in total ion scan mode are presented in Figure 2. Precursor ions in major peaks were analyzed by product ion scan mode. In addition to amino acids, acetyl, propionyl, and butyl-amino acids were also identified (Figure 2) based on *m/z* of precursor and product ions (Supplementary table S1). Peaks of modified amino acids are indicated using prefixes such as acetyl, propionyl, and butyryl, and one letter abbreviation of amino acids on the peaks. Peaks assigned to peptides are numbered. Structures of these peptides were estimated based on *m/z* of precursor and product ions as summarized in Table 2. Peak numbers of diketopiperazines and pyroglutamyl peptides are highlighted in red and green, respectively (Figure 2). The peak numbers of the aspartyl and glutamyl peptides are highlighted in dark blue and light blue, respectively (Figure 2). Peaks sharing the same number had same *m/z* of precursor ions. Better resolution of the peaks sharing the same *m/z* was achieved compared to that of the AccQ derivatives. The precursor and product ion scan analyses revealed that peaks sharing same *m/z* consisted of peptides containing Ile or Leu (peaks 23 and 24) and dipeptides with

aspartyl residues (peaks 26, 27, 28, and 29) and glutamyl residues (peaks 21, 22, 23, 24, and 25) at amino-terminus.

Presence of some α/γ-glutamyl peptides and α/β-L/D aspartyl peptides in some fermented foods has been reported (Yang et al., 2019; Nagao et al., 2024). Those isomers of glutamyl and aspartyl peptides in the VFS were identified by comparison of the retention time of synthetic standards in RP-HPLC. Representative mass chromatograms for glutamyl and aspartyl isomers in MRM mode are shown in Figure 3 and Figure 4, respectively. These data indicate presence of α/γ-glutamyl peptides and α/β-L/D aspartyl peptides in the VFS. Figure 5 shows the structure of those peptides.

Consequently, we identified 12 pyroglutamyl peptides, 5 glutamyl dipeptides with α and γ peptide bonds, 4 aspartyl dipeptides with L/D aspartyl residues and α/β peptide bonds, and 15 diketopiperazines (Table 2) and 43 unmodified dipeptides and tripeptides (Table 1). Total of 96 peptides were identified.

3.2. Contents of peptides in samples

In addition to the seven major normal peptides, the aspartyl and glutamyl dipeptides were quantified in all samples, and their levels, including all their all isomers, are displayed as a heat map in Figure 6. Contents of all γ-glutamyl peptides (γ-Glu-Met, γ-Glu-Val, γ-Glu-Ile, γ-Glu-Leu, and γ-Glu-Phe) in soy and fish sauces (0.1–1.8 mM) were higher than those of

their α-glutamyl forms (0.01-0.1 mM). In particular, γ-Glu-Val and γ-Glu-Phe were abundantly present in the VFS (> 1.4 mM). Only negligible amounts of γ-glutamyl peptides were present in lightly fermented foods, such as salami and cheese, which were used as references. All aspartyl dipeptides (Asp-Val, Asp-Ile, Asp-Leu, and Asp-Phe) and their isomers were detected in all fish and soy sauces samples. Contents of these peptides were distributed between 0.01–0.9 mM in soy and fish sauces depending on the sequences. The contents of Da-Asp-Leu and Da-Asp-Phe were lower than those of the other aspartyl peptides. Only negligible amounts of b-Asp dipeptides were present in the reference foods (salami and cheese).

Levels of pyroglutamyl peptides and diketopiperazines are displayed as heat map in Figure 7. Levels of pyroglutamyl dipeptides were much abundant (0.01–1.3 mM) compared to longer pyroglutamyl peptides in all samples. Lower but significant levels of pyroglutamyl peptides were present in salami but not in cheese.

All diketopiperazines identified in the VFS were present in all fish and soy sauces samples at different levels (0.1–11 mM). Among them, cyclo-Glu-Pro in the soy sauce (JSS-K2) showed the highest content (> 10 mM) followed by cyclo-Thr-Pro in JSS-K2 (>5 mM). A fish sauce (JFS) contained smaller levels of cyclo-Tyr-Pro, cyclo-Pro-Pro, cyclo-Val-Pro, and cyclo-Phe-Pro compared to other fish sauces. Some diketopiperazines were also present in the reference foods (salami and cheese).

3.3. Bioavailability of peptides

3.3.1. Small intestinal lumen

As shown in Table 3, the seven major normal peptides except for the aspartyl and glutamyl dipeptides did not significantly increase in the lumen of the small intestine 60 min after the administration of VFS compared to the administration of vehicle, whereas some of them (Gly-Phe, Ala-Glu, Gly, Ile, Gly-Leu, Gly-Pro/Pro-Gly, Ala-Pro, and Val-Pro) were present in the lumen even after administration of vehicle after overnight fasting. Most modified peptides except for some tri- and tetra-pyroglutamyl peptides were present in the small intestinal lumen of the vehicle group ranging from 0.04 to 1.17 μM in the effluent (10 mL). These peptides are produced from endogenous and microbial proteins. After the oral administration of VFS, only one of α-glutamyl (α-Glu-Leu) and most γ-glutamyl dipeptides except for γ-Glu-Ile significantly increased in the lumen (0.10 −5.60 μM). γ-Glu-Val and γ-Glu-Phe were two highest γ-glutamyl isopeptides in the lumen after administration of VFS and then followed by γ-Glu-Ile and γ-Glu-Leu. Most aspartyl dipeptides were significantly increased by the administration of VFS. Among them, Lβ-form of Asp-Ile, Asp-Leu, and Asp-Phe increased 5 times higher than the vehicle group and reached until 0.6 μM in the effluent (10 mL).

Most diketopiperazines, except for cyclo-Phe-Pro, significantly increased in the lumen after the administration of VFS. Levels of the diketopiperazines in the effluent of lumen were approximately 0.0–0.03 and 0.02–9.3 μM of the effluent (10 mL) after the administration of vehicle and VFS, respectively. Cyclo-Asp-Pro increased most abundantly and followed by cyclo-Asn-Pro, cyclo-Glu-Pro, and cyclo-Ser-Pro.

Most pyroglutamyl dipeptides significantly increased or tended to increase after the administration of VFS, while larger pyroglutamyl peptides did not significantly change except for pGlu-Phe-Gln.

3.3.2. Washed small intestinal tissue

We also measured the levels of VFS-derived peptides in the anterior and posterior parts of the washed small intestines of the rats. In the anterior part, the levels of indigenous glutamyl and aspartyl dipeptides were 166–6764 and 2–117 nmol/kg, respectively. In the posterior part, the levels of indigenous glutamyl and aspartyl dipeptides were 172–3556 and 2–101 nmol/kg, respectively. After oral administration of VFS, unexpectedly, some normal peptides in VFS except for Lα-Asp-Val, Lα-Asp-Ile and Lα-Val-Pro significantly increased in the intestinal tissue (Table 4). Most of β and γ isomers of aspartyl and glutamyl dipeptides, except for γ-Glu-Met, γ-Glu-Ile, γ-Glu-Leu, γ-Glu-Phe in the anterior part and Dα-Asp-Ile, Dα-Asp-Leu and Dα-Asp-Phe in both parts, significantly increased by administration of VFS compared to

the vehicle. The small intestinal tissue contained high levels of γ-Glu-Val, γ-Glu-Ile, γ-Glu-Leu and γ-Glu-Phe compared to other isopeptides after administration of VFS. Generally, levels of the glutamyl dipeptides were higher compared to those of the aspartyl dipeptides (Table 4).

As shown in Table 5, all diketopiperazines and most pyroglutamyl dipeptides except, for pGlu-Tyr and the long-chain pyroglutamyl peptides, were significantly increased in the both small intestinal tissues, while these peptides were also present in the vehicle group. Levels of the diketopiperazines in the both parts of the small intestine tissue were distributed between 1–378 and 4–3300 nmol/kg after administration of vehicle and VFS, respectively. The pyroglutamyl dipeptides were distributed between 0–318 nmol/kg and 0–1500 nmol/kg after administration of vehicle and VFS, respectively. Cyclo-Asp-Pro and pGlu-Val were the most abundant peptides in the both parts of intestinal tissue after ingestion of VFS. These data indicate that the dipeptides found in VFS including some normal dipeptides were absorbed by the small intestine.

3.3.3. Portal and peripheral blood

Blood plasma levels of glutamyl and aspartyl dipeptides, pyroglutamyl peptides, diketopiperazines, and the seven major normal peptides were examined by using LC-MS/MS in MRM mode. The seven major normal peptides including Lα forms of glutamyl and

aspartyl dipeptides did not significantly increase in blood plasma after administration of VFS. All forms of glutamyl dipeptides found in VFS were present in portal and abdominal blood plasma of rats receiving the vehicle after overnight fasting ranging from 0.8 to 266 nM. Nonetheless, γ-Glu-Val, γ-Glu-Ile, and γ-Glu-Phe increased slightly but significantly increased to 228, 90 and 271 nM in the plasma from abdominal vein blood after the administration of VFS, respectively. Detectable but small amounts of all forms of aspartyl dipeptides found in VFS were present in the portal and abdominal blood plasma of rats received vehicle ranging from 0.2 to 7 nM. Except for the normal Lα- forms, most aspartyl isopeptides significantly increased in both bloods after administration of VFS (1–15 nM). In addition, concentrations of γ-glutamyl dipeptides in the blood plasma from vehicle group were higher compared to those of α-glutamyl dipeptides in blood and all isomers of aspartyl dipeptides.

Most diketopiperazines, except the cyclo-Tyr-Pro, and some pyroglutamyl dipeptides, such as pGlu-Ile, pGlu-Tyr, pGlu-Leu, and pGlu-Pro, were significantly increased in the both bloods, while these peptides were also present in the vehicle group. After administration of vehicle and VFS, levels of the diketopiperazines and in the portal and abdominal blood were 3–400 and 24–5200 nM and pyroglutamyl dipeptides were 0.0–29 and 0.0–54 nM, respectively. Cyclo-Thr-Pro and pGlu-Pro were the most abundant diketopiperazine and pyroglutmyl peptide in the blood after administration of VFS.

4. Discussion

Fish and soy sauces are made from fish and soybean, respectively. Roasted wheat grain is generally used as fungi starter (*Aspergillus sojae*) for most Japanese-style soy sauce (*koikuchi*-type) Proteins in these ingredients are degraded by endogenous and also fungal, bacterial, and yeast proteases into amino acids and small peptides, which provides *umami* (Hakimi et al., 2022; Lioe et al., 2010; Zhao et al., 2016) and *kokumi* (Phewpan et al., 2019; Chen et al., 2023) on these products. The present study revealed presence of some modified amino acids such as *N*-acetyl, *N*-propionyl, and *N*-butyryl amino acids in addition to free amino acids in the fish and soy sauces. Furthermore, presence of some modified peptides such as γ-glutamyl dipeptides, β-aspartyl dipeptides, D-aspartyl dipeptides and amino terminal blocked peptides such as pyroglutamyl peptides and diketopiperazines (cyclic dipeptides) were detected in these samples.

It has been reported that pyroglutamyl peptides are widely present in many enzymatic digests of food proteins (Ejima et al., 2018; Miyauchi et al., 2022; Sato et al., 1998; Wijanarti et al., 2024) and fermented foods (Kiyono et al., 2016; Nagao et al., 2024; Shirako et al., 2020). The present study also demonstrates presence of pyroglutamyl peptides in fish and soy sauces. Presence of γ-glutamyl peptides in fermented foods such as cheese (Kuroda et al.,

2020) and some non-fermented foods such as legumes (Dunkel et al., 2007; Taylor et al., 2008) has been reported. In addition, glutathione, γ-glutamyl-cysteinyl-glycine, and related compounds such as γ-glutamyl-S-methyl-cysteine are widely distributed in many organisms (Lu et al., 2021). The present study shows that some fish sauces contain high levels (0.5–2 mM) of γ-glutamyl peptides such as γ-Glu-Val (1.58 mM) and γ-Glu-Phe (1.42 mM) than other foods. The α- and γ-glutamyl peptides were well resolved by reversed phase-high performance liquid chromatography without derivatization, while separation of AccQ-derivatives of α- and γ-glutamyl peptides were not easy. Nagao et al. (2024) used AccQ derivatization for detection of peptides in *miso*. There is, therefore, a possibility that γ-glutamyl dipeptides may be present in *miso*, while the presence of γ-glutamyl dipeptides in *miso* was not reported (Nagao et al., 2024). Some studies have reported that diketopiperazines are present in non-fermented foods, such as roasted cocoa (Stark and Hofmann, 2005) and chicken soup broth (Chen et al., 2004) and fermented foods, *miso* (Nagao et al., 2024). However, their contents are not so high compared to those in the fish and soy sauces (0.1–11 mM). Only a limited number of studies have reported the presence of aspartyl isopeptides in enzymatic hydrolysates of porcine liver proteins (Ejima et al., 2018) and *miso* (Nagao et al., 2024). Long-aged *miso* contains higher levels of aspartyl isopeptides than fish and soy sauce. Taken together, fish sauces are characterized by high contents of some γ-glutamyl peptides such as γ-Glu-Val and γ-Glu-Phe, while soy sauces are

characterized with high contents of some diketopiperazines, such as cyclo-Glu-Pro and cyclo-Thr-Pro.

It is well-known that glutathione, one of γ-glutamyl peptides, is synthesized by γ-glutamyl cysteine ligase and glutathione synthetase. Other γ-glutamyl peptides are generated from glutathione by γ-glutamyl transpeptidases in mammalian, bacteria, yeast, and fungi. Sofyanovich et al. (2019) reported that *Saccharomyces cerevisiae* is able to produce γ-Glu-Val. *Lactobacillus reuteri* in certain fermented foods is capable of producing γ-glutamyl dipeptides like γ-Glu-Ile and γ-Glu-Cys, which possess an exclusive *kokumi* sensation (Yang et al., 2019). These facts suggest that γ-glutamyl peptides in fish and soy sauces are synthesized by enzymes from microorganisms involved in fermentation. *Miso* is fermented without stirring, whereas the fish and soy sauce are fermented with stirring. Therefore, fermentation for the production of fish and soy sauces is considered to be more aerobic than that of *miso*. This difference may be related to the higher γ-glutamyl peptide content in the fish and soy sauces.

Diketopiperazines are generated from linear peptides through nonenzymatic processes, such as heating (Otsuka et al., 2019; Shimamura et al., 2017). Japanese soy sauce is generally heated during the final process. Soy sauce is darker in color than fish sauce, indicating that soy sauce undergoes a stronger Maillard reaction during heating. Although the detailed

manufacturing conditions for the production of fish sauce are not available, the heating process may be responsible for the higher levels of diketopiperazines in soy sauce.

Some modified peptides, such as pyroglutamyl peptides, L/D-β-aspartyl peptides, and diketopiperazines, were increased in the luminal content and small intestinal tissue after oral administration. In blood, diketopiperazines and hydrophobic L/D-aspartyl peptides were significantly increased, while only a portion of pyroglutamyl peptides showed slight increase. The present study reveals that administration of VFS significantly increased γ-glutamyl dipeptides in luminal content and small intestinal tissue. However, increase of γ-glutamyl dipeptides in blood was not so high compared to diketopiperazines and β-aspartyl peptides but higher than pyroglutamyl peptides. Possibly, part of γ-glutamyl dipeptides may be degraded or modified by some enzymes after absorbance into enterocytes. Miyauchi et al. (2022) have reported that normal peptide levels do not increase in the small intestine and blood after ingestion of rice protein hydrolysate. Surprisingly, in the present study, some normal dipeptides were increased in the small intestine, but not in the blood. This suggests that the modified peptides may inhibit exopeptidase activity in the intestine. However, once these normal peptides enter the bloodstream, they may be completely degraded by exopeptidases into amino acids.

It has been reported that γ-glutamyl dipeptides are involved in enhancing *kokumi* sensation (Ohsu et al., 2010). In addition, γ-glutamyl dipeptides such as γ-Glu-Val have been

426 reported to ameliorate TNF-α-induced vascular inflammation using a cell culture model
427 (Guha et al., 2020) and lipopolysaccharide-induced sepsis in mice model (Chee et al., 2017).
428 Furthermore, it has been reported that γ-Glu-Cys reduced postprandial hyperglycemia
429 (Muramatsu et al., 2014). Recent *in vitro* study has demonstrated that these γ-glutamyl
430 dipeptides act as an allosteric ligand of calcium sensing receptor (CaSR), which modulates
431 intracellular signaling, consequently suppresses inflammatory response (Guha and Majumder,
432 2022). Daily consumption of East Asian fish and soy sauces has potential benefits based on
433 the activation of the CaSR mentioned above. However, these studies used relatively high
434 doses of γ-glutamyl dipeptide (0.01–1 mM in culture medium and 20–50 mg/kg body weight
435 orally). Therefore, the effectiveness of daily consumption of these products via CaSR
436 activation by γ-glutamyl dipeptides needs to be examined.

437 Pyroglutamyl peptides, particularly pGlu-Leu and pGlu-Asn-Ile, have been reported
438 to improve hepatitis (Sato et al., 2013), colitis (Kiyono et al., 2016), high-fat diet-induced
439 obesity (Shirako et al., 2020), and gut microbiota dysbiosis (Shirako et al., 2019) in animal
440 models at relatively low doses (0.1–1 mg/kg). Pyroglutamyl peptides are also widely
441 distributed in other East Asian fermented foods, such as *miso* (Nagao et al., 2024) and
442 Japanese rice wine, *sake* (Kiyono et al., 2016). Therefore, pyroglutamyl peptides in fish and
443 soy sauces along with other East Asian fermented seasonings may contribute to the beneficial
444 effects in humans in these areas. Further epidemiological, observational, and interventional

studies are required to confirm this finding. It has been demonstrated that some β-aspartyl peptides exert anti-fatigue activity in animal model (Nakagawasai et al., 2021) and have inhibitory activity against angiotensin converting enzyme (Nagao et al., 2024). Unlike other ACE inhibitor peptides, present study and Nagao et al (2024) showed that β-aspartyl peptides have high bioavailability and, therefore, have potential for the antihypertensive and anti-inflammatory effects of consuming these fermented foods. Diketopiperazines have been reported to exert antioxidant, antidepressant, antimicrobial, and other beneficial effects (Taga et al., 2017). However, efficacy of diketopiperazines in doses obtained by consumption of the fermented foods remains to be examined.

5. Conclusion

The present study identified short-chain modified peptides such as γ-glutamyl dipeptides, β-aspartyl dipeptides, diketopiperazines (cyclic dipeptides), pyroglutamyl peptides and modified amino acids in fish and soy sauces in higher amounts compared to those in the reference foods (cheese and salami). Among them, γ-glutamyl dipeptides (0.5-2

464 mM) were abundantly presents in the Vietnamese fish sauce (VFS). These modified peptides
465 showed higher bioavailability compared with the normal peptides. Taken together with
466 previously reported data, these modified peptides in fish and soy sauces can be candidates of
467 bioactive peptides with health-promoting activities. Further research is necessary to elucidate
468 their effects of the doses obtained by actual consumption of these fermented foods.
469

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475

476 **Declaration of interest**

477 All authors declare that there is no conflict of interest related to this paper.
478

479 **References**
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627 **Legends for Figures:**

628 **Figure 1.** Mass spectrometry (MS) chromatograms of AccQ-derivatized compounds in the
629 VFS. A: Peaks were detected by precursor ion scan targeting the AccQ-derived product ion
630 (*m/z* 171.1) in the positive mode across the *m/z* of 225–250, 250–275, 275–300, 300–350,
631 350–400, 400–500 and 500–600. Identified amino acids, peptides, and their metabolites are
632 labelled using single-letter amino acid codes or full names. B: Enlarged MS chromatogram at

633 the scan range *m/z* 400-500. Other abbreviations: Hydroxyproline (O), β-alanine (*D),
634 ethanolamine (*S), γ-aminobutyric acid (*E), isopropanolamine (*T), prolinamide (P-NH_2),
635 taurine (Ta), and citrulline (Cit). Lysine reacted with one and two AccQ are indicated as K1
636 and K2, respectively. Ornithine, putrescine, and cadaverine reacted with one AccQ are
637 indicated as Or1, Pu1, and Ca1, respectively. Unlabelled peaks could not be assigned to
638 amino acids, peptides, or their metabolites.
639
640 **Figure 2.** MS chromatograms of non-derivatized compounds in the VFS. Peaks were
641 detected by total ion scan in the positive mode across the *m/z* of 100–200, 200–225, 225–250,
642 250–275, 275–300, and 300–350. Peaks of amino acids are presented by single-letter amino
643 acid codes. Peaks of modified amino acids with organic acids are indicated using prefixes
644 such as acetyl-, propionyl-, and butyryl- and single-letter amino acid codes. Peaks with
645 numbers were identified as peptides. Peaks with same numbers indicated presence of peptide
646 isomers with same precursor and product ions. Unlabelled peaks could not be assigned to
647 amino acids, peptides, or their metabolites. *Indicates the ion consisting of two pyroglutamic
648 acids and proton.
649
650 **Figure 3.** Representative MS chromatograms of two isomers of the underivatized α– and γ-
651 glutamyl-dipeptides in the FVS. Samples and the synthetic standard peptides were analysed
652 using RP-HPLC-MS/MS in MRM mode. αEM and γEM represent α-Glu-Met and γ-Glu-Met,
653 respectively. For other glutamyl dipeptides are shown to be similar.
654
655 **Figure 4.** Representative MS chromatograms of four isomers of underivatized aspartyl-
656 dipeptide (Lα-Asp-X, Lβ-Asp-X, Dα-Asp-X, and Dβ-Asp-X) in the FVS. X is occupied by

Val (V), Ile (I), Leu (L) and Phe (F). Samples and synthetic standard peptides were analysed using RP-HPLC-MS/MS in MRM mode.

Figure 5. Structure of α- and γ-glutamyl dipeptides (A) and α- and β- L/D aspartyl dipeptides (B). X is occupied by amino acid residues.

Figure 6. Heatmap of contents of all forms of 10 glutamyl dipeptides,16 aspartyl dipeptides, and 7 normal major peptides in the samples. Refer Figure 5 for structures of isomers.

Figure 7. Heatmap of contents of 16 pyroglutamyl peptides and 12 diketopiperazines in the samples. pGlu represents pyroglutamyl residue. The Cyclo-Tyr-Pro is a diketopiperazine consisting of Tyr and Pro residues.

Table 1. Estimated sequences of peptides in the VFS derived with AccQ based on *m/z* of product ions and precursor ions. The derivates were resolved by LC-MS/MS as shown in Figure 1A. Asterisks (*) represent immonium ions of amino acid. AccQ b1 ion was derived from AccQ moiety of the peptide derivatives. a, b, and c series and x, y and z series of product ions of peptides are present. *M/Z* is mass per charge. MW represents molecular weight of peptides without derivatization. RT is retention time.

Sequences	**RT**	**Precursor ion (*m/z*)**	**MW**	**Product ion (*m/z*)**
Gly-Pro	17.81	343	172	30 (Gly*), 70.0 (Pro*), 116.0 (y1), 171.0 (AccQ, b1), 173.3 (y2), 227.8 (b2) & 343.3
Glu-Pro	19.50	415	244	84 (Glu^{b}), 116 (y1), 171.0 (AccQ, b1), 227 (z2), 272 (a2), 272 (x2), 300 (b2) & 415
Ala-Pro	20.35	357	186	44.2 (Ala*), 70.0 (Pro*), 116.1 (y1), 171.0 (AccQ, b1), 186.8 (y2), 242.2 (b2) & 357.0
Ser-Pro	17.65	373	202	60 (Ser*), 70 (Pro*), 116 (y2), 171 (AccQ, b1), 258 (b2), 203 (y2) & 373
Pro-Pro	20.46	383	212	70.4 (Pro*), 116.1 (y1), 171.1 (AccQ, b1), 212.8 (y2), 240 (a2), 240 (x2), 267.9 (b2) & 383.0
Val-Pro	26.22	385	214	72.3 (Val*), 70.2 (Pro*), 116.2 (y1), 171.1 (AccQ, b1), 215.1 (y2), 242.1 (a2), 269.9 (b2) & 385.0
Ile-Pro	29.95	399	228	70.2 (Pro*), 86.0 (Ile*), 116.4 (y1), 171.1 (AccQ, b1), 228.8 (y2), 256.3 (x2), 284.1 (b2) & 399.0
Leu-Pro	30.47	399	228	70.6 (Pro*), 86.2 (Leu*), 116.4 (y1), 171.1 (AccQ, b1), 228.8 (y2), 256.3 (x2), 284.1 (b2) & 399.0
Glu-Pro	19.63	415	244	70.4 (Pro*), 84 (Glu^{b}) 102.2 (Glu*), 116.0 (y1), 171.1 (AccQ, b1) 187 (c1), 272 (a2), 300 (b2) & 415
Gly-Pro-Ala	17.58	414	243	44 (Gly*), 127, 155, 171.1 (AccQ, b1), 187,200, 228, 244 & 414
Ser-Pro-Asp	19.00	488	317	60 (Ser*), 171.1 (AccQ, b1), 255 (z2), 318 (y3), 355 (b3) & 488
Gly-Pro-Thr	20.35	444	273	70 (Pro*), 74 (Thr*) 114, 127, 145, 171 (AccQ, b1), 200 (a2), 217 (y2), 228 (b2) & 444
Ala-Hyp	16.26	373	202	44 (Ala*), 86,3 (Hyp*), 132.1 (y1), 171.0 (AccQ, b1), 203.0, (y2), 242.3 (b2) & 373.0
Ile-/Hyp	25.56	415	244	86.0 (Ile*), 86.0 (Hyp*), 132.4 (y1), 171.1 (AccQ, b1), 245.1 (y2), 256.1 (a2), 284.1(b2) & 415.0
Leu/Hyp	26.00	415	244	86.0 (Ile*), 86.0 (Hyp*), 132.4 (y1), 171.1 (AccQ, b1), 245.1 (y2), 256.1 (a2), 284.1(b2) & 415.0
Phe-Hyp	21.59	449	278	145, 171 (AccQ, b1), 279 (y2), 318 (b2) & 449
Gly-Pro-Hyp	16.80	456	285	70 (Pro*), 86 (Hyp*), 127 (a3), 132 (y1), 171 (AccQ, b1), 229 (y2), 286 (y3) & 456
Gly-Ile	23.00	359	188	30 (Gly*), 86 (Ile*), 128, 132 (y1), 145, 171 (AccQ, b1), 189 (y2), 200 (a2), 228 (b2), & 359

Gly-Leu	23.50	359	188	30 (Gly*), 86 (Ile*), 128, 132 (y1), 145, 171 (AccQ, b1), 189 (y2), 200 (a2), 228 (b2), & 359
Gly-Ile (Heavy type)	23.00	360	189	86/87 (Ile*), 171/172 (AccQ, b1), 190 (y2), 201 (a2), 229 (b2), & 359
Gly-Leu (Heavy type)	23.50	360	189	86/87 (Leu*), 171/172 (AccQ, b1), 190 (y2), 201 (a2), 229 (b2), & 359
Gly-His	10.14	383	212	110 (His*), 156 (y1), 171 (AccQ, b1), 200 (a1), 213 (y2), 338 (a3), 365 (b3) & 383
Pro-Glu	13.75	415	244	70 (Pro*), 102 (Glu*), 171.1 (AccQ, b1), 264.1, 244.2, 367.4 & 415
Gln-Pro	13.50	414	243	84 (Gln[b]), 116 (y1), 171 (AccQ, b1), 244 (y2) & 414
Glu-Met	21.00	449	278	84 (Glu[b]), 171 (AccQ, b1), 272 (a2), 249 (x2), 279 (y2), 300 (b2) & 449
Asp-Ile	21.50	417	246	88 (Asp*), 132 (y1), 171 (AccQ, b1), 247 (y2), 286 (b2) & 417
Asp-Leu	22.00	417	246	88 (Asp*), 132 (y1), 171 (AccQ, b1), 247 (y2), 286 (b2) & 417
Ala-Ile	24.68	373	202	44 (Ala*), 86 (Ile*), 171 (AccQ, b1), 214 (a2), 242 (b2) & 373
Ala-Leu	24.78	373	202	44 (Ala*), 86 (Leu*), 171 (AccQ, b1), 214 (a2), 242 (b2) & 373
Asn-Glu	15.20	432	261	87 (Asn*), 70 (Asn[b]), 171 (AccQ, b1), 258 (a2), 286 (b2) & 433
Val-Gly	20.30	345	174	72 (Val*), 55 (Val[b]), 171 (AccQ, b1), 242 (a2), 270 (b2) & 345
Ala-Val	12.10	359	188	44 (Ala*), 171 (AccQ, b1), 189 (y2), 215 (a2) & 359
Gly-Gly-Gly	11.10	360	189	171 (AccQ, b1), 190 (y3), 200 (a2) & 360
Gly-Met	19.70	377	206	171 (AccQ, b1),186 (c1), 207 (y2), 228 (b2) & 377
Ala-Glu	16.40	389	218	44 (Ala*), 171 (AccQ, b1), 148 (y1), 219 (y2), 243 (b2) & 389
Gly-Phe	20.35	393	222	30 (Gly*), 120 (Phe*), 166 (y1), 171 (AccQ, b1), 201 (a2), 228 (b2) & 393
Gly-Gly-Pro	15.90	400	229	30 (Gly*), 70 (Pro*), 116 (y1), 171 (AccQ, b1), 200 (a2), 173 (y2), 227 (b2) & 400
Glu-Ile	25.00	431	260	84 (Glu[b]), 86 (Ile*), 102 (Glu*), 132 (y1), 171 (AccQ, b1), 261 (y2), 300 (b2) & 431
Glu-Leu	26.00	431	260	84 (Glu[b]), 86 (Leu*), 102 (Glu*), 132 (y1), 171 (AccQ, b1), 261 (y2), 300 (b2) & 431
Glu-Phe	27.00	465	294	120 (Phe*), 171 (AccQ, b1), 278 (z2), 278 (b2), 295 (y2) & 465
Glu-Val	21.50	465	294	72 (Val*), 102 (Glu*) 171 (AccQ, b1), 229 (z2) & 417
Ala-Glu-Asn	25.50	431	260	44 (Ala*), 171 (AccQ, b1), 261 (b3) & 431
Glu-Gly	14.52	375	204	102 (Glu*), 171 (AccQ, b1), 272 (a2) & 375

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Table 2. Estimated sequences of peptides in the VFS without any derivatization. The peptides were resolved by LC-MS/MS as shown in Figure 2. Adduct ions consisting of two molecules and a proton are marked with †. One asterisk (*) represents immonium ion of amino acids. Two asterisks (**) represent related ions generated by decomposition of amino acid residues, except for the immonium ion. pGlu represents pyroglutamyl residue. MW represents molecular weight.

Sequences	Peak mark	Precursor ion (*m/z*)	MW	Product ion (*m/z*)
pGlu-dipeptides				
pGlu-Gly	1	188	187	84 (pGlu*), 30 (Gly*), 56 (pGlu**) & 76 (y1) (heavy type)
pGlu-Ala	2	201	200	84 (pGlu*), 44 (Ala*), 41, 56 (pGlu**), 90 (y1) & 155 (a2)
pGlu-Ser	3	217	216	84 (pGlu*), 60 (Ser*), 28, 41, 56 (pGlu**) & 106 (y1)
pGlu-Asp	4	245	244	84 (pGlu*), 28, 41, 56 (pGlu**), 134 (y1) & 227 (b2)
pGlu-Pro	5	227	226	84 (pGlu*), 70 (Pro*), 28, 41, 56 (pGlu**), 116 (y1), 181 (a2) & 208 (b2)
pGlu-Glu	6	259	258	84 (pGlu*), 102 (Glu*), 41, 56 (pGlu**), 84 (Glu**) 148 (y1) & 241 (b2)
pGlu-Thr	7	231	230	84 (pGlu*), 74 (Thr*), 28, 41, 56 (pGlu**), 120 (y1) & 213 (b2)
pGlu-Met	8	261	260	84 (pGlu*), 104 (Met*), 41, 56 (pGlu**), 61 (Met**), 150 (y1) 215 (a2) & 243 (b2)
pGlu-Ile	9	243	242	84 (pGlu*) 86 (Ile*), 41, 56 (pGlu**), 132 (y1), 129 (b2) & 197 (a2)
pGlu-leu	10	243	242	84 (pGlu*), 86 (Ile*), 4, 56 (pGlu**), 44 (Ile**), 132 (y1), 197 (a2), 141 (z1) & 225 (b2)
pGlu-Val	11	229	228	84 (pGlu*) 72 (Val*), 28, 41, 56 (pGlu**), 118 (y1), 183 (a2) & 211 (b2)
pGlu-Phe	12	277	276	84 (pGlu*), 120 (Phe*), 56 (pGlu**), 166 (y1) & 231 (a2)
pGlu	13	131	130	84 (pGlu*), 41, 56 (pGlu**) &130 (y1)
Two pGlu	13†	259	258	84 (pGlu*), 28, 41, 56 (pGlu**) & 130 (y1)

Glu-dipeptides				
α and γ Glu-Met	14	279	278	102 (Glu*), 104 (Met*), 84 (Glu**), 61 (Met**) & 150 (y1)
α and γ Glu-Val	15	247	246	102 (Glu*), 72 (Val*), 84 (Glu**), 41, 55 (Val**), 118 (y1) & 230 (z2)
α and γ Glu-Ile	16	261	260	102 (Glu*), 86 (Ile*), 84 (Glu**), 132 (y1) & 244 (Z2)
α and γ Glu-leu	17	261	260	102 (Glu*), 86 (Leu*), 84 (Glu**), 132 (y1) & 244 (Z2)
α and γ Glu-Phe	18	295	294	102 (Glu*), 120 (Phe *), 84 (Glu**), 166(y1) & 278 (z2)
Asp-dipeptides				
α and β-L/D-Asp-Val	19	233	232	88 (Asp*), 72 (Val*), 70 (Asp**), 55 (Val**), 118 (y1) & 187 (a2)
α and β-L/D-Asp-Ile/leu	20	247	246	88 (Asp*), 86 (Ile*), 70 (Asp**), 44 (Ile**), 132 (y1), 201 (a2) & 229 (b2)
α and β-L/D-Asp-Phe	21	281	280	88 (Asp*), 120 (Phe*), 70 (Asp**), 166 (y1) & 235 (a2)
Cyclic dipeptides				
Cyclo-Gly-Pro	22	155	154	70 (Pro*), 30 (Asp*), 70 (Asp**), 98 (z1) & 195 (b2)
Cyclo-Ala-Pro	23	169	168	70 (Pro*), 44 (Ala*) & 98 (z1)
Cyclo-Asp-Pro	24	213	212	70 (Pro*), 88 (Asp*), 70 (Asp**) & 195 (b2)
Cyclo-His-Pro	25	235	234	70 (Pro*), 110 (His*), 82, 121, 123 (His**) & 217 (b2)
Cyclo-Thr-Pro	26	199	198	70 (Pro*), 74 (Thr*), 98 (z1), 153 (a2) & 281 (b2)
Cyclo-Lys-Pro	27	226	225	70 (Pro*), 70, 84, 112 (Lys**), 98 (z1), 181 (a2) & 209 (b2)
Cyclo-Glu-Pro	28	227	226	70 (Pro*), 84 (Glu**) 116 (y1), 181 (a2) & 209 (b2)
Cyclo-Ser-Pro	29	185	184	70 (Pro*), 60 (Ser*) & 167 (b2)
Cyclo-Asn-Pro	30	212	211	70 (Pro*), 70 (Asn**) & 195 (b2)
Cyclo-Pro-Pro	31	195	194	70 (Pro*) & 98 (b1)
Cyclo-Ile-Pro	32	211	210	70 (Pro*), 86 (Ile*) & 44 (Ile**)
Cyclo-Leu-Pro	33	211	210	70 (Pro*), 86 (Ile*), 44 (Ile**) & 194 (z2)
Cyclo-Val-Pro	34	197	196	70 (Pro*), 72 (Val*) & 55 (val**)
Cyclo-Phe-Pro	35	245	244	70 (Pro*) & 116 (y1)

Cyclo-Tyr-Pro	36	261	260	70 (Pro[*]), 136 (Tyr[*]), 91 & 107 (Tyr[**])

Table 3. Contents of some normal major dipeptides in the inner content and washed tissue of small intestine and blood plasma after oral administration of vehicle and Vietnamese fish sauce (VFS). The inner content of small intestine was eluted with 10 mL PBS. Data are shown as mean ± standard deviation (n = 3). Asterisks indicate significant differences between values of vehicle and VFS using t-test (†p < 0.10, *p < 0.05, *p < 0.01).

Normal Peptides	Inner content of small intestine (μM)		Small intestinal tissue (nmol/Kg)				Blood plasma (nM)			
			Anterior		Posterior		Portal blood		Abdominal blood	
	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)
Normal major peptides										
Lα-Ala-Glu	1.9 ± 0.4	2.1 ± 0.1	764.7 ± 349.5	2866.7 ±114.1*	571.3 ± 48.3	2304.7 ± 136.5*	21.0 ± 4.0	18.3 ± 5.7	13.7 ± 3.5	22.0 ± 10.4
Lα-Gly-Phe	1.6 ± 0.7	1.4 ± 0.4	238.0 ± 66.1	748.7 ± 426.7†	247.3 ± 89.2	776.0 ± 278.6*	61.7 ± 4.5	61.7 ± 25.8	85.3 ± 13.8	81.7 ± 10.9
Lα-Gly-Ile	2.5 ± 1.3	2.6 ± 0.4	101.3 ± 7.57	294.0 ± 58.6*	110.7 ± 15.0	415.3 ± 52.5*	6.0 ± 2.0	4.3 ± 1.2	6.0 ± 1.7	5.0 ± 1.0
Lα-Gly-Leu	7.3 ± 2.6	7.1 ± 2.2	631.3 ± 136.5	2149.3 ± 493.7*	514.7 ± 209.1	1895.3 ± 390.8*	25.7 ± 17.2	29.7 ± 5.1	28.7 ± 22.5	28.0 ± 21.6
Lα-Gly-Pro	0.2 ± 0.1	0.2 ± 0.5	118.7 ± 42.7	184.0 ± 72.9	144.7 ± 14.5	422.0 ± 104.3*	45.0 ± 7.0	47.7 ± 9.3	56.3 ± 49.3	54.0 ± 43.6
Lα-Ala-Pro	0.02 ± 0.0	0.02 ± 0.0	42.7 ± 15.0	42.7 ± 11.0	18.7 ± 9.0	46.0 ± 8.0*	5.3 ± 1.5	4.3 ± 3.8	0.0 ± 0.0	0.0 ± 0.0
Lα-Val-Pro	0.1 ± 0.0	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Table 4. Contents of glutamyl dipeptides and aspartyl dipeptides in the inner content and washed tissue of small intestine and blood plasma after oral administration of vehicle and Vietnamese fish sauce (VFS). The inner content of small intestine was eluted with 10 mL PBS. Data are shown as mean ± standard deviation (n = 3). Asterisks indicate significant differences between values of vehicle and VFS using t-test (†p < 0.10, *p < 0.05, *p < 0.01).

Peptide Isomers	Inner content of small intestine (μM)		Small intestinal tissue (nmol/Kg)				Blood plasma (nM)			
			Anterior		Posterior		Portal blood		Abdominal blood	
	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)
Glu-dipeptide isomers										
α-Glu-Met	0.04 ± 0.01	0.1 ± 0.1†	1063.1 ± 502.0	1920.9 ± 224.0*	1135.1 ± 478.0	2454.7 ± 702.0*	17.7 ± 1.6	16.6 ± 2.7	14.9 ± 1.7	11.9 ± 1.8
α-Glu-Val	0.2 ± 0.1	0.2 ± 0.04	165.7 ± 78.3	352.7 ± 91.6*	172.4 ± 58.0	553.7 ± 232.0*	6.8 ± 1.3	5.8 ± 0.4	4.7 ± 0.3	3.6 ± 0.7
α-Glu-Ile	0.3 ± 0.2	0.5 ± 0.04	192.1 ± 194.9	630.3 ± 70.3*	167.5 ± 117.6	980.1 ± 510.0*	8.9 ± 2.5	4.9 ± 1.4	4.9 ± 1.5	3.5 ± 0.8
α-Glu-Leu	1.2 ± 0.5	2.1 ± 0.3*	653.7 ± 602.0	1971.9 ± 572.0*	636.9 ± 474.0	3079.5 ± 1486.0*	8.9 ± 0.6	7.9 ± 1.5	4.9 ± 1.5	4.0 ± 1.1
α-Glu-Phe	0.5 ± 0.2	0.8 ± 0.2†	277.0 ± 278.0	875.1 ± 256.0*	286.9 ± 192.0	1411.5 ± 660.0*	2.2 ± 1.3	1.7 ± 0.1	0.8 ± 1.4	1.1 ± 0.1
γ-Glu-Met	0.1 ± 0.02	0.4 ± 0.04**	3888.8 ± 2422.0	7210.0 ± 1242.0†	878.8 ± 166.7	1402.4 ± 145.2**	256.9 ±194.0	203.0 ± 90.2	196.4 ± 47.9	159.0 ± 40.1
γ-Glu-Val	0.1 ± 0.1	5.6 ± 3.5*	1424.5 ± 88.2	2354.0 ± 594.0*	623.7 ± 167.1	1885.1 ± 778.8*	167.0 ± 6.1	228.3 ± 27.5*	167.0 ± 6.1	228.3 ±27.5*
γ-Glu-Ile	0.3 ± 0.3	2.4 ± 1.8†	5638.9 ± 330.0	5933.1 ± 1556.0	339.7 ± 32.7	807.0 ± 292.1*	86.9 ± 22.6	92.7 ± 13.0	73.2 ± 0.7	89.6 ± 10.0*
γ-Glu-Leu	0.2 ± 0.03	1.7 ± 0.9*	5124.7 ± 302.0	5890.1 ± 1590.0	3555.7 ± 901.9	5879.1 ± 1198.1*	306.7 ± 95.0	230.7 ± 54.9	212.8 ± 35.4	277.0 ± 53.4
γ-Glu-Phe	0.04 ± 0.02	2.7 ± 1.6*	6763.6 ± 802.0	7513.9 ± 1760.0	2320.0 ± 441.5	4100.0 ± 2331.3*	266.3 ± 70.5	251.7 ± 35.2	205.7 ± 29.9	270.6 ±27.7*
Asp-dipeptide isomers										

Table 5. Contents of diketopiperazines and pyroglutamyl dipeptides in the inner content and washed tissue of small intestine and blood plasma after oral administration of vehicle and Vietnamese fish sauce (VFS). The inner content of small intestine was eluted with 10 mL PBS. Data are shown as mean ± standard deviation (n = 3). Asterisks

Lα-Asp-Val	0.1 ± 0.01	0.2 ± 0.03*	83.5 ± 41.1	448.7 ± 391.6	52.5 ± 14.2	204.9 ± 98.5†	0.3 ± 0.0	0.2 ± 0.2	0.5 ± 0.4	0.3 ± 0.4
Dα-Asp-Val	0.02 ± 0.01	0.1 ± 0.01*	50.2 ± 8.3	275.5 ± 66.4*	34.2 ± 6.4	116.9 ± 44.0*	3.2 ± 2.3	2.5 ± 1.6	1.5 ± 0.1	3.9 ± 0.4**
Lβ-Asp-Val	0.1 ± 0.01	0.4 ± 0.14*	11.7 ± 4.2	171.5 ± 43.3**	7.3 ± 0.04	61.6 ± 2.6**	3.8 ± 1.1	10.5 ± 3.1**	2.0 ± 0.2	6.1 ± 1.7*
Dβ-Asp-Val	0.01 ± 0.0	0.03 ± 0.01*	2.4 ± 0.5	15.9 ± 3.9**	2.2 ± 0.4	7.2 ± 2.6*	0.6 ± 0.4	3.7 ± 0.7*	0.4 ± 0.4	3.7 ± 1.9*
Lα-Asp-Ile	0.04 ± 0.01	0.1 ± 0.01*	58.1 ± 18.5	118.2 ± 48.2†	32.34 ± 3.7	47.1 ± 11.3†	0.5 ± 0.3	0.3 ± 0.6	0.1 ± 0.2	0.3 ± 0.1
Dα-Asp-Ile	0.01 ± 0.0	0.01 ± 0.0*	50.6 ± 2.5	89.7 ± 62.2	13.4 ± 4.4	40.6 ± 20.4†	0.9 ± 0.2	1.0 ± 0.4	1.3 ± 0.0	2.2 ± 0.5*
Lβ-Asp-Ile	0.01 ± 0.0	0.1 ± 0.03**	21.1 ± 2.1	81.4 ± 24.8**	17.7 ± 6.3	64.9 ± 15.9**	7.0 ± 0.9	1 0.2 ± 1.5*	6.6 ± 0.2	9.9 ± 1.3*
Dβ-Asp-Ile	0.01 ± 0.0	0.01 ± 0.01*	3.9 ± 0.6	20.8 ± 5.4**	2.4 ± 0.5	6.2 ± 0.9**	0.5 ± 0.0	2.1 ± 0.6**	0.3 ± 0.0	2.9 ± 0.6**
Lα-Asp-Leu	0.0 ± 0.0	0.01 ± 0.0*	8.2 ± 1.5	37.6 ± 9.7*	4.8 ± 1.5	12.8 ± 1.2*	0.0 ± 0.0	0.04 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Dα-Asp-Leu	0.01 ± 0.0	0.01 ± 0.0*	8.7 ± 3.7	55.2 ± 71.9	4.7 ± 0.8	6.9 ± 2.9	4.7 ± 1.8	6.3 ± 1.5	6.9 ± 0.2	8.6 ± 0.7*
Lβ-Asp-Leu	0.02 ± 0.01	0.3 ± 0.2*	107.2 ± 4.0	210.9 ± 66.1*	51.5 ± 18.9	128.2 ± 24.7**	4.9 ± 0.8	14.9 ± 1.9*	4.0 ± 0.5	10.1 ± 2.5*
Dβ-Asp-Leu	0.01 ± 0.0	0.1 ± 0.04*	25.7 ± 0.8	69.2 ± 27.5*	14.3 ± 3.1	52.3 ± 19.4*	7.1 ± 1.4	10.9 ± 1.6*	8.0 ± 1.6	14.5 ± 3.9*
Lα-Asp-Phe	0.06 ± 0.01	0.1 ± 0.2*	116.7 ± 55.7	238.5 ± 33.2*	101.5 ± 42.8	70.8 ± 13.4	0.7 ± 0.4	0.3 ± 0.2	0.2 ± 0.3	0.3 ± 0.2
Dα-Asp-Phe	0.01 ± 0.0	0.01 ± 0.0**	9.5 ± 2.9	10.4 ± 1.8	3.8 ± 0.8	6.9 ± 2.2†	0.3 ± 0.02	0.4 ± 0.0*	0.0 ±0.0	0.5 ± 0.1*
Lβ-Asp-Phe	0.02 ± 0.01	0.2 ± 0.1*	16.6 ± 3.5	92.4 ± 29.6**	26.5 ± 0.8	69.3 ± 27.5*	3.4 ± 0.6	5.1 ± 0.8*	2.1 ± 0.7	5.9 ± 2.3*
Dβ-Asp-Phe	0.01 ± 0.0	0.1 ± 0.03*	8.9 ± 2.3	61.2 ± 11.9**	11.9 ± 0.0	21.8 ± 0.4**	4.6 ± 1.0	9.3 ± 1.5**	4.6 ± 0.8	11.2 ± 4.1*

indicate significant differences between values of vehicle and VFS using t-test (†p < 0.10, *p < 0.05, *p < 0.01).

Modified peptides	Inner content of small intestine (μM)		Small intestinal tissue (nmol/Kg)				Blood plasma (nM)			
			Anterior		Posterior		Portal blood		Abdominal blood	
	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)	Vehicle	Treatment (60 mins)
Diketopiperazines										
Cyclo-Tyr-Pro	0.01 ± 0.0	0.02 ± 0.0*	29.7 ± 11.9	128.7 ± 10.1*	7.4 ± 1.7	20.7 ± 4.9*	459.3 ± 163.9	414.7 ± 166.5	368.7 ± 83.5	398.7 ± 166.7
Cyclo-Glu-Pro	0.04 ± 0.0	2.6 ± 1.3*	55.9 ± 34.6	622.0 ± 315.7*	136.5 ± 6.9	954.7 ± 348.1*	175.7 ± 48.0	901.3 ± 221.8**	108.0 ± 6.6	424.0 ± 107.6**
Cyclo-Asn-Pro	0.04 ± 0.0	5.6 ± 3.6*	114.1 ± 24.2	972.8 ± 461.6*	138.5 ± 20.2	3015.9 ± 1652.4*	385.3 ± 53.7	1359.7 ± 170.7**	252.0 ± 47.6	888.0 ± 217.2**
Cyclo-Thr-Pro	0.02 ± 0.0	0.4 ± 0.2*	77.9 ± 19.0	654.1 ± 183.4*	99.4 ± 17.0	547.2 ± 124.1*	419.7 ± 94.4	5175.3 ± 723.6**	316.0 ± 64.4	3666.7 ± 461.9**
Cyclo-Pro-Pro	0.01 ± 0.0	0.02 ± 0.0**	20.0 ± 1.4	53.5 ± 5.1*	25.5 ± 2.9	54.7 ± 6.2*	175.0 ± 11.5	619.3 ± 155.9**	125.0 ± 22.5	442.3 ± 57.6**
Cyclo-Ser-Pro	0.01 ± 0.01	1.5 ± 0.9*	378.3 ± 19.1	1242.3 ± 340.9*	248.3 ± 41.5	1571.3 ± 555.6**	335.7 ± 53.5	4514.7 ± 757.7**	238.0 ± 54.3	3233.3 ± 305.5**
Cyclo-Ile-Pro	0.01 ± 0.0	0.04 ± 0.01*	22.7 ± 6.9	143.9 ± 4.6**	44.7 ± 8.8	172.7 ± 36.7**	97.0 ± 13.7	860.3 ± 306.0**	45.3 ± 10.0	553.6 ± 115.5**
Cyclo-Leu-Pro	0.01 ± 0.0	0.03 ± 0.0**	74.8 ± 7.7	316.9 ± 94.3*	101.1 ± 9.6	216.5 ± 31.3**	74.0 ± 10.6	785.3 ± 192.8**	46.0 ± 3.0	598.0 ± 72.1**
Cyclo-Val-Pro	0.01 ± 0.0	0.1 ± 0.0**	63.3 ± 10.3	343.3 ± 6.5**	48.7 ± 5.7	229.8 ± 18.7**	167.3 ± 12.7	2366.7 ± 453.1**	150.3 ± 45.9	1900.0 ± 200.0**
Cyclo-Phe-Pro	0.002 ± 0.0	0.003 ± 0.0	33.8 ± 8.6	192.9 ± 62.6*	21.5 ± 7.6	67.5 ± 19.4*	47.7 ± 5.5	141.3 ± 45.2*	28.3 ± 11.7	74.0 ± 24.8*
Cyclo-Asp-Pro	0.4 ± 0.03	9.3 ± 5.9*	160.8 ± 10.7	918.7 ± 464.3*	205.6 ± 33.5	3202.7 ± 1791.0*	224.3 ± 73.4	869.3± 406.6*	214.7 ± 68.4	590.3 ± 149.9**
Cyclo-Ala-Pro	0.01 ± 0.0	0.1 ± 0.0*	104.7 ± 8.4	286.5 ± 3.2**	58.5 ± 13.4	215.7 ± 18.5**	271.3 ± 10.1	2064.0 ± 325.7**	222.7 ± 46.1	1866.7 ± 208.2**
Pyroglutamyl peptides										

pGlu-Phe-Gln	0.0 ± 0.0	0.02 ± 0.0*	0.5 ± 0.3	1.3 ± 0.3	2.4 ± 0.4	3.3 ± 1.8	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Gln-Pro	0.2 ± 0.2	0.1 ± 0.3	40.8 ± 38.1	27.2 ± 6.7	156.5 ± 29.8	198.2 ± 63.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Val-Gln	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.8	0.8 ± 0.2	19.3 ± 1.5	18.4 ± 12.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Gln-Leu-Leu	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	0.7 ± 0.2	3.7 ± 5.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Leu-Ser-Glu	0.0 ± 0.0	0.0 ± 0.0	00.0 ± 00.0	00.0 ± 00.0	00.0 ± 00.0	00.0 ± 00.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Leu-Ser	0.0 ± 0.0	0.0 ± 0.0	1.04 ± 0.6	0.9 ± 0.3	21.5 ± 6.2	26.3 ± 5.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Leu-Leu	0.0 ± 0.0	0.00 ± 0.00	0.3 ± 0.2	0.4 ± 0.1	8.1 ± 5.4	12.5 ± 2.9	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Pro	0.1 ± 0.4	1.5 ± 1.2†	15.8 ± 7.5	306.6 ± 237.2†	240.0 ± 35.6	880.0 ± 66.8*	28.6 ± 1.8	53.6 ± 6.2*	22.6 ±1.6	51.5 ± 18.2*
pGlu-Gln	0.1 ± 0.2	0.7 ± 0.2**	25.8 ± 3.8	130.3 ± 41.4**	260.0 ± 50.5	540.0 ± 117.4*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Ala	0.0 ± 0.0	0.9 ± 0.6*	0.01 ± 0.0	190.6 ± 121.2*	280.0 ± 240.0	760.0 ± 200.0†	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
pGlu-Leu	0.1 ± 0.0	0.4 ± 0.2*	12.4 ± 0.8	78.7 ± 42.4*	344.0 ± 76.9	822.0 ± 294.0*	18.0 ± 2.3	17.3 ± 4.1	16.8 ±0.7	17.9 ± 0.1*
pGlu-Val	0.02 ± 0.01	1.1 ± 0.8†	3.8 ± 2.01	225.4 ± 161.7†	173.6 ± 102.6	1448.0 ± 658.0*	4.2 ± 7.2	14.6 ± 24.9	4.0 ± 6.9	14.5 ± 8.6
pGlu-Tyr	0.5 ± 0.1	0.9 ± 0.2†	108.9 ± 22.4	175.9 ± 43.9†	318.0 ± 99.8	384.0 ± 98.9	13.4 ± 7.5	10.0 ± 6.4	3.6 ± 0.6	3.5 ± 2.5
pGlu-Ile	0.03 ± 0.02	0.4 ± 0.3†	4.9 ± 0.4	76.1 ± 57.8†	87.2 ± 29.3	358.0 ± 162.2*	8.0 ± 1.9	9.3 ± 6.1	7.1 ± 2.4	9.20 ± 1.1
pGlu-Phe	0.02 ± 0.01	0.2 ± 0.1*	4.4 ± 1.9	38.2 ± 21.8*	91.0 ± 27.8	310.0 ± 187.8†	3.5 ± 0.5	6.0 ± 7.5	1.5 ± 0.4	3.7 ± 4.2

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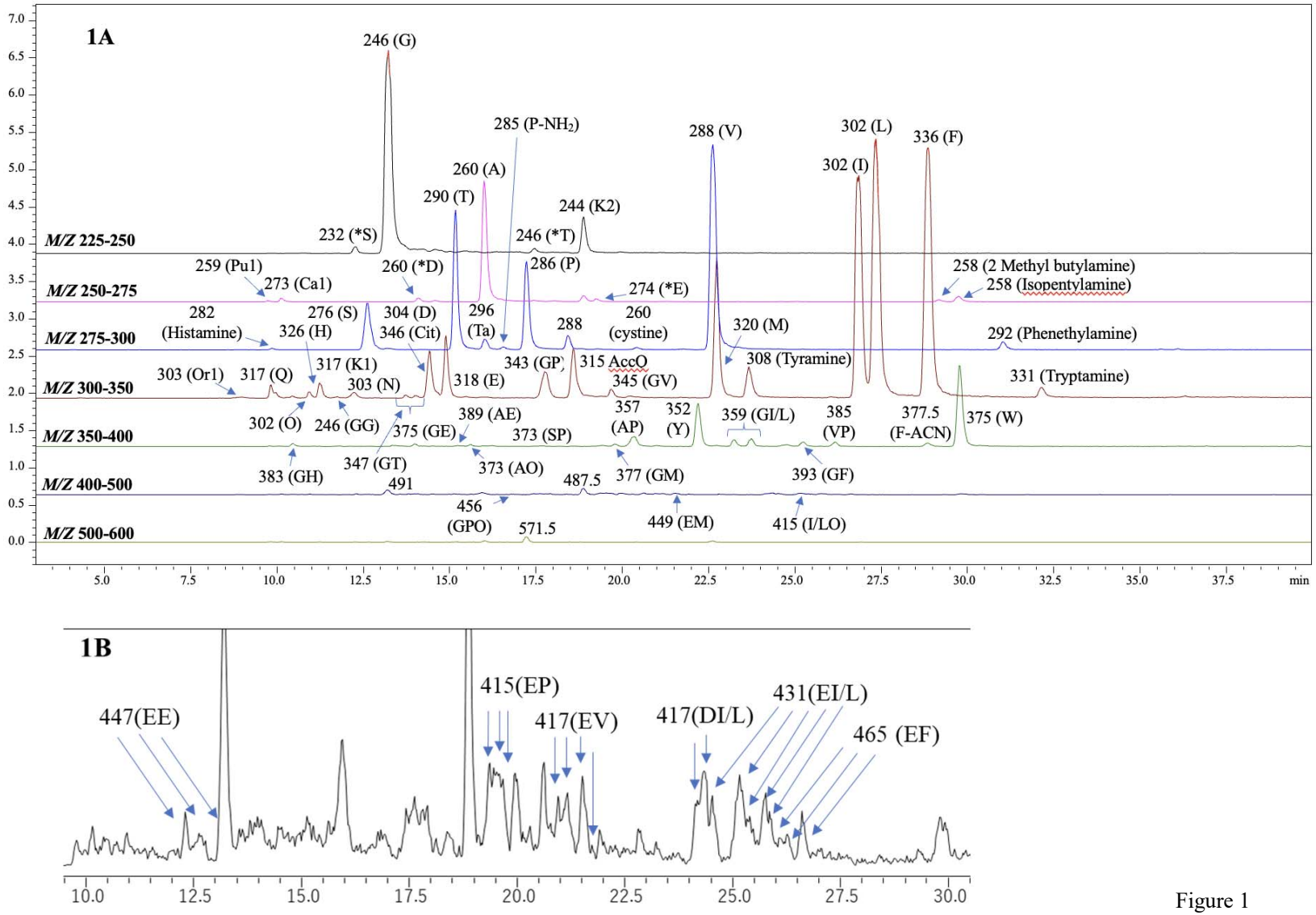

Figure 1

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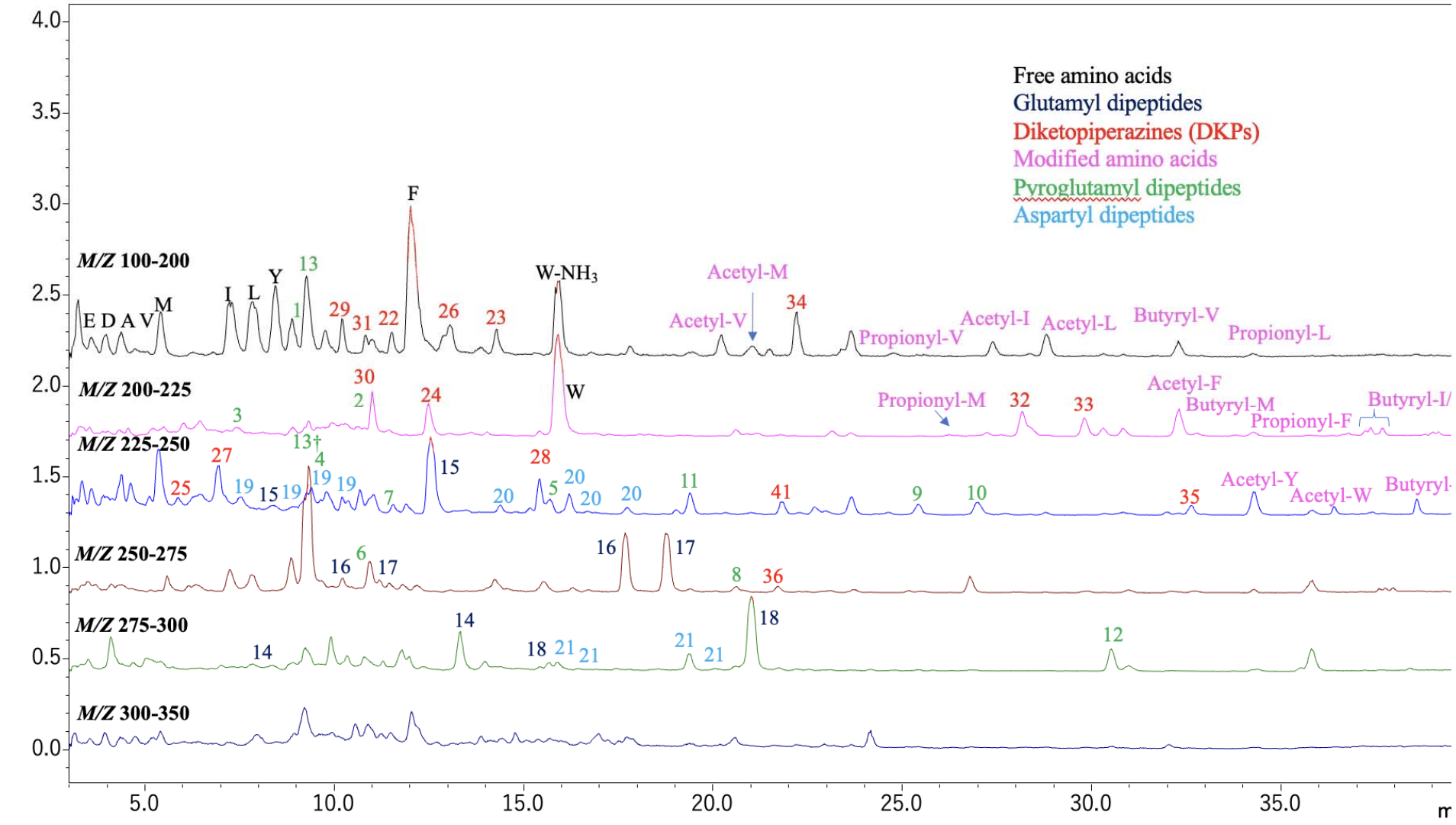

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Figure 2

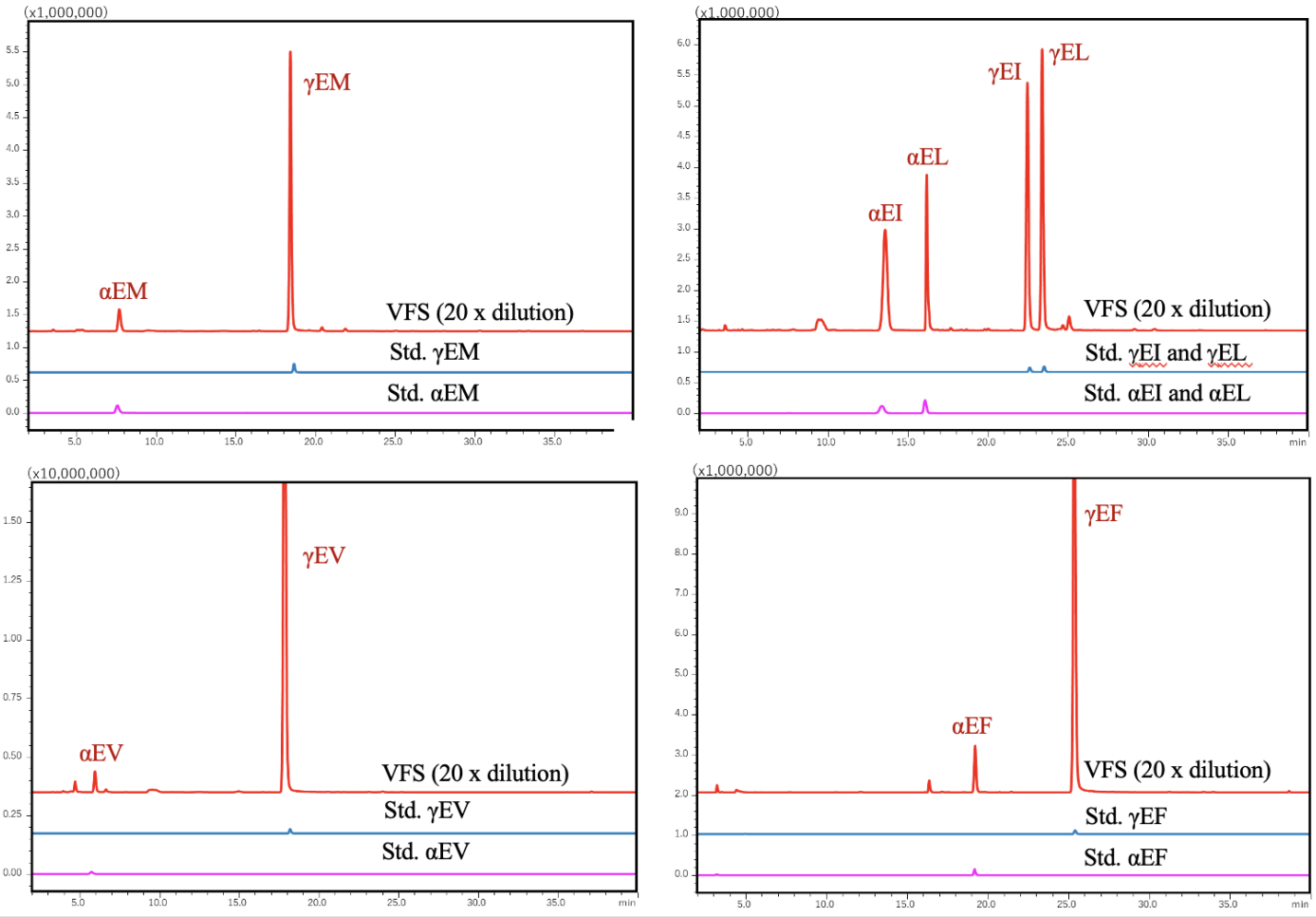

Figure 3

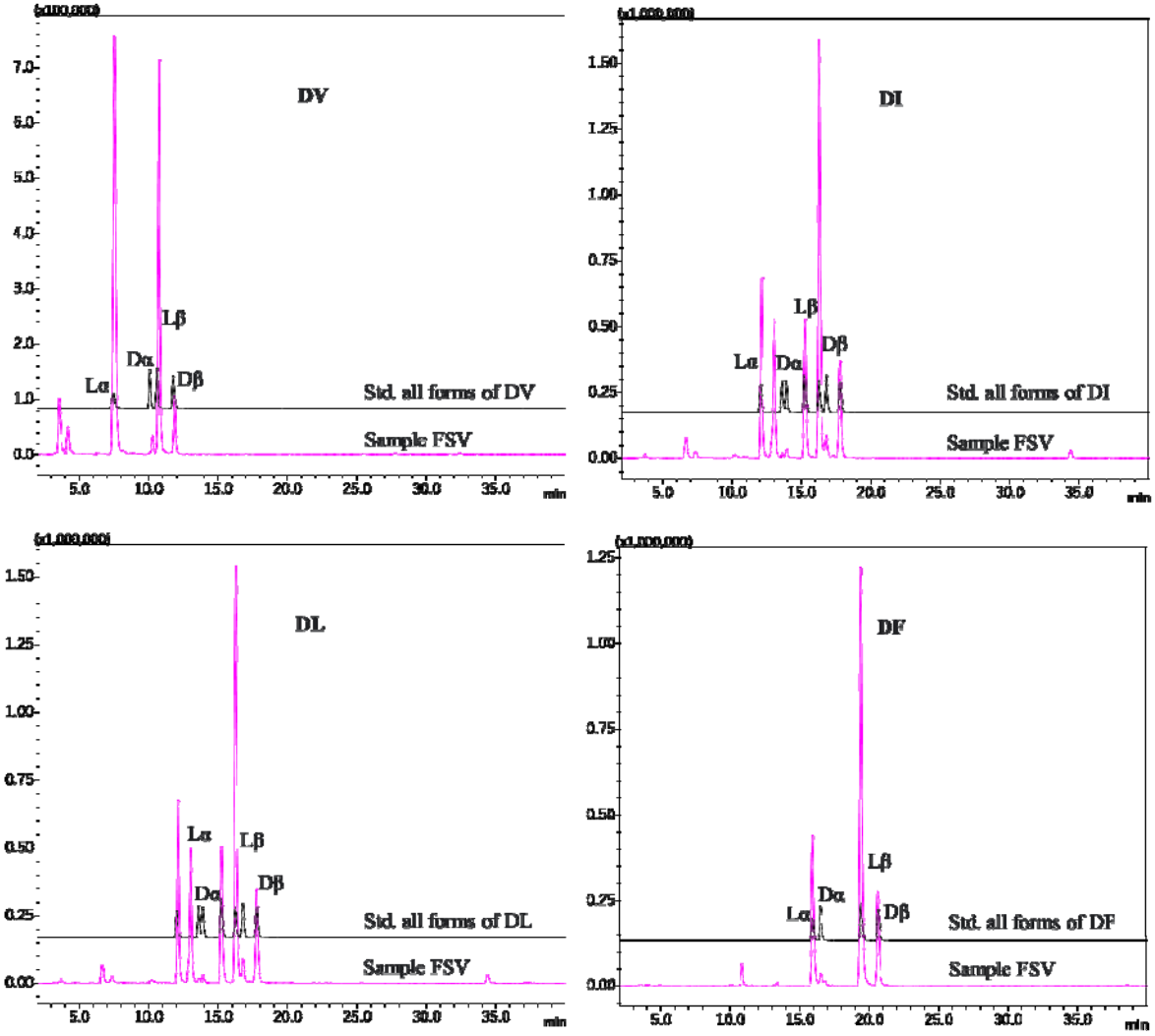
DV
Lα
Dα
Lβ
Dβ
Std. all forms of DV
Sample FSV
DI
Lα
Dα
Lβ
Dβ
Std. all forms of DI
Sample FSV
DL
Lα
Dα
Lβ
Dβ
Std. all forms of DL
Sample FSV
DF
Lα
Dα
Lβ
Dβ
Std. all forms of DF
Sample FSV
min

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Figure 4

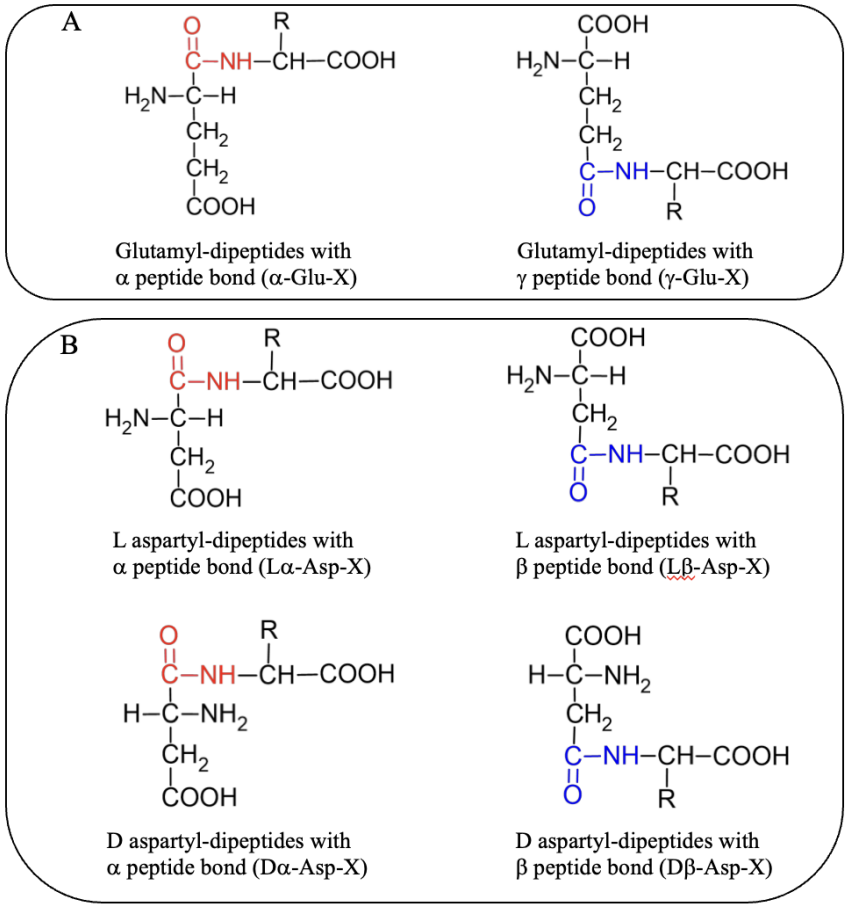

Figure 5

F


Fish sauces
Soy sauces
Fermented meat and milk
VFS
TFS
JFS
JSS-K1
JSS-K2
JSS-T
Salami
Cheese
Concentration (μM)
1600
800
0
α-Glu-Met
γ-Glu-Met
α-Glu-Val
γ-Glu-Val
α-Glu-Ile
γ-Glu-Ile
α-Glu-Leu
γ-Glu-Leu
α-Glu-Phe
γ-Glu-Phe
Lα-Asp-Val
Lβ-Asp-Val
Dα-Asp-Val
Dβ-Asp-Val
Lα-Asp-Ile
Lβ-Asp-Ile
Dα-Asp-Ile
Dβ-Asp-Ile
Lα-Asp-Leu
Lβ-Asp-Leu
Dα-Asp-Leu
Dβ-Asp-Leu
Lα-Asp-Phe
Lβ-Asp-Phe
Dα-Asp-Phe
Dβ-Asp-Phe
Lα-Ala-Glu
Lα-Gly-Phe
Lα-Gly-Ile
Lα-Gly-Leu
Lα-Gly-Pro
Lα-Ala-Pro
Lα-Val-Pro

Figure 6


Fish sauces
Soy sauces
Fermented meat and milk
VFS
TFS
JFS
JSS-K1
JSS-K2
JSS-T
Salami
Cheese
Concentration (μM)
11200
5600
0
pGlu-Phe-Gln
pGlu-Gln-Pro
pGlu-Val-Gln
pGlu-Gln-Leu-Leu
pGlu-Leu-Ser-Glu
pGlu-Leu-Ser
pGlu-Leu-Leu
pGlu-Pro
pGlu-Glu
pGlu-Gln
pGlu-Ala
pGlu-Leu
pGlu-Val
pGlu-Tyr
pGlu-Ile
pGlu-Phe
Cyclo-Tyr-Pro
Cyclo-Glu-Pro
Cyclo-Asn-Pro
Cyclo-Thr-Pro
Cyclo-Pro-Pro
Cyclo-Ser-Pro
Cyclo-Ile-Pro
Cyclo-Leu-Pro
Cyclo-Val-Pro
Cyclo-Phe-Pro
Cyclo-Asp-Pro
Cyclo-Ala-Pro