

The Contribution of Peptides and Amino Acids to the Taste of Foodstuffs

Jiro Kirimura, Akira Shimizu, Akimitsu Kimizuka, Tsunehiko Ninomiya, and Noboru Katsuya

The tastes of individual amino acids were characterized as being sweet, salty, sour, bitter, or MSG-like. The contribution of amino acids to the taste of various foodstuffs is discussed. The tastes of various peptides were characterized as being sour, bitter, or

practically tasteless. Peptides from sake, soy sauce, and partial hydrolyzates of soybean protein were isolated and their tastes, as well as other properties, described.

The importance of amino acids in contributing to the taste of foodstuffs was first recognized by Ikeda at Tokyo University in 1908 (Ikeda, 1908). He discovered that monosodium L-glutamate (MSG) was the essential component of the taste-imparting ingredients of traditional Japanese food seasoners, such as sea tangle (a type of seaweed). At that time the taste of traditional Japanese foods such as sake, miso, and soy sauce were presumed to be due to the amino acids which were released from the natural proteins during the course of fermentation. In addition, the taste of cheese had been known, in Europe and the United States, to be characterized by amino acids formed during the ripening process.

Since then, many studies on the taste of amino acids in foods and on their production by extraction, fermentation, or by chemical syntheses have resulted in mass production of various amino acids, and in a consequent reduction of their price. Today, amino acids are used in food processing not only to enhance the nutritive value of many processed foods such as cereals, but also to enhance or improve otherwise the taste of natural characteristics of many foodstuffs.

The taste of peptides and their relative contribution to the taste of foods have been in the past the topic of extensive investigations; studies have been undertaken on the distribution, isolation, and elucidation of the chemical structures of peptides and some of their properties in foods. This paper deals with the role that some specific amino acids and peptides play in determining the taste of certain foodstuffs.

TASTE OF INDIVIDUAL AMINO ACIDS

Free amino acids in natural food extractives are important taste contributors. The term "extractives" as used in this paper is defined as the mixtures that can be extracted from foodstuffs with warm or hot water. Food extractives are believed to consist mainly of such substances as free amino acids, peptides, nucleotides, and organic acids and their esters, bases, sugars, and salts. Although it is the combination of several factors, among them the complex of all the organoleptic components present, that determine the taste of a food, extractives are the principal contributor. It is well known that MSG and 5'-nucleotides are mutually synergistic and play an important role in taste.

The taste characteristics of individual amino acids have been studied by many investigators including the author's group (Yoshida *et al.*, 1966) and that of Solms (Solms

et al., 1965), in which quantitative evaluations were reported. In the author's laboratories, a panel consisting of 100 people was chosen by screening approximately 1000 employees of the Ajinomoto Co., Inc., by means of a sensitive test. The age of the panelists, which included both men and women, ranged from 19 to 40 years. The taste intensities of individual amino acids were estimated by comparing them to that of solutions of sodium chloride at concentrations of from 0.2000 to 5.123 grams per 100 ml.

The nature of the tastes were characterized as being sweet, salty, sour, bitter, MSG-like, or otherwise; and their intensities measured using a 0 to 10 scale. The results of the sensory tests showed good agreement among the panelists.

Figure 1 illustrates the nature and the intensity of the taste of arginine, serine, glutamic acid, and alanine. Arginine has a bitter taste accompanied by slight sweetness, whereas serine has a sweet taste accompanied by sourness and a MSG-like taste. Glutamic acid is a combination of the sour taste with a taste like MSG. In alanine the sweet taste is combined with a slight taste like MSG. In general, the tastes of the individual amino acids are complex and need to be described by more than one taste characteristic. A classification of amino acids according to their organoleptic characteristics and their threshold values is summarized in Table I.

Of the amino acids tested, the taste intensities of alanine, arginine, glutamic acid, serine, and threonine increased as

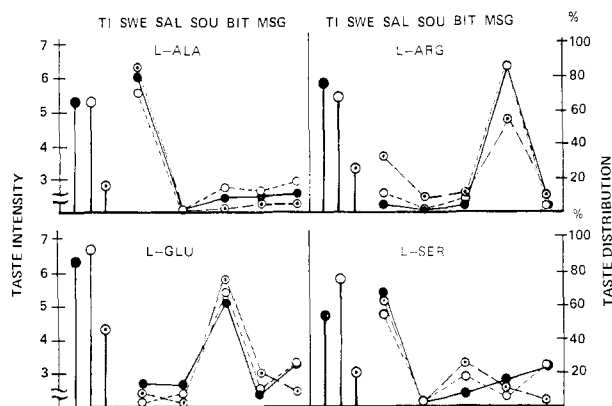


Figure 1. Taste characteristics of some amino acids

—●— Solid; —○— High concentration (in water); —□— Low concentration (in water)
TI: Relative taste intensity compared with 0.2 to 5.123% sodium chloride solution
SWE: Sweetness; SOU: Sourness; BIT: Bitterness; MSG: Taste like monosodium L-glutamate
Values of high and low concentration (g./dl.): Ala 0.5, 5.0; Arg 0.2, 1.0; Glu 0.025, 0.2; Ser 1.5, 15.0

Central Research Laboratories, Ajinomoto Co., Inc., Kawasaki, Japan

Table I. Threshold Values and Organoleptic Characteristics of Amino Acids

Amino Acids	Sweet Amino Acids					MSG ^b
	T.V., ^a Mg/Dl.	SWE ^b	SAL ^b	SOU ^b	BIT ^b	
Hyp	50	***			**	
Lys·HCl	50	**			**	
Ala	60	***				
Gly	130	***				
Ser	150	***		*		*
Gln	250	*				*
Thr	260	***		*	*	
Pro	300	***			***	
Sour and MSG-like Amino Acids						
Asp	3			***		*
Glu	5			***		**
His·HCl	5			***		
Asn	100			**	*	
Glu·Na	30	*	*			***
Asp·Na	100		**			**
Bitter Amino Acids						
His	20				**	
Arg·HCl	30				***	
Met	30				***	*
Val	40				***	
Arg	50				***	
Ile	90				***	
Phe	90				***	
Trp	90				***	
Leu	190				***	

^a T.V.: threshold value.

^b Asterisks denote relative taste intensities.

Table II. Free Amino Acid Content of Extractives (Ohara, 1966) (Mg% on Wet Basis)

Amino Acids	Mackerel	Tri-choloma			Green Tea ^b
		Uni ^a	Shimeji	Purple Laver	
Asp	9.8	4	53	230	136
Thr	9.6	68	21	78	61
Ser	6.9	130	51	53	81
Glu	20	103	68	640	668
Pro	5.4	26	13	62	18
Gly	54	842	12	125	47
Ala	37	261	33	1092	25
Val	14	154	9.1	21	6.1
Met	7.3	47	1.7	0	0.6
Ile	9.6	100	6.5	14	47
Leu	14	176	11	20	34
Tyr	6.6	158	8.6	0	4
Phe	9.2	79	19	15	10
Trp	2.2	39	2.6	0	12
His	563	54	25	0	6.7
Lys	22	215	119	24	7.4
Arg	6.1	316	41	90	142

^a Unripe gonad of sea urchin, taurine 105 mg%.

^b On moisture-free basis, theanine 1727 mg%.

their concentrations were increased. Taste analyses were performed also using solid amino acids, but little difference was observed between aqueous and solid samples except in the cases of aspartate, serine, and threonine. The taste intensification resulting from the addition of MSG to foods is a consequence of the flavor contributory effect of MSG by itself and also of the synergism between 5'-nucleotides existing in foods and added MSG. This synergism is also observed between 5'-inosinate and either C₇ monosodium monoamino dicarboxylates, homocysteic acid, tricholomic acid, ibotenic acid, or DL-threo-β-oxyglutamate.

Table III. Taste Threshold Values of Some Dipeptides and Amino Acids

Peptides	T.V., %	Amino Acids	T.V., %
γ-L-Glu-L-Glu	0.0025	L-Glu	0.005
L-Leu-L-Leu	0.10	L-Leu	0.19
Gly-Gly	^a	Gly	0.11

^a Little or no taste at 1%.

TASTE OF AMINO ACID MIXTURES

Recently, in connection with studies on the inherent taste of free amino acids and their distribution in foods, the taste of various combinations of amino acids has been studied. Not only does the distribution of free amino acids differ from food to food, but quantitative changes within each food may be seasonal. Table II shows qualitative and quantitative data on the free amino acid content of five different foods (Ohara, 1966). In mackerel, histidine accounts for about 70% of the total free amino acids. In uni, a very tasty Japanese product made from the unripe gonad of the sea urchin, glycine predominates, followed by arginine, lysine, alanine, serine, glutamic acid, and taurine. Similarly, the predominant amino acid in *Tricholoma shimeji* (edible fungi) is lysine; in purple laver (a type of seaweed) alanine and glutamic acid predominate; theanine and glutamic acid are the major amino acids of green tea. Some of these amino acids *per se* are essential for the taste of some foods. Thus, methionine is the most specific component of the taste of uni, and glycine is very important in contributing to the distinct taste of lobster and crab.

In beef extract, however, no specific amino acid characterizes its taste but, rather, all the free amino acids combine to give the complex taste sensation.

In our laboratories a hydrolyzed protein concentrate, Ajimate F-40, was developed as a replacement for beef extracts, on the basis of studies on the contribution of free amino acids to the taste of foodstuffs.

TASTE OF INDIVIDUAL PEPTIDES

The authors have previously observed that the taste intensities of peptides are generally weak, compared to those of the corresponding free amino acids (Kimizuka *et al.*, 1963). However, as shown in Table III, the threshold values of L-Leu-L-Leu and γ-L-Glu-L-Glu were approximately half those of L-Leu and L-Glu, respectively.

Sixty different peptides were tested in 0.2% aqueous solutions by 12 trained panel members to evaluate their taste characteristics, and the following are some generalizations resulting from this work.

The taste characteristics of peptides are complex, but can be classified into three groups: Compounds in Group 1 have a sour taste; those in Group 2 have a bitter taste; and those in Group 3 have little or no taste. As shown in Table IV, peptides such as L-Ala-L-Asp, γ-L-Glu-L-Glu, and Gly-L-Asp-L-Ser-Gly are sour, peptides such as L-Leu-L-Leu, L-Arg-L-Pro, and L-Val-L-Val are bitter, and peptides such as L-Lys-Glu, L-Phe-L-Phe, and Gly-Gly-Gly-Gly have little or no taste.

It appears that a relationship exists between the patterns of component amino acids and the taste of dipeptides. Dipeptides which tasted sour contained two acidic amino acids, acidic- and neutral-amino acids, or acidic- and aromatic-amino acids.

Table IV. Taste of Peptides in 0.2% Aqueous Solution

- Group 1. Sourness**
 Gly-L-Asp, Gly-L-Glu
 L-Ala-L-Asp, L-Ala-L-Glu
 L-Ser-L-Asp, L-Ser-L-Glu
 L-Val-L-Asp, L-Val-L-Glu
 L-Asp-L-Ala, L-Asp-L-Asp
 L-Glu-L-Ala, L-Glu-L-Asp, L-Glu-L-Glu
 L-Glu-L-Phe,^a L-Glu-L-Tyr^a
 γ-L-Glu-Gly,^b γ-L-Glu-L-Ala,^b γ-L-Glu-L-Asp^b
 γ-L-Glu-L-Glu^b
 L-Phe-L-Asp, L-Phe-L-Glu
 L-Trp-L-Asp, L-Trp-L-Glu
 Gly-L-Asp-L-Ser-Gly
 L-Pro-Gly-Gly-L-Glu
 L-Val-L-Val-L-Glu

- Group 2. Bitterness**
 Gly-L-Ile, Gly-L-Met, Gly-L-Phe, Gly-L-Try
 L-Ala-L-Phe
 L-Val-L-Ala, L-Val-L-Val, L-Val-L-Leu
 L-Leu-Gly, L-Leu-L-Leu, L-Leu-L-Tyr
 L-Lys-Gly, L-Lys-L-Ala
 L-His-L-His
 L-Val-L-Val-L-Val
 L-Arg-L-Pro

- Group 3. Having Little or No Taste**
 Gly-Gly, Gly-L-Ala, Gly-L-Ser, Gly-L-Thr
 Gly-L-Asp(NH₂), Gly-L-Pro
 L-Ala-Gly, L-Ala-L-Ala
 L-Val-Gly
 L-Lys-L-Glu
 L-Phe-L-Phe^c
 L-Pro-Gly, L-Pro-L-Ala
 Gly-Gly-Gly-Gly

^a Accompanied by bitterness and astringency.
^b Accompanied by astringency.
^c Taste in solid state.

It is presumed that the sour taste is caused by hydrogen ions arising from the dissociation of dipeptides. The order of the taste intensity is 1) >2) >3).

Dipeptides which tasted bitter contained neutral amino acids with either large alkyl groups (≥ C₃) or a combination of large (≥ C₃) and small (≤ C₂) alkyl groups, neutral and aromatic amino acids, or neutral and basic amino acids.

Dipeptides having little or no taste were composed of

Table V. Effect of Peptides on the Stability of Soybean oil

Peptides (0.02%)	Peroxide Value ^a	
	5 Hrs.	10 Hrs.
None	83.0	241.4
Gly-L-Leu	16.9	136.2
β-Ala-3-Methyl-L-His	25.3	145.4
Gly-Gly	51.7	319.7
γ-L-Glu-L-Glu	59.4	252.2
Gly	58.6	228.6
Butyl hydroxy anisole	22.9	141.5

^a Official and Tentative Method of the American Oil Chemists' Society (1947).

two amino acids with small alkyl groups, acidic- and basic-amino acids, or two aromatic amino acids (Figure 2).

It is interesting that dipeptides composed of sweet amino acids, Gly-Gly, Gly-L-Ala, and Gly-L-Pro, are almost tasteless. The disappearance of sweetness may be a result of chain lengthening. The combination of an acidic and a basic amino acid, such as L-Lys-L-Glu and L-Arg-L-Glu, is also tasteless. This may be due to interactions between the amino groups and the carboxyl groups in the dipeptide molecule.

Dipeptides composed of two aromatic amino acids, such as L-Phe-L-Phe, had essentially bitter tastes, but this was not thoroughly evaluated because of the low solubilities of these peptides in water. The taste characteristics and taste intensities of dipeptides are summarized in Figure 2.

Another interesting aspect of some of these peptides was that they showed rather strong antioxidant activity, Gly-L-Leu being the most effective. Antioxidant activity was determined by means of the active oxygen method, in which the stability of a vegetable oil was determined by measuring peroxide values after 5 and 10 hours. Dipeptides were found to be more effective stabilizers of vegetable oils than of animal fats (Table V).

PEPTIDES IN BREWED BEVERAGES AND FOODSTUFFS

It is important to correlate chemical structure and taste of peptides for better utilization of peptides in foodstuffs. The authors have also studied the relationship between

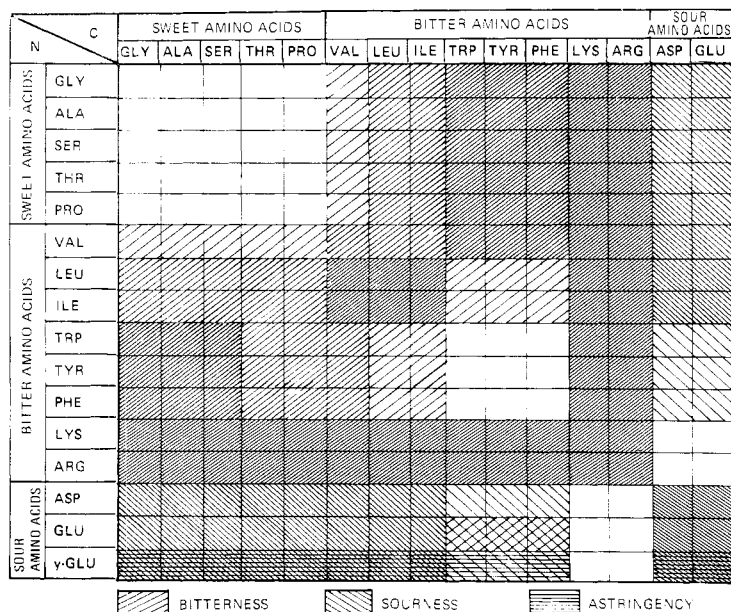


Figure 2. Taste of dipeptides

Upper left N/C
 N = N-terminal amino acid
 C = C-terminal amino acid

Table VI. Effect of Peptides on "Body" of Synthetic Sake (Tajima *et al.*, 1968)

Sample	Sensory Score
Control synthetic sake	5.0
+0.03% L-Val-L-Glu	6.8
+0.03% L-Pro-L-Glu	5.8
+0.03% L-Pro-L-Asp	5.8
+0.03% γ -L-Glu-L-Glu	5.2
+0.03% L-Val-L-Val	5.4
+0.03% Gly-L-Leu	7.0
Natural sake	10.0

amino acid composition and molecular weight, on one hand, and taste, on the other, especially in the case of peptides of higher molecular weight.

The distribution of peptides in natural foodstuffs, such as beef and whale extracts, cheese, seaweeds, etc., and the isolation of peptides therefrom have been studied by many investigators. Since peptides occur in relatively large amounts in some typical Japanese beverages and foodstuffs, such as sake, miso, and soy sauce, the peptides of these foods have been the objects of studies in Japan (Tajima and Sato, 1964; Takahashi and Nose, 1958; Takeuchi and Yoshii, 1967).

Sake. Sake is a traditional Japanese alcoholic beverage which is made from rice by mixed fermentation using fungi, yeast, and bacteria. The unique taste of sake is said to be derived mainly from components of rice, such as hydrolyzates of protein, starch, etc. The content of free amino acids in sake is approximately 0.3%, and they are very important contributors to the unique taste of sake. The peptides in sake amount to from 20 to 30% of the free amino acid content. Molecular sieve chromatography with Dowex-50 columns was used to separate groups of peptides in sake (Takahashi and Nose, 1958), and about 70% of the water-soluble nitrogen in sake consists of amino acids and di- or tripeptides and small amounts of higher molecular weight peptides (Figure 3). The only dipeptides isolated and crystallized were Val-Gln and Ile-Gln (Tajima and Sato, 1964). Artificial sake consumed in Japan is made by blending known ingredients found in natural sake, and thus it is similar to natural sake in taste. It is made by blending alcohol, glucose, succinic acid, lactic acid, inorganic phosphates, sodium chloride, MSG, glycine, and alanine. To this artificial sake, 0.03% of dipeptides was added by Tajima *et al.* (1968), and the effect of dipeptides on the taste was evaluated by a trained panel consisting of six members. All the dipeptides which were added to artificial sake improved the body, compared with the control, and caused the taste of artificial sake to come closer to that of natural sake (Table VI). It appears that peptides are not the principal tasty substances in sake, but rather that they contribute to the complexity and balance of the taste of natural sake. Addition of dipeptides to artificial sake also resulted in an increased buffering capacity of the product. As shown in Figure 4, Gly-L-Leu and L-Pro-L-Glu increased the buffer action at pH > 9, and L-Val-L-Glu showed strong buffer action at pH 7 to 8. In general, peptides have complex dissociation constants, compared to those of amino acids, and this property may result in the fine regulation of pH and may be related to the ability exhibited by peptides to improve the body of artificial sake.

Miso. Miso is a brewed foodstuff made from soybean

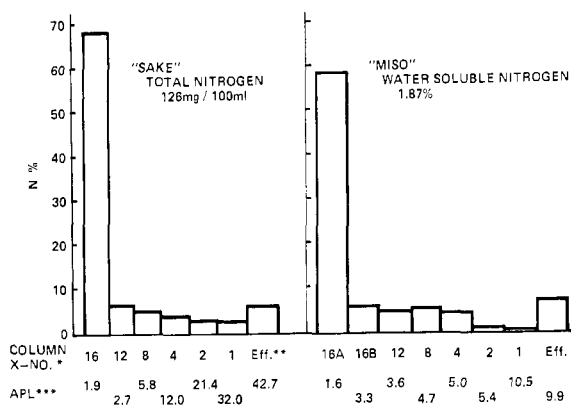


Figure 3. Distribution patterns of peptides in "sake" and "miso" (Takeuchi and Nose, 1958)

* : Cross-linking number of Dowex-50 column
 ** : Final effluent through molecular sieve chromatography
 *** : Average peptide length

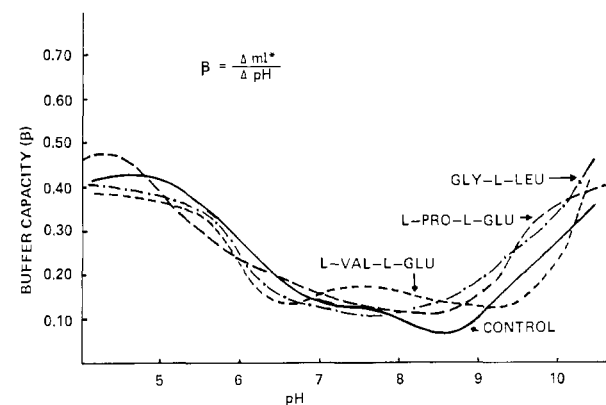


Figure 4. Effect of peptides on the buffer capacity on synthetic sake

* Δ ml. = Quantity of 1/10N NaOH or 1/10N HCl added (Lehmann, 1922)

and/or rice. There are three main types of miso with respect to the composition of the raw materials—i.e., soybean miso (soybean 100%), sendai miso (rice/soybean *ca.* 5.5), and shiro miso (rice/soybean *ca.* 22). In all three types of ripened miso, approximately the same distribution pattern of peptides was observed, regardless of the difference in raw materials. As shown in Figure 3, the average peptide length (APL) is less than 5.0 (Takeuchi and Yoshii, 1967). The amino acid composition of the major peptides of the hydrolyzate was determined, and it was found that they were acidic peptides containing aspartic acid, glutamic acid, and glycine.

Soy Sauce. PEPTIDE CONTENT OF SOY SAUCE. The total nitrogen content of Japanese soy sauce is generally 1.5%, one-half of which is in the form of free amino acids and about 12 to 15% in peptide form. The APL of peptides in soy sauce is from 3.5 to 3.8 (Table VII).

AMINO ACID COMPOSITION AND APL OF PEPTIDES. The amino acid content of the peptides was determined, and the results showed that in soy sauce larger amounts of aspartic acid, leucine, and glycine, and smaller amounts of glutamic acid and valine were present in peptide form than were present as free amino acids (Figure 5).

The nitrogen distribution of dialyzed soy sauce was examined by molecular sieve chromatography using six different cross-linked Dowex-50 resins, and most of the

Table VII. N-Distribution and APL of Soy Sauce A

Components	ND ^a (%)	APL ^b
Free amino acids	53	1.3
Peptides	13	3.8
Pyroglutamic acid	3	1.0
NH ₃ -N and unknown	31	0
Soy sauce A	100	1.75

^a ND: Nitrogen distribution.
^b APL: Average peptide length
 = $\frac{\text{(N of amino acids and/or peptides)}}{\text{amino-N}}$

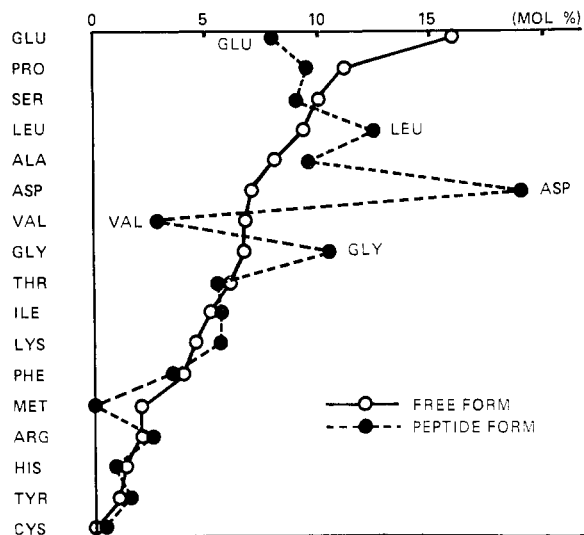


Figure 5. Amino acid composition of soy sauce A

nitrogen was in the form of low molecular weight peptides (Figure 6).

PREPARATION OF SOY SAUCE PEPTIDES. In studies on the contribution of peptides to the taste of soy sauce, peptide fractions were separated from soy sauce by dialysis and molecular sieve chromatography (Figure 7).

Ten liters of soy sauce which had been naturally brewed for months were dialyzed in Visking's cellulose acetate tubing (17/8-inch inflated diameter) against distilled water at 20° C. for 12 hours. The diffusate was then passed through three Dowex-50 columns (X-16, X-4, and X-1), and two peptides having APL's of 3.8 and 4.1 were isolated from the X-4 and X-1 columns, respectively. Two different peptides were isolated, in a similar manner, from 8 liters of a soybean protein solution (2.5%) that was hydrolyzed

Table VIII. Analysis of Peptide Fractions from Soy Sauce and Protein Hydrolyzate

Origin	Name	APL	T-N, % ^a	NH ₄ -Cl, % ^a	Ash, % ^a	Sp. Visc. (η/η) ^b
Soy sauce A	Peptide A	3.8	11.5	2.3	0.2	1.4
	Peptide B	4.1	14.9	1.5	0.4	1.45
Pronase hydrolyzate	Peptide C	4.4	16.5	7.5	0.4	1.4
	Peptide D	6.2	16.1	2.6	0.4	1.35

^a Dry basis.
^b 9% aqueous solution.

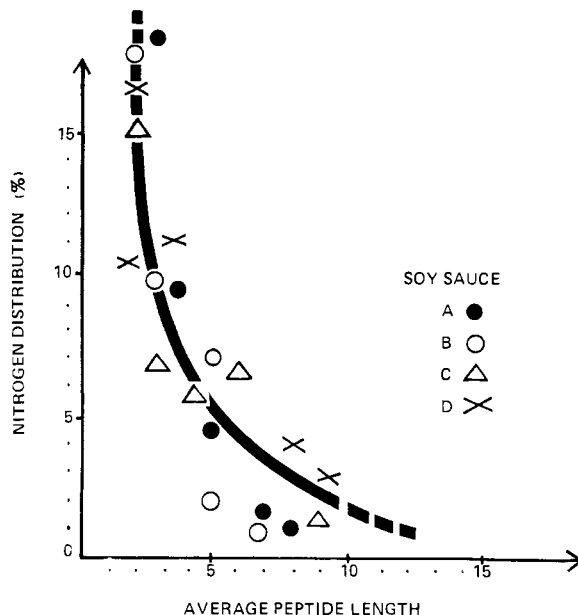


Figure 6. N-distribution of dialyzed soy sauce

by 4 grams of Pronase AF (a neutral proteinase from *Streptomyces griseus*) at pH 8.0, 55° C., and for 15 hours. These conditions of enzymic hydrolysis are quite different from those employed in the commercial preparation of soy sauce in which raw materials—soybean and wheat—are hydrolyzed at pH 6 at 20 to 25° C. for 15 hours with a proteinase from *Aspergillus sp.*

ORGANOLEPTIC AND OTHER PROPERTIES OF THE PREPARATIONS. The results in Table VIII show that the four peptides contained small amounts of NH₄Cl and ash; however, these compounds did not effect the sensory tests.

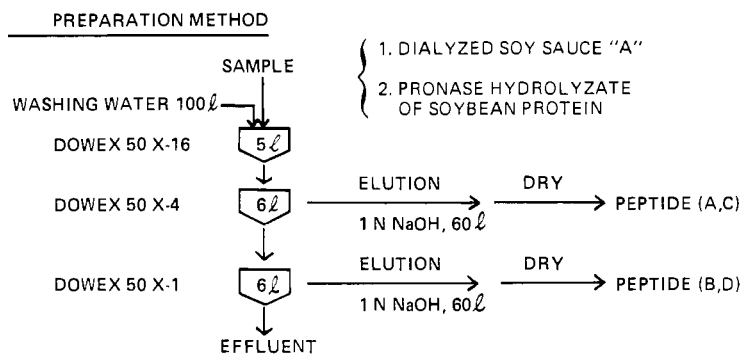


Figure 7. Preparation of peptide fractions from soy sauce and a soybean protein hydrolyzate

Table IX. Relationship Between Peptides, MSG, and Taste

pH 5.0; Panel size 9, trained
MSG at pH 5.0; RL 15^b; TL 60^b; DL 20^c

Triangle Test

X (X,Y,Y)		Y		(Mg./100 ml. of distilled water)	
MSG	Peptide	MSG	Peptide	Peptide B	Peptide C
30	62	0	62	<i>d</i>	<i>d</i>
6	62	0	62	N.s. ^e	N.s. ^e
60	62	60	0		<i>f</i>
60	31	60	0	<i>g</i>	N.s.
60	6	60	0	N.s.	N.s.

Duo-trio Test

X (X):(Y,Y')		Y		(Mg./100 ml. of 0.5% NaCl soln.)	
MSG	Peptide	MSG	MSG	Peptide B	Peptide C
15	31	15	40	N.s. ^e	<i>d</i>
15	31	15	50	<i>d</i>	
40	31	40	15	N.s. ^e	N.s. ^e
65	31	65	15	N.s. ^e	N.s. ^e
85	31	85	15	<i>f</i>	<i>g</i>

- ^a RL: Threshold value.
- ^b TL: Limen at which specific taste can be clearly detected.
- ^c DL: Differential limen.
- ^d Significant at the 1% level.
- ^e N.s.: Not significant.
- ^f Significant at the 5% level.
- ^g Significant at the 0.1% level.

For each peptide the specific viscosity of a 9% aqueous solution (1.4% N) was approximately 1.4. This value was lower than that of a MSG solution adjusted to 1.4% nitrogen and much lower than that of whole soy sauce (specific viscosity: 2.2), and thus the peptides have no effect on the viscosity of soy sauce. The high viscosity of whole soy sauce is probably due to carbohydrates.

The contribution of peptides to the buffering capacity of whole soy sauce was slight (Figure 4).

TASTE OF THE PREPARATIONS. Aqueous solutions of all four peptides tasted bitter and astringent. The thresholds were from 8 to 60 mg. per 100 ml., bitterness and astringency being easily detected at concentrations of 62 mg. per 100 ml. or higher (Figure 8).

To test what effect, if any, the peptide had on the taste of NaCl, a 0.1% solution of peptide was added to a NaCl solution (1.00 ± 0.015%). The solution exhibited the same saltiness as that in a solution containing 1.00% of NaCl alone, and hence the peptide had no effect on saltiness of NaCl.

Sensory tests were performed to determine the relationship between peptides B and C and MSG (Table IX). Taste differences were not significant between a solution containing 62 mg. per 100 ml. of peptide alone and a peptide solution containing subthreshold amounts of MSG or between a solution containing 60 mg. per 100 ml. of MSG alone and a solution containing subthreshold amounts of peptide. Differential limen (DL) of MSG in the mixture of MSG and either peptide was larger than that in MSG alone, indicating that the peptides depressed the intensity of the taste of MSG. The same results were obtained using the peptides and IMP or GMP.

The bitter and astringent taste of the peptides was similar to that of other soy sauce components such as monosodium succinate, sodium acetate, sodium lactate, and ammonium chloride; but there were significant

Table X. Relationship Between Peptides and Other Components of Soy Sauce Having Bitter or Astringent Taste

pH 5.0, aqueous solution;
Panel size 10, trained

Triangle Test

(X,Y,Y) { X = Peptide (TL) + Component (1/10 TL)
 { Y = Peptide (TL)

Peptide Conc., Mg./dl.	Component Name (RL)	Conc.	Significance	
			Peptide B	Peptide C
62	Na-succinate (16)	13	<i>a</i>	N.s. ^b
62	Na-acetate (63)	13	<i>c</i>	<i>c</i>
62	Na-lactate (63)	25	<i>c</i>	<i>d</i>
62	Na-levurate (31)	6	<i>d</i>	<i>a</i>
62	NH ₄ Cl (16)	6	<i>a</i>	<i>a</i>

- ^a Significant at the 5% level.
- ^b N.s.: Not significant.
- ^c Significant at the 0.1% level.
- ^d Significant at the 1% level.

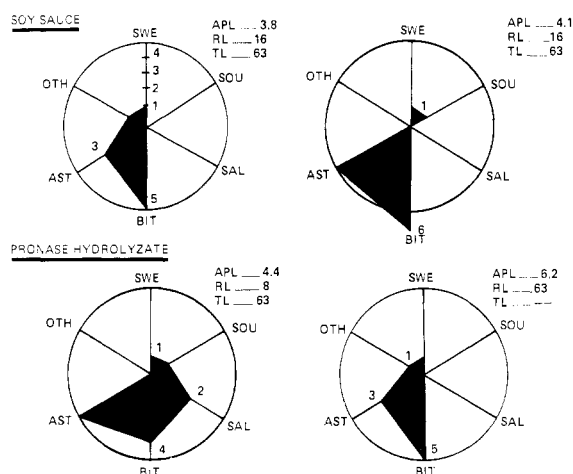


Figure 8. Taste profiles of peptide preparations

Radii represent taste intensities 100 mg./dl., pH 5.0-5.2, panel size 10, trained
AST: Astringency, OTH: Others
APL: Average peptide length
RL: Threshold value (mg./dl.)
TL: Limen at which specific taste can be clearly detected

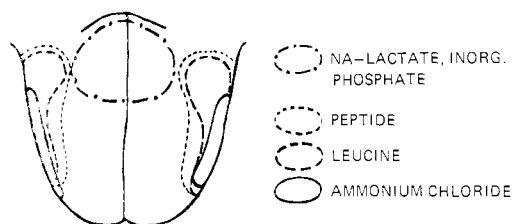


Figure 9. Area of the tongue stimulated by various components of soy sauce

differences in taste between solutions containing 62 mg. per 100 ml. of peptide alone and solutions containing subthreshold amounts of soy sauce components (Table X).

Sensory tests were performed to evaluate the effect of adding peptides to various soy sauce preparations. Imitation soy sauce (HVP liquid, Ajieki Type A, an acid hydrolyzate of soybean meal) had an unpleasant taste in the presence of peptide B compared to HVP alone; but a half-imitation soy sauce (a mixture of HVP and brewed soy sauce) had a more pleasant taste with added peptide

Table XI. Effect of Peptides on Mixtures of HVP and Soy Sauce

pH 4.8 to 5.0; T-N 0.15 g./dl.; NaCl 1.8 g./dl.; panel size 10

Triangle Test

(X,Y,Y) X: Testing base + Peptide B 160 mg./dl.
Y: Testing base only

Testing Base		Significance	Effect of Peptide
Nitrogen Ratio HVP:Soy sauce A	Additives (Leu, NH ₄ Cl, NaH ₂ PO ₄ , and Lactate)		
10:0	0	*** ^a	Unpleasant
6:4	0	***	Pleasant
6:4	Equivalent to 1/10 dilution of soy sauce A	***	Unpleasant

^a *** = Highly significant.

Table XII. Partial Hydrolysis of Soybean Protein^a

	HCl Concentration, N		
	0.03	0.01	0.006
A. P. L. ^b	3.8	7.7	11
Liberated Asp, ^c %	64	70	90

^a 105° C., 72 hours, 1 part protein to 250 parts HCl (w./v.).

^b Average peptide length.

^c Liberated Asp/total liberated amino acids × 100.

than that without peptide. The half-imitation soy sauce, however, tasted less pleasant upon addition of peptide B and additives, such as leucine, NH₄Cl, NaH₂PO₄, and lactate (in the same amounts as contained in brewed soy sauce) than that without peptide added (Table XI).

Peptide added to the sauce stimulated both sides of the tongue (Figure 9). Although leucine imparted bitterness and astringency and NH₄Cl imparted saltiness and astringency, both stimulated the same area of the tongue. On the other hand, phosphate and lactate stimulated the central area of the tongue. When compounds stimulated comparable areas of the tongue, the taste of the sauce to which peptide had been added was more acceptable than sauce containing compounds which stimulated different areas of the tongue.

Finally, although the effect of peptides on the taste of soy sauce was different for each case mentioned above, these peptides were generally concluded to be bitter and astringent, as opposed to the universal taste enhancers, such as MSG, IMP, and GMP.

BITTER PEPTIDES IN PARTIAL HYDROLYZATES OF SOYBEAN PROTEIN

Peptides were prepared by extraction of natural products, chemical synthesis, and partial hydrolysis of protein using enzymes or other hydrolytic reagents. In particular, the hydrolytic action of very dilute hydrochloric acid on soybean protein was studied. Dilute hydrochloric acid

was used to achieve a selective cleavage of protein at both sides of aspartyl residues. As shown in Table XII, the lower the concentration of hydrochloric acid, the more selective was the cleavage of aspartyl residues. The partial hydrolyzate had an APL of 3.8 and tasted slightly bitter.

Bitter peptides are a common product in the partial enzymic hydrolysis of soybean proteins. Fujimaki *et al.* (1968) identified the structures of seven peptides isolated from a peptic hydrolyzate of soybean protein and found that with the exception of one peptide, the amino- or carboxyl-terminal amino acid of these peptides was leucine. These bitter peptides can detract from the taste of foods, but a treatment using aminopeptidase, which hydrolyzes leucine-containing peptides, was effective in improving the taste of foods (Minamiura *et al.*, 1968).

SUMMARY

Based on the analytical and sensory results reported in this paper, the contributions of amino acids to the taste of foodstuffs can be summarized as follows: some amino acids contribute to the inherent tastes of foodstuffs themselves, some specific patterns of amino acid mixtures intensify the taste of foodstuffs and increase the mouthfulness without losing their inherent tastes, and the buffer action of amino acids can also contribute to the taste of foodstuffs.

The relationship between the chemical structure of dipeptides and their tastes may be utilized for improving foodstuffs as it points the way to the selective use of dipeptides in foods. Although further studies are required, it is presently concluded that peptides contribute to both the complexity and favorable balance of the taste of foodstuffs.

LITERATURE CITED

- Fujimaki, M., Yamashita, M., Okazawa, Y., Arai, S., *Agr. Biol. Chem.* **32**, 794 (1968).
Ikeda, K., *J. Tokyo Chem. Soc.* **30**, 820 (1908).
Kimizuka, A., Sakurai, K., Kirimura, J., Presented at the Meetings of Agricultural Chemical Society of Japan, October 1963.
Lehmann, G., *Biochem. Z.* **133**, 30 (1922).
Minamiura, Y., Matsumura, Y., Yamamoto, T., Fukumoto, J., Abstracts of papers, p. 95, 39th Annual Meeting of Agricultural Chemical Society of Japan, 1968.
Official and Tentative Methods of the American Oil Chemists' Society, Vol. I, A.O.C.S. Tentative Method Cd 12-57, 1947.
Ohara, M., "The Taste of Food," (in Japanese), Korin-zensho, Vol. 9, Korin Shoin, Tokyo, 1966.
Solms, J., Vutaz, L., Egli, R. H., *Experientia* **21**, 692-4 (1965).
Tajima, O., Sato, J., *Rept. Inst. Phys. Chem. Res.* **40**, 259 (1964).
Tajima, O., Shimizu, A., Kirimura, J., Institute of Physical and Chemical Research, Tokyo, unpublished data, 1968.
Takahashi, A., Nose, K., *J. Ferment. Technol.* **36**, 248 (1958).
Takeuchi, T., Yoshii, H., *J. Ferment. Technol.* **45**, 29-33 (1967).
Yoshida, M., Ninomiya, T., Ikeda, S., Yamaguchi, S., Yoshikawa, T., Ohara, M., *J. Agr. Chem. Soc. Japan* **40**, 295 (1966).

Received for review December 31, 1968. Accepted March 27, 1969. Presented in Symposium on Importance of Nonvolatile Compounds in Flavor, Division of Agricultural and Food Chemistry, 156th Meeting, ACS, Atlantic City, N. J., Sept. 1968.