ARTICLES

Convenient Synthesis of L-Ornithyltaurine·HCl and the Effect on Saltiness in a Food Material

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A new convenient synthesis of a salty peptide, ornithyltaurine (Orn-Tau), was designed. Compared with a conventional synthesis, the process was shortened by two steps and the purification was made much easier in the new one. Consequently, Orn-Tau was easily obtained and subjected to a further series of experiments. The product produced a good saltiness without Na^+ in the presence of HCl, and the saltiness became stronger as the amount of added HCl increased. The best quality of saltiness was obtained by adding 1.2 equiv of HCl to the Orn-Tau solutions. The Orn-Tau·1.2HCl was equally salty to NaCl on a molar basis and also had an enhancing effect on saltiness of NaCl. The intake of Na^+ could be cut by 95% in a model system and by 50% in a food material (soy sauce) by using Orn-Tau·1.2HCl, which have the most excellent saltiness and Na^+ dietary effect of our NaCl substitutes at the present time.

Keywords: *Salt substitute; L-ornithyltaurine; sodium ion dietary effect; new large scale synthesis*

INTRODUCTION

In 1984, our research group reported that a series of basic dipeptides, such as Orn-Tau·HCl, ornithyl-β-alanine·HCl (OBA·HCl), and some others, produced saltiness (Tada et al., 1984). Since this finding, we have continuously studied the properties of their salty taste and attempted to find other new salty compounds. In 1988, we found a new salty compound with a more simple structure, glycine ethyl ester·HCl (Gly-OEt·HCl) (Kawasaki et al., 1988). The next year, it was found that the saltiness of OBA and Gly-OEt was controlled by the amount of HCl, and when 1.3 equiv of HCl was added, the strongest saltiness was obtained. The saltiness of OBA·1.3HCl was 1.4 times stronger than that of NaCl on a molar basis. The saltiness of Gly-OEt·1.3HCl was stated to be equally salty to NaCl on a molar basis. When both salty compounds were mixed with NaCl, the sourness originated by HCl was decreased and taste qualities reached a practical level. In these mixed solutions, the intake of Na^+ could be cut by 75% and 50% by using OBA·1.3HCl and Gly-OEt·1.3HCl, respectively. Lys·HCl and Orn·HCl, though themselves did not have saltiness, enhanced the saltiness of NaCl and the intake of Na^+ could be cut by 25% in the mixed solutions (Tamura et al., 1989). More recently, we found that an *O*-aminoacyl sugar, which is a complex of amino acids and sugars, had an excellent enhancing effect of the NaCl saltiness and the intake of Na^+ could be cut by 90% (Tamura et al., 1993).

Though we have studied saltiness for more than 10 years and found some salty compounds described above, the saltiness of the first finding, Orn-Tau·HCl, is still the best. We employed Orn-Tau·HCl as a typical salty compound without Na^+ and examined its effect when added to a practical food material. As a food material for this study, soy sauce was selected. Soy sauce is one of the most popular liquid fermentation seasonings made from soybeans in Japan and is used not only as a sauce for *Sashimi*, *Susi*, etc. but also as a base for most Japanese foods. A typical Japanese person intakes about 27mL of soy sauce containing about 16% (2.7 M) NaCl everyday. In the soy sauce, many kinds of constituents of food materials such as inorganic ions, amino acids, sugars, acids, and alcohols were contained, and so we might reasonably evaluate the effect on a food material of Orn-Tau·HCl.

For the examinations, we had estimated that a considerable amount of Orn-Tau·HCl would be required, which by a conventional synthesis would be difficult to obtain. We sought to settle the problem by devising a new convenient synthesis. The first problem was the purification. After coupling dibenzyloxycarbonylornithine [*Z*-Orn(*Z*)-OH] with taurine (H-Tau-OH), an extraction with ethyl acetate was carried out for the purification. However, owing to the sulfonyl group of Tau, the extraction was very difficult even at pH 2. To prevent a low yield, we might perform the extraction many times under conditions of saturation with NaCl at drastically low pH. These operations needed a large amount of ethyl acetate and special excellent techniques to avoid contamination of Na^+ and deterioration of the taste quality. At one time, a research group reported that the saltiness of Orn-Tau was due to the contaminated Na^+ (Huynh-ba and Philippossian, 1987). This shows the difficulty of these operations. A second problem is the coupling method of *Z*-Orn(*Z*)-OH and Tau. In the conventional synthesis, an active ester method was used for the coupling, because there are

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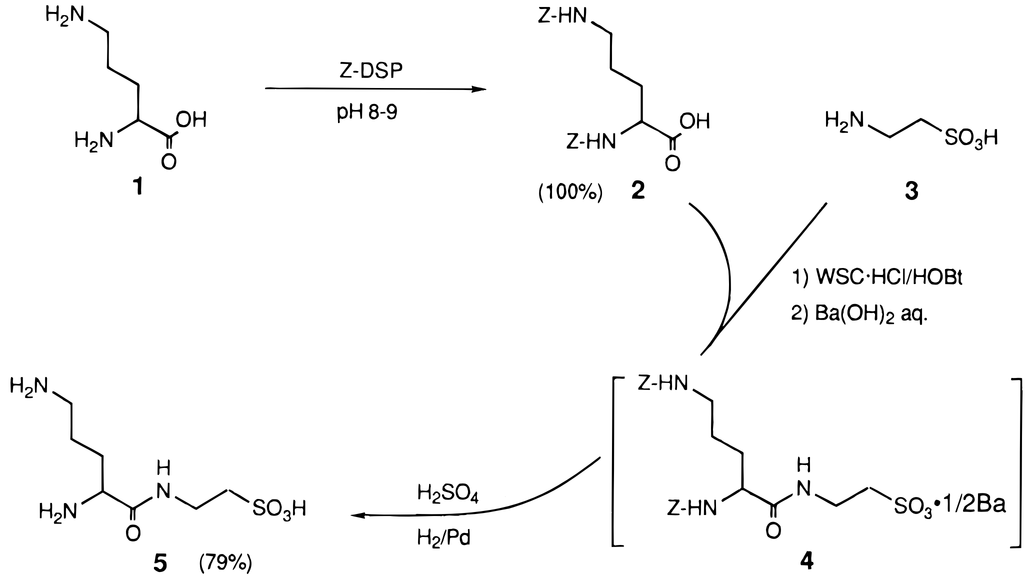
Scheme 1



no appropriate sulfonyl-protecting groups without taste deterioration. In the active ester method, synthetic steps and operations increased.

In Scheme 1, a new synthetic route is shown. The two problems are solved in this method. In this paper we report the convenient synthesis and the taste properties and effects on saltiness in soy sauce of the Orn-Tau·HCl.

MATERIALS AND METHODS

Materials. L-Ornithine (H-Orn-OH) was provided from Ajinomoto Inc. and 4-(benzyloxycarbonyloxy)phenyldimethylsulfonium methylsulfate (*Z*-DSP; Kouge et al., 1987) from Sanshin Chemical Industrial Co., Ltd., Yamaguchi. Taurine (H-Tau-OH) was purchased from Tokyo Kasei Kogyo Co., Ltd. Water soluble carbodiimide (WSC·HCl) and 1-hydroxybenztriazolehydrate (HOBt·H_2O) were from Kokusan Chemical Works, Inc. Barium hydroxide octahydrate [$Ba(OH)_2$·$8H_2O$], palladium carbon, and all solvents were from Katayama Chemical Industrial Co., Ltd. Commercial soy sauce and all materials of soy sauce sample were prepared by Takeda Syokuryo Co., Ltd.

Synthesis of Orn-Tau. *(a) General Procedures.* All melting points are uncorrected. Thin-layer chromatography was carried out on Merck silica gel G with two solvent systems: (1) 1-butanol/acetic acid/pyridine/water (4:2:2:1 v/v); (2) chloroform/methanol/acetic acid (50:10:2 v/v). Spots of materials possessing a free amino group on a thin-layer plate were detected by spraying ninhydrin and those of amino group blocked materials by spraying 25% hydrogen bromide in acetic acid and then ninhydrin. The optical rotations were measured on a Union PM-101 polarimeter, and microanalyses were carried out by Sanshin Chemical Industrial Co., Ltd., Yamaguchi. Prior to analyses, the compounds were dried over phosphorus pentaoxide at 66 °C (2 mmHg) for 8 h.

(b) Orn-Tau (**5**). *Z*-Orn(*Z*)-OH (**2**) was prepared using *Z*-DSP (Kouge et al., 1987). To an aqueous solution (100 mL) of **2** (20.0 g, 50 mmol), H-Tau-OH (8.1 g, 65 mmol), and HOBt (0.76 g, 5.0 mmol), was added triethylamine (6.9 mL, 50 mmol) at room temperature. After 0.5 h of stirring, WSC·HCl (12.4 g, 65 mmol) was added to the solution. After 8 h of stirring, 0.2 N aqueous solution of $Ba(OH)_2$·$8H_2O$ (125 mL, 25 mmol) was added to the reaction mixture and the barium salt of the protected dipeptide derivative was precipitated. The precipitate was collected and washed with deionized water (2 L) several times.

When **4** was isolated, the washed precipitate was dissolved in hot ethanol/water (50 mL, 9:1 v/v). $BaCO_3$, which was slightly produced by CO_2 in the air, was filtered off, and the filtrate was added to ether (250 mL). The precipitated product was collected and dried under reduced pressure: yield 23.6 g (82%); mp 89 °C; $[\alpha]^{25}_D$ −3.0 (*c* 1, DMF); R_{f1} 0.67, R_{f2} 0.15. Anal. Calcd for $C_{46}H_{56}O_{16}N_6S_2Ba$: C, 48.02; H, 4.91; N, 7.31. Found: C, 47.89; H, 5.01; N, 7.21.

When not isolated, to a suspension of the precipitate in water (200 mL) was added 1 N H_2SO_4(aq) (35 mL). The mixture was stirred for 0.5 h and then hydrogenated in the presence of palladium carbon at room temperature for about 6 h at atmospheric pressure. The catalyst and precipitation of $BaSO_4$ was filtered off and washed with water several times. H_2SO_4(aq) (0.1 N, 92 mL) was added to the filtration until formation of $BaSO_4$ was complete. $BaSO_4$ were removed and the filtrate lyophilized. The final product was obtained as a hygroscopic solid: yield 11.5 g (79%); hygroscopic; $[\alpha]^{25}_D$ −0.1 (*c* 1, H_2O); R_{f1} 0.14, R_{f2} 0. Ba^{2+} in the final product was spectrophotometrically determined by dimethylsulfonazo III (Budesinsky et al., 1967).

Preparation of Sample Solutions. *(a) Preparation of Orn-Tau·nHCl Solutions (n = 0−1.3).* Orn-Tau·*n*HCl solutions for sensory analyses were prepared by adding HCl solutions to HCl-free Orn-Tau solutions (see Table 1). HCl-free Orn-Tau was obtained by the new convenient synthesis.

Table 1. Influence of HCl on the Salty Taste of Orn-Tau[a]

HCl (equiv)	pH	score[b]	sourness
0	8.9	0	
0.11	8.0	0	
0.67	7.0	1	
1.00	6.4	2	±
1.10	6.1	2.5	+
1.20	5.9	3	+
1.30	3.4	3.5	++

[a] Concentration of Orn-Tau is 30 mM. [b] Score shows saltiness strength. Scores +3, +2, +1 are equal to the saltiness of 0.25%, 0.125%, 0.063% NaCl solutions, respectively.

Table 2. Strength of the Salty Taste of Orn-Tau·1.2HCl[a]

Orn-Tau·1.2HCl(aq) (mM)	score[b]	NaCl(aq) (mM)	score[b]
120 (2.88%, pH 5.1)	+6	107.0 (0.625%)	+6
100 (2.40%, pH 5.2)	+5	85.6 (0.5%)	+5
60 (1.44%, pH 5.3)	+4	64.2 (0.375%)	+4
45 (1.08%, pH 5.4)	+3.5		
30 (0.72%, pH 5.3)	+3	42.8 (0.25%)	+3
15 (0.36%, pH 5.4)	+1	10.7 (0.063%)	+1

[a] Threshold value of Orn-Tau·1.2HCl is 7.5 mM. [b] Score shows saltiness strength. Scores of +6, +5, +4, +3, +2, +1 are equal to the saltiness of 0.625%, 0.5%, 0.375%, 0.25%, 0.125%, 0.063% NaCl solutions, respectively.

Table 3. Saltiness of Orn-Tau·1.2HCl with Added NaCl

combination			
Orn-Tau·1.2 HCl	NaCl	pH	saltiness
100 mM (2.40%)	0.0% (0 mM)	5.2	+5, contains weak sourness
60 mM (1.44%)	0.063% (10.7 mM)	5.2	+5, contains very weak sourness
30 mM (0.72%)	0.125% (21.4 mM)	5.3	+5
15 mM (0.36%)	0.375% (64.2 mM)	5.3	+5
0 mM (0%)	0.5% (85.6 mM)	6.7	+5

Table 4. Weight Percentage of Sodium Ions in Sample Solutions Containing Orn-Tau·1.2HCl and Sodium Chloride

Orn-Tau·1.2 HCl	NaCl (%)	Na^+ contents[a] (% w/w)	ratio of Na^+ [b] (%)
100 mM (2.40%)	0.0	0	0
60 mM (1.44%)	0.063	1.7	4.3
30 mM (0.72%)	0.125	5.8	15
15 mM (0.36%)	0.375	20.1	51
0 mM (0%)	0.5	39.4	100

[a] Na^+ contents = Na^+/(NaCl + Orn-Tau) × 100. The weight of Na^+ in each sample solution was divided by the total weight of additives. [b] Ratio of Na^+ = Na^+ contents/39.4 × 100. Ratio of the weight percentage of Na^+ in the sample solution compared with a solution containing only 0.5% of NaCl.

(b) Preparation of Mixed Solutions of Orn-Tau·1.2HCl and NaCl. Mixed solutions listed in Table 3 were prepared by adding NaCl to Orn-Tau·1.2HCl solutions.

(c) Preparation of Contents-Modified Soy Sauce Samples Containing Orn-Tau·1.2HCl. We requested a soy sauce material containing only 6.4% NaCl for Takeda Syokuryo Co., Ltd. (Muramatu et al., 1990). We called this soy sauce "pre-soy sauce". Though the glutamate and ethanol contents of pre-soy sauce were a little higher than those of the commercial soy sauce, pre-soy sauce amended to 16% NaCl had the same taste and flavor as the commercial one (Muramatu et al., 1993). NaCl and Orn-Tau·1.2HCl were added to pre-soy sauce to prepare contents-modified soy sauce with three additive ratios (60%, 50%, and 33% mol/mol). The sample containing Orn-Tau·1.2HCl with additive ratio 60% was prepared as follows: Orn-Tau·1.2HCl was added to pre-soy sauce (NaCl concentration is 1.12 M), and the Orn-Tau·1.2HCl concentration of the sample was adjusted to 1.65 M. In this sample, the total concentration of Orn-Tau·1.2HCl and NaCl was 2.77 M, which was the same as the NaCl concentration of commercially available soy sauce. The sample with additive ratio 50% was prepared as follows: NaCl was added to pre-soy sauce so that the NaCl concentration became 1.38 M. Orn-Tau·1.2HCl was then added, and the Orn-Tau·1.2HCl concentration of the sample was adjusted to 1.39 M. Total concentration of the two was 2.77 M. The sample with additive ratio 33% was also prepared in a similar way and contained 1.85 M NaCl and 0.92 M Orn-Tau·1.2HCl. To apply these contents-modified soy sauces to the sensory test, total concentrations of NaCl and Orn-Tau·1.2HCl were adjusted to the concentrations shown Table 5.

Sensory Analysis. All samples were evaluated by a panel of four or five people in terms of their characteristics and taste strength. After rinsing the mouth, each person kept the solution in his/her mouth for about 10 s to evaluate the character and taste intensity of the sample. All panel members gave the same evaluation for the taste characteristics, while there were some personal variations in the taste strength evaluation owing to personal and experimental conditions. However, these personal deviations in the taste strength were eliminated by tasting a standard NaCl solution and comparing these results at the same time. The saltiness strength was evaluated on a score of 0−10. Score 10 was judged to be equivalent in salty taste to a reference 192.6 mM (1.125%) NaCl. Score of from 9 to 1 indicated a descending degree of saltiness, compared with the reference solution: score 9, 8, 7, 6, 5, 4, 3, and 2 meant that the saltiness of the sample solution was the same as that of 171.2 mM (1.0%), 149.8 (0.875%), 128.4 mM (0.75%), 107.0 (0.625), 85.6 mM (0.50%), 64.2 mM (0.375), 42.8 mM (0.25%), and 21.4 mM (0.125%) NaCl, respectively; score 1 was the saltiness of the threshold value of NaCl (0.063%); score 0 indicated tastelessness or other tastes.

Table 5. Saltiness and Taste Quality of Orn-Tau·1.2HCl adding Low-Sodium-Chloride-Containing Soy Sauce

concn[a] (mM)	saltiness[b]/taste quality[c]			
	A	B	C	D
192.6	8/++	9/+++	9.5/+++	10/+++
171.2	7.5/++	8.5/+++	9/+++	9/+++
149.8	7/++	7.5/+++	8/+++	8/+++
128.4	6.5/++	6.5/+++	7/+++	7/+++
107.0	5.5/++	5.5/+++	6/+++	6/+++
85.6	5/++	5/+++	5/+++	5/+++
64.2	4.5/++	4/+++	4/+++	4/+++
42.8	3.5/++	3/+++	3/+++	3/+++
21.4	2.5/++	2/+++	2/+++	2/+++
10.7	1.5/++	1/+++	1/+++	1/+++

[a] Total concentration of NaCl and Orn-Tau. [b] A, the ratio of Orn-Tau to the total concentration is 60%; B, 50%; C, 33% (mol/mol); D, commercial soy sauce. [c] Taste quality of the samples was evaluated on a scale of +++ and ++: +++, equivalent to reference commercial soy sauce in quality; ++, slightly inferior to commercial one.

The quality of contents-modified soy sauce sample solutions was evaluated. A sample solution was given a scale of +++ if the taste quality was judged equal to that of commercially available soy sauce. Scale ++ indicates that the quality is a little inferior to that of traditional soy sauce. The procedure for the sensory analysis was described in our previous paper (Ishibashi et al., 1987) in detail.

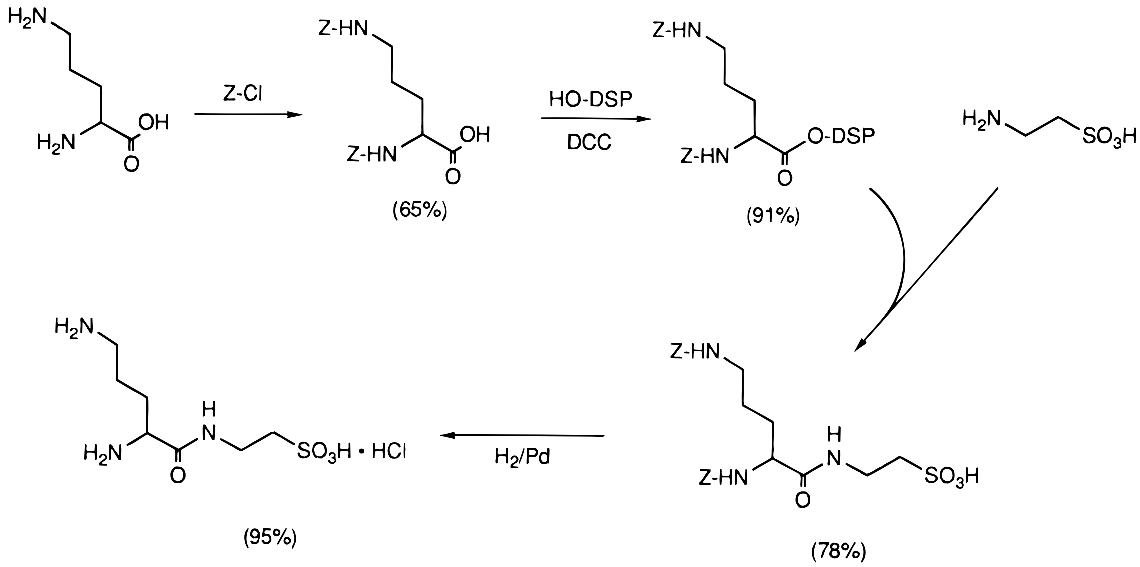
RESULTS AND DISCUSSION

New Convenient Synthesis of Orn-Tau. A conventional synthesis of Orn-Tau·HCl is shown in Scheme 2. Even in 3 mmol scale, very careful techniques and great cost were required to obtain such high yields. Therefore, this method was not suitable for large scale synthesis.

In the new method (Scheme 1), we could directly couple *Z*-Orn(*Z*)-OH with H-Tau-OH by the WSC-HOBt method (Konig et al., 1970). This coupling proceeded readily in water without blocking the sulfonyl group and side reactions did not occur. Subsequently, an aqueous solution of $Ba(OH)_2·8H_2O$ was added to the reaction mixture and *Z*-Orn(*Z*)-Tau-OH was selectively precipitated as a barium salt [*Z*-Orn(*Z*)-Tau·$^1/_2$Ba]. The precipitate washed several times with deionized water was added to 1 N H_2SO_4(aq) (0.7 equiv of the starting material) and then deblocked by hydrogenation. After $BaSO_4$ and the catalyst were removed, Ba^{2+} was completely precipitated as $BaSO_4$ by adding 0.1 N H_2SO_4(aq). $BaSO_4$ was filtrated off, and solid Orn-Tau was obtained by lyophilization. Ba^{2+} was not detected in the final product.

In this method, two problems in conventional synthesis were solved and the following advantages for large scale synthesis were found: All operations could be performed in a water solvent system. Water is superior to the other solvents from the point of safety, cost, and easy control of the reaction condition by pH.

Scheme 2



Our synthetic process was shortened by two steps compared to the conventional one, and the purification was made much easier. The final product did not contain any counteranion such as HCl and so was an ideal sample for the examinations to determine the taste properties of Orn-Tau.

Taste Behavior of Orn-Tau. *(a) Optimum HCl Equivalent for Orn-Tau Saltiness.* For evaluating the character and taste strength, 30 mM Orn-Tau solutions containing 0−1.3 equiv of HCl were prepared. We carried out sensory analyses of these solutions and found that solutions containing <0.67 equiv of HCl did not produce the salty taste. At pH 7, Orn-Tau containing 0.67 equiv of HCl produced a very weak salty taste and the saltiness strength became stronger as the amount of HCl was increased. However, when more than 1.3 equiv of HCl was added, it was difficult to discriminate the saltiness from the sourness originating from HCl. We obtained the strongest saltiness by adding 1.3 equiv of HCl, but Orn-Tau having 1.2 equiv of HCl produced the most excellent saltiness, which contained a slightly sour taste (see Table 1). Orn-Tau·1.2HCl was equally salty as NaCl on a molar basis. This taste behavior is similar to OBA, except for the minimum equivalent of HCl for saltiness production (Tamura et al., 1989). The saltiness quality of Orn-Tau·1.2HCl is much superior to that of OBA·1.3HCl, and the saltiness was still excellent even in high concentration compared with other NaCl substitutes (Table 2). It is thought that this difference is caused by a sulfonic acid group of Orn-Tau, which is the only difference between Orn-Tau and OBA.

In the new synthesis of Orn-Tau, contamination of Na^+ could be held to a minimum and Orn-Tau exhibited excellent salty taste. It was confirmed that the saltiness of Orn-Tau was produced without Na^+.

(b) Taste Behavior of Mixed Solutions of Orn-Tau·1.2HCl and NaCl. We prepared mixed solutions of Orn-Tau·1.2HCl and NaCl in several ratios as shown in Table 3. These ratios were employed according to the results of sensory analysis of OBA as previously reported (Tamura et al., 1989). From the results of sensory analysis, it was found that all mixed solutions of each series possessed the same saltiness strength and the sourness arising from excess HCl was weakened by adding NaCl even though the pH was still acidic. The sourness of 60 mM Orn-Tau·1.2HCl almost completely disappeared when 0.063% NaCl was added. In other words, the saltiness of 0.063% NaCl (score +1) was increased to +5 by adding 60 mM Orn-Tau·1.2HCl. We calculated how much the proportion of Na^+ was reduced by adding Orn-Tau·1.2HCl. The results are summarized in Table 4. In a solution containing only 0.5% NaCl, the weight percentage of Na^+ is 39.4%. In the case of solution of 60 mM Orn-Tau·1.2HCl and 0.063% of NaCl, the weight percentage of Na^+ is 1.7% of the total amount of additives. The weight percentage of Na^+ in the solution containing 60 mM Orn-Tau·1.2HCl and 0.063% NaCl is only 4.3% of that containing 0.5% NaCl. Therefore, the percentage of Na^+ can be reduced by around 95% in this model system.

Effect on Saltiness in Pre-Soy Sauce of Orn-Tau·1.2HCl. To determine the effect on a practical food material, we added the Orn-Tau·HCl to pre-soy sauce and prepared contents-modified soy sauce samples containing Orn-Tau·1.2HCl. By comparing commercially available soy sauce and the contents-modified soy sauce samples, we examined the Na^+ dietary effect and the influence of Orn-Tau·1.2HCl on the quality of soy sauce.

Results of sensory analysis of the samples are listed in Table 5. The 60% additives ratio samples produced almost the same saltiness as did the commercially available soy sauce in low concentration. In high concentration, however, the saltiness and taste quality were not satisfied. The deterioration of taste quality was mainly caused by the sourness of Orn-Tau·1.2HCl, and it is thought that the saltiness was diminished by influence of constituents in pre-soy sauce. When 50% and 33% additive ratio samples were tasted, the same level of saltiness and taste quality as those of commercially available soy sauce were reproduced. We judged that taste quality of 50% additive ratio sample reached a practical level. In a food material, using Orn-Tau·1.2HCl, Na^+ from NaCl could be reduced 50% from that of commercially available soy sauce.

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