

罐詰水産食品に 5'-Ribonucleotides の利用

橋田 度・毛利 威徳・志賀 岩雄

Application of 5'-Ribonucleotides to Canned Sea-Foods*

WATARU HASHIDA, TAKENORI MOURI and IWAO SHIGA

SUMMARY

Effect of 5'-ribonucleotides on enhancing taste of canned sea-foods was investigated. Short-necked clam (*Venerupis semidecusata*), oyster (*Ostrea giges*), red crab (*Acanthodes armatus*), and whale meat were experimentally packed with small amounts of 5'-ribonucleotides in lacquered tin cans and processed according to commercial procedures.

The paired-preference test and ranking test were employed for evaluating sensory qualities of the canned foods stored for 6 months at room temperature. It was found that taste is enhanced at the following levels: 0.04 or 0.08% of Ribotide (1:1-mixture of 5'-IMP·Na₂ and 5'-GMP·Na₂) for short-necked clam, 0.04 or 0.08% of Ribotide for red crab meat, 0.1% of 5'-IMP·Na₂, 0.1% of 5'-GMP·Na₂, or 0.2% of Ribotide for corned beef-style whale meat. To measure stabilities of added 5'-ribonucleotides during heat processing and storage, amounts of residual 5'-ribonucleotides were determined with 5'-nucleotidase. The added nucleotides were found to be fairly stable. Canned red crab meat showed a retention of 77 to 88% of added nucleotides during storage for 28 days, and canned short-necked clam a retention of 52 to 61% during storage for 189 days.

INTRODUCTION

The flavor-enhancing effects of 5'-nucleotides, especially 5'-IMP and 5'-GMP, have been established. Their application as food seasonings is extensively reported (Shimazono, 1964, 1965; Kuninaka *et al.*, 1964; Ohara *et al.*, 1964; Hashida, 1964; Sawyer, 1966). Results on canned vegetables have been published (Hashida *et al.*, 1966). The present paper describes the application of 5'-nucleotides to canned sea-foods.

Studies have been made on the distribution of 5'-nucleotides in sea-foods (Saito, 1960, 1961; Jones, 1960; Fujita *et al.*, 1960; Kobayashi, 1966; Arai, 1966; Tomiyama *et*

* Reprinted from FOOD TECHNOLOGY, Volume 22, Number 11, November 1968, Pages: 1436

al., 1966; Spinelli *et al.*, 1966).

Shimazono (1964) summarized the results on distribution patterns of nucleotides and divided the sea-foods into two categories, IMP-type and AMP-type. Usually, 5'-IMP has been reported to be the major component of nucleotides in fish muscle and whale meat which are designated as IMP-type. In aquatic invertebrates such as shellfish, cuttlefish and prawn belonging to the AMP-type, considerable amount of 5'-AMP has been found but no measurable amount of 5'-IMP.

Addition of 5'-IMP to materials, in which 5'-IMP is not inherently contained, might cause modification of the original taste. In the cases of short-necked clam, oyster and red crab, extremely careful investigations will be needed to determine whether the flavor caused by the use of 5'-IMP is acceptable. On the other hand, when 5'-IMP is inherently contained in materials, 5'-IMP will be more readily acceptable in order to compensate its loss during processing or to enhance the original flavor. From these viewpoints, the addition of 5'-IMP to whale meat is considered to be promising.

The present investigation was undertaken to study the effect of 5'-IMP · Na₂ and/or 5'-GMP · Na₂ applied to four kinds of canned sea-foods produced in relatively large volumes in Japan. The nucleotides effectively enhanced taste of canned short-necked clam, red crab and corned whale meat, and were fairly stable in the course of heat processing and long-term storage.

MATERIALS AND METHODS

Sodium salts of 5'-IMP, 5'-GMP and "Ribotide" (registered trade name for 1:1-mixture of 5'-IMP · Na₂ and 5'-GMP · Na₂ as flavor enhancer from Takeda Chemical Industries) and 5'-nucleotidase used for analyzing 5'-nucleotides were supplied by Takeda Chemical Industries, Ltd., Osaka, Japan.

Canning. Four kinds of canned sea-foods were processed by commercial practices as follows: boiled short-necked clam, Yanagawa Plant, Hayashikane-Sangyo Co.; red crab, Kurayoshi Plant, Tottori Canning Co.; boiled oyster, Head Plant, Aohata Canning Co.; and corned beef-style whale meat, Senoue Plant, Teihoku Shokuryo Co.

Ribotide, 5'-IMP · Na₂, 5'-GMP · Na₂ or monosodium glutamate (MSG) was added separately or in combination at the prescribed concentrations for the total contents, and the cans were sterilized conventionally. The cans were stored for 6 months at room temperature, and were opened at scheduled time for the sensory test and the determination of remaining 5'-nucleotides.

Sensory test. Both or either the paired-preference test and/or the ranking test were conducted for evaluating the quality of canned foods according to the methods used on vegetables previously described (Hashida *et al.*, 1966).

Assay of 5'-ribonucleotide. The canned foods were separated into the solids and liquid, and extracted with cold perchloric acid. Individual 5'-nucleotide in the extract was determined by means of a Dowex 1×8 column according to a procedure proposed

by Bergkvist *et al.* (1954) and modified by Nakajima *et al.* (1961 a).

The total amount of 5'-nucleotides remaining in the solids or liquid of the canned foods was determined enzymatically by the method of Nakajima *et al.* (1963), which had been previously examined for its application to the analysis of the marine products: Ten grams of boiled short-necked clam were homogenized with cold perchloric acid and centrifuged. The supernatant liquid was neutralized to pH 7.0 with 5N-KOH.

The precipitate was removed, and the supernatant was made up to 200 ml with distilled water. Portions (5, 10, 20, 25 and 50 ml) of the prepared extract were treated with active carbon and was assayed with 5'-nucleotidase. As shown in Table 1, there was no consistent drift in the content of 5'-nucleotide among the amounts of the extract corresponding to 0.5, 1.0, 1.25 and 2.5g of clam meat.

In Table 2 are shown results of recovery experiments in which 0.21 to 0.62 μ mol of Ribotide was added to 0.5g of red crab meat or 1.0g of short-necked clam meat and assayed with 5'-nucleotidase. The recovery of 5'-nucleotides from short-necked clam was more than 92%, and that from red crab was between 91 to 95%. This method was thus proved to be adequate for the present purpose. It is desirable to make a correction by using the recoveries obtained from test runs at the same assay periods.

Table 1 Constancy of assay for the total amount of 5'-nucleotide at ascending levels of clam meat.

Samples, Clam meat extract (ml)	Total 5'-nucleotide (μ mol)	Total 5'-nucleotide (μ mol/g of clam meat)	Mean value
5	0.150	0.600	} 0.453 ± 0.013
10	0.233	0.466	
20	0.437	0.437	
25	0.576	0.461	
50	1.195	0.448	

Table 2 Recovery of Ribotide added to short-necked clam and red crab.

Materials (Meat)	Added amount of Ribotide (5'-phosphoric acid, μ mol)	Analytical value (5'-phosphoric acid, μ mol)	Recovery value (5'-phosphoric acid, μ mol)	Recovery rate (%)
Short-necked clam 1 g	0	0.437		
	0.392	0.798	0.361	92.1
Red crab 0.5g	0	0.396		
	0.206	0.591	0.195	94.5
	0.412	0.769	0.373	90.5
	0.618	0.954	0.558	90.5

RESULTS AND DISCUSSION

Nucleotides in canned foods. Distribution of 5'-nucleotides in raw materials and their behaviors during processing, which were considered to greatly influence the effectiveness of the foreign nucleotides, were investigated prior to the studies on the application of the flavor-enhancers.

Distribution of 5'-nucleotide in materials. The amounts of individual nucleotides in perchloric acid extracts of the raw materials are shown in Table 3.

Table 3 The amounts of nucleotides in materials.

Sea-foods		Concentration in $\mu\text{mol/g}$						
		5'-CMP	5'-AMP	5'-UMP	5'-IMP	5'-GMP	ADP	ATP
Short-necked clam (<i>Venerupis semidecusata</i>)	fresh	+ ¹	0.31	0.05	0	0	0.50	0.63
	boiled	+	1.25	0.13	0	0	0.80	0.22
Red crab (<i>Acanthodes armatus</i>)	fresh	+	0.14	0.16	0	0	0.28	0.08
	boiled	+	0.52	0.33	0	0	0.15	0.03
Oyster (<i>Ostrea giges</i>)	fresh	+	0.61	0.96	0	0	0.72	0.43
	boiled	+	0.37	0.04	0	0	0.41	0.20

¹+ indicates a trace amount.

As a result, 5'-AMP and 5'-UMP were found to be the major components of 5'-nucleotides in short-necked clam, red crab and oyster. Trace of 5'-CMP was detected in some cases, but 5'-GMP and 5'-IMP were negligible.

It has been found by Nakajima *et al.* (1961 b) that approximately 2 mg/g of 5'-IMP is contained in whale meat.

Changes of nucleotide during canning process. Short-necked clam was shelled after blanching in boiled water for 7 min. Stripped meat was rinsed and dipped over night in ample water. Clam meat was packed in 65.4×101.1 mm cans, each can containing 210g of solid and 100g of 4% NaCl solution. 210g of prepared meat was equivalent to 194g of raw meat. Samples from each stage were extracted with cold perchloric acid and assayed with 5'-nucleotidase for the total amount of 5'-nucleotides. The increase in the total amount of 5'-nucleotides was observed during heat-processing of clam meat (Table 4).

A chromatographical study indicated that the increase in the total amount of 5'-nucleotides could be interpreted as the increase of AMP (a poor flavor-enhancer) formed by the degradation of ATP during heat-processing.

Red crab meat was also canned in lacquered 74.1×39.2 mm cans, and after sterilization the cans were opened and the total amount of 5'-nucleotides was measured. It was found that the total amount of 5'-nucleotides increased during heat-processing of crab meat similarly to the case of short-necked clam.

Table 4 Change of the total 5'-nucleotide in canning process of short-necked clam.

	Content, equivalent amount (g/can)	Total 5'-nucleotide ($\mu\text{mol/g}$)	Total 5'-nucleotide ($\mu\text{mol/can}$)
Raw meat	194	0.40	78
Stripped meat, after blanching	166	1.48	246
Meat, after rinsing and dipping	210	0.45	95
Solid, after canning sterilization	138	0.74	102
Liquid, after canning sterilization	166	0.74	124

Canned foods with added Ribotide and 5'-nucleotides. *Canned short-necked clam.* Stripped and prepared clam meat was packed in 65.4×101.1 mm cans, each can containing 230g of solid and 80g of seasoning solution with Ribotide of the following concentrations:

- No. 1, control, no Ribotide added
- No. 2, Ribotide added at 0.02% of total content
- No. 3, Ribotide added at 0.04% of total content
- No. 4, Ribotide added at 0.08% of total content

The cans were opened 12, 99, and 189 days after sterilization. The contents of canned clams were separated into the solids and the liquid, and the taste and the determination of remaining Ribotide were carried out.

As shown in Table 5, in samples stored for 12 or 99 days, the solids enriched with

Table 5 Effect of addition of Ribotide on canned short-necked clam (a paired-preference test).

Storage period (days)	No. of judgments preferring no Ribotide	No. of judgments preferring Ribotide	% of judgments preferring Ribotide	χ^2	Statistical Significance ¹
No Ribotide vs. 0.02% Ribotide					
12	19	17	47
99	9	15	63	1.5	...
189	3	12	80	5.4	*
No Ribotide vs. 0.04% Ribotide					
12	15	22	60	1.3	...
99	9	15	63	1.5	...
189	3	12	80	5.4	*
No Ribotide vs. 0.08% Ribotide					
12	4	16	80	7.2	**
99	5	19	79	8.1	**
189	3	12	80	5.4	*

... Indicates no significance at 5% level.

* Significant at 5% level.

** Significant at 1% level.

0.08% Ribotide tasted better than the Ribotide-free solids at a 1% level of significance. But the effect of 0.02 or 0.04% Ribotide on the solids was not clear even at a 5% level of significance. In samples stored for 189 days, the solids with 0.02%, 0.04% or 0.08% Ribotide tasted better than the Ribotide-free at the 5% level of significance.

The ranking totals given by a panel on the solids of cans receiving 3 different levels of Ribotide are shown in Table 6. It can be seen that a large number of the tasters preferred Sample 4 (0.08% Ribotide) as the best and Sample 3 (0.04% Ribotide) as the 2nd, when these were examined after the storage of 99 or 189 days. However, no remarkable difference between 3 samples was recognized after 12-day storage.

Table 7 shows the remaining Ribotide level per can, after 12 and 189 days of storage, determined as 5'-nucleotide by enzymatic method. In the samples held for 12 days, 5'-nucleotide levels in liquid were higher than those in solids. As Ribotide was added

Table 6 Ranking totals of canned short-necked clam.

Storage period (days)	No. of tasters	Ranking total of sample No.2 (0.02% Ribotide)	Ranking total of sample No.3 (0.04% Ribotide)	Ranking total of sample No.4 (0.08% Ribotide)	Statistical significance
12	20	40	46	34	---
99	20	47	44	29	**
189	15	40	28	22	**

--- Indicates no significance at 5% level.

** Significant at 1% level.

Table 7 Retention of 5'-nucleotide in canned short-necked clam.

			Total contents per can (g)	5'-nucleotide ($\mu\text{mol}/100\text{g}$)	5'-nucleotide ($\mu\text{mol}/\text{can}$)	
After 12-days storage						
No. 1 control,	solid		164	43.4	71	} 145
added no Ribotide	liquid		145	51.3	74	
No. 3 added	solid		163	66.5	108	} 307
0.04% Ribotide	liquid		147	135.0	199	
No. 4 added	solid		164	85.0	139	} 399
0.08% Ribotide	liquid		144	180.8	260	
After 189-days storage						
No. 1 control,	solid		156	34.7	54	} 127
added no Ribotide	liquid		147	49.8	73	
No. 2 added	solid		160	51.3	82	} 195
0.02% Ribotide	liquid		140	80.5	113	
No. 3 added	solid		167	72.5	121	} 277
0.04% Ribotide	liquid		137	113.9	156	
No. 4 added	solid		169	109.1	185	} 387
0.08% Ribotide	liquid		138	146.2	202	

into the seasoning liquid, the differences of the levels may indicate insufficient penetration of Ribotide from liquid into solids.

In samples of 189 days of storage, certain decrease in Ribotide content was observed in the liquid, accompanying the increase in the solids. Thus, it is assumed that Ribotide added to the seasoning liquid only slowly penetrates into the clam meat in the duration of the storage period.

Even without Ribotide being added, a fairly large amount of 5'-nucleotide was detected because of the endogenous 5'-AMP and 5'-UMP in the material (cf. Table 3).

The stability of added Ribotide in Samples 2, 3, and 4 during 189 days of storage was calculated as follows:

The amount of Ribotide remaining was estimated by comparing the 5'-nucleotide content of Samples 2, 3, and 4 with that of Sample 1 (Ribotide not added), respectively.

$$\text{Sample 2} - \text{Sample 1} = 195 - 127 = 68 \mu\text{mol}$$

$$\text{Sample 3} - \text{Sample 1} = 277 - 127 = 150 \mu\text{mol}$$

$$\text{Sample 4} - \text{Sample 1} = 387 - 127 = 260 \mu\text{mol}$$

As the amount of Ribotide added (0.02, 0.04, and 0.08%) corresponds to 124 μmol in Sample 2, 248 μmol in Sample 3, and 496 μmol in Sample 4, the retention of added Ribotide after 189 days of storage are:

$$(68/124) \times 100 = 55\% \text{ in Sample 2}$$

$$(150/248) \times 100 = 61\% \text{ in Sample 3}$$

$$(260/496) \times 100 = 52\% \text{ in Sample 4}$$

The remaining rates of Ribotide after 12-day storage were 65% in Sample 3 and 51% in Sample 4.

From the results obtained on the sensory tests for canned clams, it is concluded that the additions of 0.08% and 0.04% of Ribotide are highly effective on enhancing the flavor, whereas, 0.02% Ribotide is less effective.

Canned boiled oyster. To each of lacquered 65.4×81.3 mm cans containing 200 g of oyster and 50g of brine (4% salt solution) were added as follows: no nucleotide (control), 0.04% 5'-IMP (No. 1), 0.04% 5'-GMP (No. 2), 0.04% Ribotide (No. 3), and 0.08% Ribotide (No. 4).

The sterilized cans were stored at room temperature. They were tested after 45, 96, 98, 210 and 236 days of storage. The contents were divided into solids and liquid, and the sensory test was carried out on the solids, and remaining nucleotides in both solids and liquid were determined.

Taste of the samples to which 0.04% of 5'-IMP or 0.04% of 5'-GMP had been added was compared with the control by means of the paired-preference test. More than 60% of tasters preferred oysters with added 5'-IMP or 5'-GMP after 45 to 210 days of storage, though, the evaluations, when assessed by the χ^2 method, were not significant at the 5% level.

The ranking test was conducted by employing 15 tasters on 3 kinds of samples stored for 45 days to which, respectively, nil (control), 0.04% and 0.08% Ribotide had

Table 8 Retention of 5'-nucleotide in canned boiled oyster.

		Total contents per can (g)	5'-nucleotide ($\mu\text{mol}/100\text{g}$)	5'-nucleotide ($\mu\text{mol}/\text{can}$)	
After 98-days storage					
No. 0 control,	solid	158	146	231	} 369
added no nucleotide	liquid	92	150	138	
No. 1 added	solid	158	188	297	} 493
0.04% 5'-IMP	liquid	92	214	196	
No. 2 added	solid	158	188	297	} 503
0.04% 5'-GMP	liquid	92	224	206	
No. 3 added	solid	158	183	289	} 494
0.04% Ribotide	liquid	92	223	205	
No. 4 added	solid	158	238	376	} 626
0.08% Ribotide	liquid	92	272	250	
After 236-days storage					
No. 0 control,	solid	158	144	228	} 363
added no nucleotide	liquid	92	149	137	
No. 1 added	solid	158	184	291	} 488
0.04% 5'-IMP	liquid	92	214	197	
No. 2 added	solid	158	181	286	} 487
0.04% 5'-GMP	liquid	92	218	201	
No. 3 added	solid	158	181	286	} 478
0.04% Ribotide	liquid	92	208	192	

been added before packing. The ranking totals were: 35 for the control, 31 for the samples with 0.04% Ribotide added, and 24 for those with 0.08% Ribotide. Evaluation of these data, also agreed with the paired preference test indicating no significant difference at the 5% level.

The amount of Ribotide, designated as the total amount of 5'-nucleotide present, per can is shown in Table 8. In samples held for 98 and 236 days, 5'-nucleotide levels in the liquid were higher than those in the solids of corresponding samples, indicating the penetration of 5'-nucleotide into oyster meat is limited during the storage periods. The rates of remaining Ribotide, calculated as described previously, are:

after 98 days of storage,

61, 65, 63 and 64% in Samples 1, 2, 3 and 4, respectively,

after 236 days of storage,

61, 60, and 58% in Samples 1, 2 and 3, respectively.

Canned red crab. Red crab meat prepared under conventional method was packed in lacquered 74.1×39.2 mm cans, each can containing 105 g of solids, 2 g of NaCl and the flavor enhancing substances. The marks of samples were:

No. 1, control, no Ribotide added

No. 2, Ribotide added at 0.04% of total content

No. 3, Ribotide added at 0.08% of total content

No. 4, Ribotide and MSG added at 0.04 and 0.1%, respectively.

Table 9 Effect of Ribotide added on canned red crab (a paired-preference test).

Storage period (days)	No. of judgments preferring no Ribotide	No. of judgments preferring Ribotide	% of judgments preferring Ribotide	χ^2	Statistical significance
No Ribotide vs. 0.04% Ribotide					
28	4	13	77	4.8	*
168	14	26	65	3.6	...
No Ribotide vs. 0.08% Ribotide					
28	1	16	94	13.2	***
78	2	14	88	9.0	**
168	11	28	72	7.4	**
No Ribotide vs. 0.04% Ribotide plus 0.1% MSG					
28	0	17	100	∞	***
78	0	16	100	∞	***
168	10	30	75	10.0	**

... Indicates no significance at 5% level.

* Significant at 5% level.

** Significant at 1% level.

*** Significant at 0.1% level.

As shown in Table 9, the results of the paired-preference test indicate that most tasters preferred Samples 2, 3, and 4 to Sample 1. The effect of Ribotide was clear at the 1% or 0.1% level of significance in Samples 3 and 4 during a period of half a year. Significance reached higher levels with the addition of 0.08% of Ribotide. One-tenth per cent MSG was found to be synergistically effective when used together even with the lower level of Ribotide (0.04%).

Three samples of canned crab meat receiving 0.04% Ribotide, 0.08% Ribotide and 0.04% Ribotide plus 0.1% MSG were ranked in the order of preference by the panel. As shown in Table 10, samples with 0.08% Ribotide and 0.04% Ribotide plus 0.1% MSG were preferred at high levels of significance to the samples with 0.04% Ribotide.

The amount of remaining Ribotide was determined as above described (Table 11). A certain amount of 5'-nucleotide was detected in the controls, indicating that 5'-AMP and 5'-UMP are originally contained in the material. The rates of Ribotide remaining

Table 10 Ranking totals of canned red crab.

Storage period (days)	No. of tasters	Ranking total of sample No.2 (0.04% Ribotide)	Ranking total of sample No.3 (0.08% Ribotide)	Ranking total of sample No.4 (0.04% Ribotide plus 0.1% MSG)	Statistical significance
28	15	42	28	20	**
78	15	44	28	18	**
168	20	55	31	34	**

** Significant at 1% level.

Table 11 Retention of 5'-nucleotide in canned red crab.

No.	Additives		Total content (g/can)	5'-nucleotide ($\mu\text{mol/g}$)	5'-nucleotide ($\mu\text{mol/can}$)
	Ribotide (%)	MSG (%)			
After 28-days storage					
1	0	0	105	0.725	76
2	0.04	0	105	1.430	150
3	0.08	0	105	2.040	214
4	0.04	0.1	105	1.340	141
After 78-days storage					
1	0	0	105	0.722	76
2	0.04	0	105	1.390	146
3	0.08	0	105	1.900	200
4	0.04	0.1	105	1.350	142
After 168-days storage					
1	0	0	105	0.714	75
2	0.04	0	105	1.333	140
3	0.08	0	105	1.860	195
4	0.04	0.1	105	1.418	149

in cans stored for 28 days were calculated in the similar manner described in the case of short-necked clam:

$$\text{Sample 2} - \text{Sample 1} = 74 \mu\text{mol}$$

$$\text{Sample 3} - \text{Sample 1} = 138 \mu\text{mol}$$

$$\text{Sample 4} - \text{Sample 1} = 65 \mu\text{mol}$$

As the amounts of Ribotide added were 84, 168, 84 μmol in Samples 2, 3, and 4, respectively, the percentages of Ribotide remaining were figured to be 88, 82 and 77%, respectively.

The percentages of Ribotide remaining after 78 days of storage were 83, 74 and 79% in Samples 2, 3 and 4, respectively.

As mentioned above (Table 3), red crab meat does not contain inherent measurable amounts of 5'-IMP and 5'-GMP. Addition of Ribotide to the crab meat, however, seems to be readily acceptable judging from the results from sensory tests. Thus, the appropriate quantity of Ribotide, i.e., 0.04%, added simultaneously with MSG, seems reasonable from the viewpoint not only of fortified taste but also of over-all desirable flavor. Stability of Ribotide added to canned red crab is relatively high.

Canned whale meat (corned beef-style). Whale meat was packed in 99.1×120.9 mm cans in the similar manner to corned beef: whale meat trimming was mixed well with shortening, gelatin, egg white, fat and other ingredients and MSG and/or 5'-nucleotides of the amounts prescribed below:

No. 0, 0.5% MSG, no 5'-nucleotide

No. 1, 0.2% MSG, no 5'-nucleotide

No. 2, 0.2% MSG, 0.1% 5'-IMP

No. 3, 0.2% MSG, 0.1% 5'-GMP

No. 4, 0.2% MSG, 0.1% Ribotide

No. 5, 0.2% MSG, 0.2% Ribotide

The cans were stored at room temperature and opened about 1, 3 and 6 months after processing. The content was minced and mixed uniformly. The taste of the minced samples was evaluated by the paired-preference test and ranking test.

As seen in Table 12, the paired-preference test shows that more than 59% of tasters preferred Samples 2 and 3 to Sample 1 stored for half a year. The effect of 5'-nucleotides was detected at the 5% level of significance among samples stored for 29 or 97 days.

Table 12 Effect of 5'-nucleotide added on canned whale meat
(a paired-preference test).

Storage period (days)	No. of judgments preferring no nucleotide	No. of judgments preferring nucleotide	% of judgments preferring nucleotide	χ^2	Statistical significance
No Nucleotide (No. 1) vs. 0.1% 5'-IMP (No. 2)					
29	12	24	67	4.0	*
97	13	19	59	1.1	...
198	14	20	59	1.1	...
No Nucleotide (No. 1) vs. 0.1% 5'-GMP (No. 3)					
29	11	25	69	5.4	*
97	11	25	69	5.4	*
198	13	21	62	1.9	...

... Indicates no significance at 5% level.

* Significant at 5% level.

The ranking totals on four of the 6 samples after 31 days of storage were 35, 48, 38 and 29 for Samples 0, 1, 4, and 5, respectively. The only small difference between Samples 0 and 4 may be interpreted as showing that the effect of 0.5% of MSG is similar to 0.2% MSG plus 0.1% Ribotide.

In another series of sensory tests, the flavors of the minced meat diluted five times with water into soup were compared. By the paired-preference test, the effect of addition of 5'-IMP or 5'-GMP was recognized in higher level of significance on the diluted soup than on the minced meat. Table 13 shows the ranking totals given by the panel on the soup samples. It can be seen that a large number of tasters preferred Samples 0 and 5 to Sample 1 opened after 31, 100 and 200 days of storage. The effect of added Ribotide was obvious at the 1% level of significance.

The amount of 5'-nucleotide remaining after 30 days of storage is shown in Table 14. The remaining rates of 5'-nucleotides after 30, 97 and 200 days of storage were 67 to 72%, 60 to 72%, and 52 to 71%, respectively.

From the results obtained from the sensory tests for canned whale meats, it is clear that the flavor is enhanced with the addition of 0.1% of 5'-IMP, 5'-GMP or Ribotide.

Table 13 Ranking totals of canned whale meat.

Storage period (days)	No. of tasters	Ranking total of				Statistical significance
		Sample No. 0 (0.5% MSG, no Ribotide)	Sample No. 1 (0.2% MSG, no Ribotide)	Sample No. 4 (0.2% MSG, 0.1% Ribotide)	Sample No. 5 (0.2% MSG, 0.2% Ribotide)	
31	15	34	46	49	21	**
100	20	43	70	49	38	**
200	20	40	72	46	42	**

Whale meat was diluted five times into soup and tasted.

** Significant at 1% level.

Table 14 Retention of 5'-nucleotide in canned whale meat.

No.	Additives	5'-nucleotide $\mu\text{mol/g}$			Recovery (%)
		Analysed value	Recovered value	Added amount	
0	0.5% MSG no 5'-nucleotide	1.00			
1	0.2% MSG no 5'-nucleotide	0.98			
2	0.2% MSG 0.1% 5'-IMP	2.43	1.45	2.04	71
3	0.2% MSG 0.1% 5'-GMP	2.36	1.38	2.07	67
4	0.2% MSG 0.1% Ribotide	2.36	1.38	2.00	69
5	0.2% MSG 0.2% Ribotide	3.84	2.86	4.00	72

It has been shown that 5'-GMP is contained in only small amounts in whale meat. Nevertheless, the nucleotide was found to be effective in enhancing the taste the same as 5'-IMP which is contained abundantly in meat. Also, it was adjudged that the addition of 0.2% MSG and 0.2% Ribotide yields a better result as compared with the addition of 0.5% MSG alone.

Generally, the effect of 5'-nucleotide was felt more strongly in the soup than in the minced meat.

Stability of 5'-IMP, 5'-GMP or Ribotide was rather high in canned whale meat: about 70% of the nucleotide added was found to stand sterilization and storage.

要 旨

5'-イノシン酸 (5'-IMP), 5'-グァニル酸 (5'-GMP) よりなる所謂核酸系調味料がひろく食品加工に応用されている。缶詰野菜類に添加して効果のあることは既に前報 (本誌第8号167頁(1968))

に報告した。

本報では水産食品として、あさり、かき、ずわいがに、コンビーフ風鯨肉の缶詰に核酸系調味料を添加した場合の旨味増強効果と調味料の成分 5'-ヌクレオチドの安定性について報告する。製造後6カ月間室温に貯蔵した場合の缶詰食品の風味を2点撰択試験と順位法による官能検査で評価した。添加した5'-ヌクレオチドの安定性は5'-ヌクレオチダーゼを用いる酵素法によって測定した。

核酸系調味料は次の缶詰食品で顕著な効果を示した。即ちあさりに0.04あるいは0.08%のリボタイド(5'-IMPと5'-GMPの等量混合物)、ずわいがにに0.04あるいは0.08%のリボタイド、コンビーフ風鯨肉に0.1%の5'-IMP、0.1%の5'-GMPあるいは0.2%のリボタイドを添加した場合、明らかに旨味を増強した。

添加されたヌクレオチドは缶詰の加熱殺菌、その後の貯蔵期間を通じてかなり安定であり、ずわいがに缶詰では28日後に77~88%、あさり缶詰では189日後に52~61%の残存率を示した。

ABBREVIATIONS USED

AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; IMP, inosine monophosphate; GMP, guanosine monophosphate; CMP, cytidine monophosphate; UMP, uridine monophosphate.

REFERENCES

- Arai, K. 1966. Nucleotides in the muscles of marine invertebrates. *Bull. Japan. Soc. Sci. Fisheries.* 32, 174.
- Bergkvist, R. and Deutch, A. 1954. Ion exchange chromatography of nucleotides polyphosphates. *Acta. Chem. Scand.* 8, 1877.
- Fujita, T. and Hashimoto, Y. 1960. Inosinic acid content of foodstuffs III. Marine Products. *Bull. Japan. Soc. Sci. Fisheries.* 26, 907.
- Hashida, W. 1964. Chemical seasonings. *Japan Food Science* 3 (12), 33.
- Hashida, W., Mouri, T. and Shiga, I. 1966. Application of 5'-ribonucleotides to canned vegetables. *Food Technol.* 20 (7), 95.
- Jones, N.R. 1960. The separation and determination of free purines, pyrimidines and nucleoside in cod muscle. *The Analyst* 85, 111.
- Kobayashi, K. 1966. The acid-soluble nucleotides of fish muscle. *Bull. Japan. Soc. Sci. Fisheries.* 32, 166.
- Kuninaka, A., Kibi, M. and Sakaguchi, K. 1964. History and development of flavor nucleotides. *Food Technol.* 18 (3), 29.
- Nakajima, N., Ichikawa, K., Kamada, M. and Fujita, E. 1961a. Food chemical studies on 5'-ribonucleotides. I. On the 5'-nucleotides in foods. I. Determination of the 5'-ribonucleotides in various stocks by ion exchange chromatograph. *J. Agr. Chem. Soc. Japan.* 35, 797.
- Nakajima, N., Ichikawa, K., Kamada, M. and Fujita, E. 1961 b. Food chemical studies on 5'-

- ribonucleotides. II. On the 5'-ribonucleotides in Foods. (2) 5'-Ribonucleotides in fishes, shellfishes and meats. *J. Agr. Chem. Soc. Japan.* 35, 803.
- Nakajima, N., Ichikawa, K., Yoshimura, I., Kuriyama, C., Kamada, M. and Fujita, E. 1963. Food chemical studies on 5'-ribonucleotides. III. Enzymatic determination of 5'-ribonucleotides in food. *J. Agr. Chem. Soc. Japan.* 37, 558.
- Ohara, M. and Komata, Y. 1964. Chemical seasonings. *Japan Food Science* 3 (12), 15.
- Saito, T. 1960. Taste of fishes, fishery products and inosinic acid. *Kagaku (Chemistry), Japan* 15, 101.
- Saito, T. 1961. Adenosine triphosphate and the related compounds in the muscles of aquatic animals. *Bull. Japan. Soc. Sci. Fisheries.* 27, 461.
- Sawyer, F.M. 1966. Interaction of flavor modifying effects of monosodium glutamate and 5'-ribonucleotides in processed foods. Paper presented at meeting of American Chemical Soc., Sept. 15.
- Shimazono, H. 1964. Distribution of 5'-ribonucleotides in foods and their application to foods. *Food Technol.* 18 (3), 36.
- Shimazono, H. 1965. A new flavor enhancer, the 5'-ribonucleotides. *Food Manufacture* 40 (11), 53.
- Spinelli, J. and Kemp, B.J. 1966. Rapid measurement of inosine monophosphate and total adenosine nucleotides in fish tissue. *J. Agr. Food Chem.* 14, 176.
- Tomiyama, T., Kobayashi, K., Kitahara, K., Shirasaki, E. and Ohba, N. 1966. A study on the change in nucleotides and freshness of carp muscle during the chill storage. *Bull. Japan. Soc. Sci. Fisheries.* 32, 262.
- Ms. rec'd 8/23/67; revised 6/17/68; accepted 7/2/68.

Appreciation is expressed to Prof. Dr. S. Teramoto, Osaka University, and Mr. U. Inamoto, ex-president of Toyo Food Institute, for their encouragements in this work. The authors thank Hayashikane-Sangyo Co., Tottori Canning Co., Aohata Canning Co., and Teihoku Shokuryo Co. for the courtesy in experimental canning. This work was supported by Takeda Chemical Industries, Japan. The authors are grateful to Dr. H. Shimazono, Mr. Y. Sumita, Mr. S. Hori, Mr. H. Sugibayashi and others of Takeda Chemical Industries for their advice and interest. The authors acknowledge the technical assistance of Miss J. Terada and Miss K. Hata.