缶詰野菜に5′-Ribonucleotidesの利用

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APPLICATION OF 5′-RIBONUCLEOTIDES TO CANNED VEGETABLES

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SUMMARY

The minimal levels of 5′-ribonucleotides needed to improve the sensory qualities of canned vegetables were assessed by the organoleptic test, and their stability during heat processing and storage was assayed by the method that uses 5′-nucleotidase. Several vegetable items were experimentally packed in tin cans and processed according to commercial practices with 5′-ribonucleotides at the following levels : 0.08% for green peas, 0.025% for asparagus, 0.01% for mushrooms, and 0.02% for green beans, etc.

Canned samples stored for several months were submitted to taste test. Vegetables with added 5′-ribonucleotides tasted much better at a high level of significance, and the additives were retained fairly well even in canned samples stored six months at room temperature.

INTRODUCTION

Along with the theoretical elucidation of their properties (Kuninaka, 1960 ; Honjo *et al.*, 1963), numbers of the nucleic acid group such as 5′-inosinic acid and 5′-guanylic acid, each of which is one of the essential tastegiving substances originally present in foods, have been manufactured on a large scale as flavor enhancers. These substances have already been well accepted as seasoning agents for home use, but studies on the application of these agents in the food industry should have made further advancement. Shimazono (1964 a, b) recently reviewed the application of 5′-ribonucleotide to foods.

The distribution of 5′-ribonucleotides in vegetables and mushrooms was reported by Bergkvist (1958a, b), Nakajima *et al.* (1961), Fujita *et al.* (1961a), and Hashida *et al.* (1963, 1964a). Their distribution patterns in vegetables and mushrooms were different from those in meat. These foods contained appreciable amounts of 5′-AMP and 5′-UMP but not measurable amounts of 5′-IMP. Remarkable amounts of 5′-GMP were found in Shii-take (*Lentinus edodes*) and Shimezi (*Lyophyllum aggregatum*), but it was not found in mushrooms (material for canning, *Psalliota bisporus*). Glutamic acid has long been recognized as a potent flavor-enhancing substance. The synergistic effects of 5′-IMP and 5′-GMP with monosodium glutamate were recently reported by many authors (Kuninaka, 1960 ; Fujita *et al.*, 1961 b : etc.). It was thus assumed that when 5′-IMP and 5′-GMP were added to vegetables containing appropriate amounts of glutamate,

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their flavor enhancing activity would be greater than in glutamate-free product. Glutamate content should be taken into consideration before adding 5'-ribonucleotides.

We have been studying application of these substances to improve the taste of canned foods, particularly for enhancement of their taste, and this paper reports application of the agents to canned agricultural products. Experiments were carried out on eight kinds of water-boiled canned agricultural foods such as green peas, asparagus, mushrooms, and green beans, etc., which were produced in relatively large quantities.

MATERIALS AND METHODS

Sodium salt of 5'-ribonucleotide (Ribotide, registered trade name of Takeda Chemical Industries for this substance as a flavor enhancer : 1 : 1 mixture of 5'-IMP•Na_2 and 5'-GMP•Na_2) and the enzyme 5'-nucleotidase, used in assaying 5'-nucleotides, were supplied through the courtesy of Takeda Chemical Industries, Ltd., Osaka, Japan.

Agricultural canned foods, namely bamboo shoot, asparagus, mushrooms, carrots (baby food), and green beans were experimentally produced at the author's laboratory, and green peas, sweet corn, and tomato juice were obtained from markets. These canned foods were used as samples for chemical analysis after making them into a porridge form with an electric mixer. These samples were also used as the controls in sensory tests before Ribotide was added. For chemical analysis, both solids and liquids of each canned food were mixed together, except with canned mushrooms, whose liquid portion was replaced by water. Salt (NaCl), reducing sugar, total sugar, total nitrogen, amino nitrogen, and acidity were determined by conventional methods. Glutamic acid was determined by decarboxylation method employing the enzyme from *Escherichia coli*.

After preliminary estimation of the optimal addition level of Ribotide, the foods were canned experimentally. Ribotide was dissolved in the liquid at twice the optimal concentration for the total contents of each can, and the cans were sterilized by conventional method. The cans so produced were stored for 6 months and then opened for sensory test and also for determinatin of the Ribotide level remaining. Green peas were canned at the Hamaguchi factory of Sanshin Food Co., and asparagus at the Head Office factory of Shinkai Asparagus Co., and tomato juice at the Kurayoshi factory of Tottori Canning Co. The other foods were canned at the authors' laboratory.

Sensory test. The panel of tasters comprised about 40 persons at the college and laboratory who were involved in food science. A paired-preference test was carried out by questioning the tasters as to their preference for the Ribotide-free control or the sample to which Ribotide had been added at various concentrations, and the levels of significance of their judgments were assessed, based on the χ^2 method. A ranking test was also conducted on a few different addition levels of Ribotide so as to determine the optimal concentration of the addition of Ribotide. The tasters were asked to rank the several samples in order of preference.

Assay of 5'-ribonucleotide. The remaining Ribotide level in the solid and liquid of the canned foods was quantitatively determined from the phosphoric acid liberated by 5'-nucleotidase prepared from bull semen (Nakajima *et al.*, 1963).

RESULTS AND DISCUSSION

Chemical analysis of canned foods. Results of the chemical analysis are shown in Table 1. It appeared that the dominant taste components in these vegetables were: salt in green peas; salt, sugar, amino acid, and acidity in asparagus; salt, sugar in sweet corn; salt in mushrooms; sugar, acidity in carrots; salt in green beans; salt, sugar, and acidity in tomato juice. Significant amounts of glutamic acid were found in sweet corn, asparagus, and tomatoes. The percent glutamic acid in the vegetables was approximately the same as reported by Hac *et al.*(1949) and Ohara and Komata (1964).

Table 1 Components of canned foods.

	Bamboo shoots	Green peas	Asparagus	Sweet corn	Mushrooms	Carrots (baby food)	Green beans	Tomato juice
pH:	5.2	4.8	4.4	6.2	5.6	5.2	5.2	4.0
Moisture (%):	94.8	87.2	91.5	81.7	93.2	84.5	94.8	92.1
In soluble fraction								
Salt, %	0.01	1.23	0.62	0.52	0.51	0.09	0.92	0.66
Reducing sugar, %	0.14	0	2.21	0.41	trace	2.97	1.43	3.6
Total sugar, %	0.33	trace	2.27	11.5	trace	4.89	1.86	3.6
Total nitrogen, %	0.102	0.052	0.144	0.061	0.199	0.132	0.069	0.123
Amino nitrogen, %	0.030	0.006	0.050	0.026	0.015	0.037	0.031	0.030
Glutamic acid, %	0.008	0.004	0.055	0.10	0.022	0.035	0.018	0.14
Titratable acidity N/10-ml/100ml	2.4	3.4	15.9	6.2	2.0	11.8	8.9	59.8

Preliminary addition of Ribotide. As shown in Table 2, no significant preference was indicated between the Ribotide-free and the 0.006% Ribotide asparagus samples. However, 75% of the 59 tasters preferred the 0.05% Ribotide over the Ribotide-free sample. These evaluations, when assessed by the χ^2 method, gave a 1% significance level for the 0.0125% addition, and a 0.1% significance level of the 0.05% Ribotide.

Table 2 The effect of added Ribotide on asparagus.

Addition level of Ribotide (%)	No. of tasters who judged Ribotide-free sample to be better (A)	No. of tasters who judged Ribotide-added sample to be better (B)	% of judgments preferring Ribotide-added sample $\frac{B}{A+B}\times 100$	χ^2	Statistical significance
0.006	18	28	61	2.2	···
0.0125	27	54	67	9.0	**
0.025	26	49	65	7.1	**
0.05	15	44	75	14.3	***

···: not significant at 5% level
*: significant at 5% level
**: significant at 1% level
***: significant at 0.1% level

The three samples of asparagus containing 0.0125, 0.025%, and 0.05% Ribotide and the control were also ranked for preference by a panel of 20 tasters. All three samples containing Ribotide were preferred over the control with the lowest level, 0.0125% Ribotide, being preferred over the two higher levels. Thus it may be deduced therefrom that the optimal addition level of Ribotide to asparagus is 0.0125%, and that when the possible breakdown of Ribotide by sterilization is taken into consideration, twice the level (i.e. 0.025%) will be desirable.

Table 3 The effect of added Ribotide on mushrooms.

Addition level of Ribotide (%)	No. of tasters who judged Ribotide-free sample to be better (A)	No. of tasters who judged Ribotide-added sample to be better (B)	% of judgments preferring Ribotide-added sample $\frac{B}{A+B}\times 100$	χ^2	Statistical significance
0.001	17	33	66	5.1	*
0.0025	12	46	79	19.9	***
0.005	4	25	86	15.2	***
0.01	8	48	86	28.6	***

…: not significant at 5% level
*: significant at 5% level
**: significant at 1% level
***: significant at 0.1% level

As given in Table 3, the number of tasters preferring the mushroom samples containing Ribotide increased as the addition level was raised.

Sensory tests were performed on samples of other agricultural products first made into a porridge form and Ribotide then added at various levels. The results are summarized in Table 4.

Table 4 The minimal addition level of Ribotide at which its taste-improving effect can be appreciated at a 5% or 0.1% level of significance.

Kinds of foods	Minimal addition level which gives a 5% level of significance (concentration, %)	Minimal addition level which gives a 0.1% level of significance (concentration, %)
Bamboo shoots		0.005
Green peas		0.04
Asparagus	0.0125	0.05
Sweet corn		0.01
Mushrooms	0.001	0.0025
Carrots (baby food)	0.025	
Green beans	0.005	0.02
Tomato juice	0.005	

A level of 0.0063% was reported by Fujita *et al.* (1961b) to be the threshold value of Ribotide in an aqueous solution. As shown in Table 4, a taste-improving effect of Ribotide could be distinctly recognized when it was added to green peas, tomato juice,

and bamboo shoots at a level around its threshold value. However, such effect could be noted when Ribotide was added to mushrooms even at a level lower than the threshold value. In this case, however, it will be necessary to consider the synergistic taste-giving effect of the 5'-nucleotides and glutamic acid normally present in mushrooms at a fairly high level. Green beans and carrots, in contrast, needed Ribotide added at about 5-10 times the threshold level. The optimal addition level of Ribotide should therefore be determined with due rigard to the effects of the natural composition of each agricultural product.

canned foods with added Ribotide. *Canned green peas.* Green peas were canned in No. 4 cans (Japan standard, 74.1×113.0 mm) with each can containing 285 g of solid and 170 g of 1% NaCl solution and the following Ribotide levels :

No. 1, control, no Ribotide added

No. 2, Ribotide 0.08% of total content

No. 3, Ribotide and monosodium glutamate (MSG) atrespective concentraions of 0.04 and 0.1 %.

The cans were opened 10, 111, and 180 days after sterilization, and taste tests and determination of remaining Ribotide level were carried out.

As indicated in Table 5, a paired preference test on the solids of the samples showed that more tasters preferred samples 2 and 3 over sample 1. This resultwas supported by a ranking test.

Table 5 The effect of addition of Ribotide on canned green peas (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. 0.08% Ribotide					
10	6	17	74	5.2	*
111	18	34	65	4.9	*
189	8	35	81	8.6	**
No Ribotide vs. 0.04% Ribotide plus 0.1% MSG					
10	3	20	87	6.3	*
111	12	40	77	15.1	***
189	5	38	88	12.7	***

The fact that the 5'-nucleotide content of the solid was found to be about one-half of the liquid 10 days after addition, indicated insufficient penetration of Ribotide into the solid. After 189 days' storage at room temperature, almost the same levels of Ribotide were found in both the solid and the liquid, suggesting satisfactory penetration. Since green peas are high in starch content and sterilized under high temperature, the stability of Ribotide might be lower in green peas than in asparagus (*cf.* next item).

Canned asparagus. Asparagus was canned in No. 7 cans(special long, 65.3×138.9mm)

with each can containing 315 g of solid and 120g of 3% NaCl solution, and the following Ribotide levels :

No. 1, control, no Ribotide added

No. 2, Ribotide at 0.025% of total content

No. 3, Ribotide and MSG at respective concentrations of 0.0125 and 0.1%

The ranking total given by a panel of 20 tasters on the solids of these three samples is shown in Table 6. It can be seen that an overwhelmingly large number of the tasters preferred samples 2 and 3 to sample 1. No remarkable difference between samples 2 and 3 may be interpreted to indicate that the Ribotide level could be reduced by half when MSG is added at a level of 0.1%.

Table 6 Ranking test of canned asparagus.

Storage period (days)	Ranking total of sample No. 1 (no Ribotide added)	Ranking total of sample No. 2 (Ribotide 0.025%)	Ranking total of sample No. 3 (Ribotide 0.0125% and MSG 0.1%)	Statistical significance
3	56	36	28	**
101	57	29	34	**
184	57	30	33	**

Table 7 The remaining 5'-nucleotide level in canned asparagus.

		Total contents per can (g)	5'-nucleotide (μmol. per 100g)	5'-nucleotide (μmol. per can)	
After 3 days' storage					
No. 1 control, added no Ribotide	solid	300	18.7	56	88
	liquid	131	24.6	32	
No. 2 added 0.025% of Ribotide	solid	297	56.1	167	241
	liquid	131	57.0	74	
No. 3 added 0.0125% of Ribotide and 0.1% of MSG	solid	300	34.4	104	160
	liquid	131	41.9	56	
After 184 days' storage					
No. 1 control, added no Ribotide	solid	300	19.0	57	85
	liquid	131	21.8	28	
No. 2 added 0.025% of Ribotide	solid	305	.....	.....	
	liquid	126	45.3	57	
No. 3 added 0.0125% of Ribotide and 0.1% of MSG	solid	303	34.3	103	152
	liquid	127	38.8	49	

Table 7 shows the Ribotide level per can remaining, after 3 and 184 day's storage, determined as 5'-nucleotide by enzymatic method. No large difference in 5'-nucleotide content was found between the solids and the liquid of samples 2 and 3. These facts implied that Ribotide so added readily penetrated the solid from the liquid, spread uniformly, and remained at a desirable level in the solid, exerting a taste-improving effect.

Cans opened 3 days after sterilization showed a remarkable amount of 5'-nucleotides, even in cans without added Ribotide, as shown in the second column of Table 7. This

might be attributed to 5'-AMP and 5'-UMP inherently present in the material (Hashida *et al.*, 1964a).

The stability of added Ribotide was calculated with sample 2 and 3 after 3 days' storage;

The amount of 5'-ribonucleotide remaining was obtained by comparing the 5'-nucleotide content of sample 2 with that of sample 1, and also that of sample 3 with that of sample 1.

sample 2−sample 1 = 241 − 88 = 153 μmol

sample 3−sample 1 = 160 − 88 = 72 μmol

This difference corresponded to the amount of Ribotide added, i.e., 109 mg(218 μmol.) in sample 2, and 54 mg (108 μmol.) in sample 3 (0.5 mg of Ribotide used in this experiment is equivalent to 1 μmol. of 5'-nucleotide). The remaining level of Ribotide after 3 days' storage was:

153/218×100=70% in sample 2

72/108×100=67% in sample 3

Only a slight difference in 5'-nucleotide content was found between samples opened after 3 days and 184 days of storge. It was assumed that breakdown of Ribotide during storage at room temperature need not to be taken into consideration.

Canned mushrooms. To each of 3 lacquered No. 8 cans (65.3×52.7 mm) containing 85 g of blanched mushrooms and 60 g of brine (2.5% salt solution) were added no Ribotide (No. 1), 0.01% Ribotide (No. 2), and 0.005% Ribotide and 0.1% MSG (No. 3). The cans were opened 9, 57, and 190 days after sterilization for 18 min at 120°C, and a sensory test was carried out and remaining Ribotide determined.

In samples stored 57 or 190 days, solid enriched with Ribotide tasted better at a high level of significance than Ribotide-free solid, as shown in Table 8. But the effect of Ribotide was not clearly detected at a 5% level of significance in samples stored 9 days. The reason may be that the level of Ribotide was less in solid than in liquid, owing to difficulty of penetrating the solid.

The remaining Ribotide was determined as the 5'-nucleotide content of each can

Table 8 The effect of addition of Ribotide on canned mushrooms (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. 0.01% Ribotide					
9	19	31	62	2.9	.....
57	2	18	90	12.8	***
190	12	32	73	9.1	**
No Ribotide vs. 0.005% Ribotide plus 0.1% MSG					
9	19	30	61	2.5	.....
57	4	16	80	7.2	**
190	12	31	72	8.4	**

Table 9 5'-nucleotide content of canned mushrooms (58 days after canning).

		Total content per can (g)	5'-nucleotide (μmol./100g)	5'-nucleotide (μmol./can)
No. 1, control Ribotide unadded	solid	82	25.7	21.1 } 38.2
	liquid	63	27.2	17.1 }
No. 2, added 0.01% of Ribotide	solid	86	41.4	35.6 } 59.7
	liquid	59	40.8	24.1 }
No. 3, added 0.005% of Ribotide and 0.1% of MSG	solid	84	33.7	28.2 } 48.7
	liquid	60	34.2	20.5 }

(Table 9). In samples held 58 days, Ribotide levels were nearly equal in both the solids and the liquid, showing satisfactory penetration. 5'-Nucleotide was found even when Ribotide was not added, because of 5'-AMP and 5'-UMP originally present in the material. The amount of 5'-nucleotide remaining was calculated as described previously.

sample 2−sample 1=21.5 μmol.

sample 3−sample 1=10.5 μmol.

Such difference corresponded to the amount of Ribotide added, i.e., 14.5 mg (29.0 μmol.) in sample 2 and 7.25 mg (14.5 μmol.) in sample 3. The remaining level of Ribotide after 58 days' storage was:

21.5/29.0×100=74% in sample 2

10.5/14.5×100=73% in sample 3

Other agricultural products. Blanched green beans were packed in plain No. 7 cans (65.4×101.1 mm), each containing 100 g of solid, 120 g of 2.0% NaCl aqueous solution, and Ribotide at 0 or 0.02%. A larger number of tasters preferred beans with added Ribotide (1% level of significance) after 6 - 175 days' storage. The Ribotide level of 0.02% could be reduced by half when MSG was added at 0.1 % level. Even after 6 months of storage, 64-69% of initially added Ribotide was found.

In tomatoes (juice) and sweet corn, further investigation may be needed becasuse the range of optimal addition levels of Ribotide to them were very narrow. Excessive addition not only wasted Ribotide but harmed the flavor of sweet corn.

Some considerations on actual canning. The addition level of Ribotide should be decided after consideration of its synergistic effect with glutamic acid, which is naturally present in many foods.

Although the taste-improving effect of Ribotide is clear, its effect on the natural flavor of food must be considered.

Addition of Ribotide should be decided only after proper consideration is given to the specific use of each food, since there are a variety of uses of the so-called canned foods; for example, bamboo shoots are used as a material for cooking, asparagus is eaten without further cooking, and green peas are used by some principally for the "coloring" effect.

It may be considered that the decomposition of 5'-ribonucleotide occurred mainly during heat-sterilization (Hashida *et al.*, 1964b), with only a slight decrease in other canning processes.

要　　旨

5'-イノシン酸（5'-ＩＭＰ）や5'-グアニル酸（5'-ＧＭＰ）の呈味作用が明らかになって，これからなる調味料が食品添加物として，食品加工の面でも大いに使用されるようになった．

農産食品には元来5'-ＩＭＰは殆んど含まれていないが，本報では5'-ＩＭＰ，5'-ＧＭＰを添加した農産缶詰を試作し，その風味に対する効果をしらべると共に，添加したこれらヌクレオチド類の缶詰製造時および製造後貯蔵中の安定性を検討した．農産缶詰の大部分は有力缶詰製造工場の御厚意によって試作して頂いたものである．

5'-ヌクレオチドの分析は5'-ヌクレオチダーゼを用うる酵素法，缶詰食品の風味については当短大職員をパネルとする官能検査によった．農産缶詰すなわちグリンピース，アスパラガス，マッシュルーム，グリンビーンズにはそれぞれ0.08%，0.025%，0.01%，0.02%のリボタイド（商品名，5'-ＩＭＰと5'-ＧＭＰの等量混合物）が添加された．

缶詰を1，3，6カ月間室温で貯蔵し，開缶して旨味の増強効果を2点選択法あるいは順位法でしらべたが，前記の缶詰では5%またはより高度の有意差で旨味が強くなることがみとめられた．缶詰に添加した5'-ヌクレオチドは比較的安定で，マッシュルームで70%以上，アスパラガスで62～70%，グリンビーンズで64～70%の残存率がみとめられた．

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