Effects of Polyphosphates on Extractability of Agar in the Cooking Process of Seaweeds—I. Relationship between Variety of Polyphosphates and Extractability of Agar

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Polyphosphates such as sodium or potassium pyrophosphate, tripolyphosphate, hexametaphosphate and metaphosphate were effective in increasing agar extractability when they were added in the cooking process of *Gelidium pacificum* OKAMURA and alkali-treated *Gracilaria verrucosa* (HUDSON) PAPENFUSS, at 1×10^{-3} M concentration levels. There was a tendency for the effect of polyphosphate on agar extraction to become greater with increasing degree of condensation of phosphate. The results were especially remarkable with alkali-treated *Gracilaria*. Monobasic salts of orthophosphate had some effect on extractability of agar from *Gracilaria*, but tribasic orthophosphates were ineffective with both *Gelidium* and *Gracilaria*.

Application of polyphosphates in the manufacture of agar has been recently developed by the author to obtain a whitening effect on the finished product.¹⁾ In an industrial application, it was observed at a few factories that there is a softening effect on the raw material caused by the addition of polyphosphates. On the other hand, a similar softening effect on beans and peas has been known and has been applied in food industries.²⁾ With respect to the softening effects on such plant structures, it is expected that agar extractability may be improved by adding polyphosphates to cooking water containing seaweeds.³⁾ It has also been noticed that the whitening effect on agar, or the chelating action on ionic iron, of the commercial polyphosphates is very different among the finished products depending on their chemical composition, commercial lots, brands and factories.⁴⁾

With this background, the present investigation has been carried out in order to obtain information about the relationship between variety of polyphosphates and their effect on agar extraction in the cooking process of two typical agar seaweeds.

Experimental

Phosphates Ten kinds of phosphates (Table 1) were used for the experiments. (With the help of general information on the Japanese industrial polyphosphates,²⁾ the condensation degree of sodium metaphosphate of No. 7, was estimated to be three.)

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Table 1. Phosphates used for the experiments.

No.	Chemical formula	pH*
1	$\text{Na}_3\text{PO}_4 \cdot 12 \text{H}_2\text{O}$	10.14
2	$\text{Na}_2\text{HPO}_4 \cdot 12 \text{H}_2\text{O}$	8.22
3	$\text{NaH}_2\text{PO}_4 \cdot 2 \text{H}_2\text{O}$	5.38
4	$\text{Na}_4\text{P}_2\text{O}_7 \cdot 10 \text{H}_2\text{O}$	9.30
5	$\text{Na}_5\text{P}_3\text{O}_{10} \cdot 6 \text{H}_2\text{O}$	9.20
6	$(\text{NaPO}_3)_6$	6.48
7	$(\text{NaPO}_3)_n^{**}$	7.31
8	K_3PO_4	9.62
9	KH_2PO_4	5.45
10	$\text{K}_4\text{P}_2\text{O}_7 \cdot 3 \text{H}_2\text{O}$	9.32

* pH of 1×10^{-3} M aqueous solution.

** See the text.

Agar seaweeds *Gelidium pacificum* OKAMURA, Ōbusa in Japanese, was harvested at Maehama beach, Ōse, Shizuoka State in July, 1965. The crude dry seaweeds in commercial style were washed by kneading in water to remove foreign matter and air dried at the laboratory yard. The moisture content of the refined dry seaweeds was 16.6%. (The yield of refined dry seaweeds from crude dry seaweeds was about 86%.)

Gracilaria verrucosa (HUDSON) PAPENFUSS, Ogonori in Japanese, which was harvested at Akkeshi Bay, Hokkaido State in 1966, was treated with alkali, and then refined. The unrefined seaweeds in commercial style were soaked in an alkaline solution which was composed of 2% of NaOH and 0.05% of CaCl_2 , and held at 95°C for 4 hr.⁵⁾ The weight ratio of unrefined seaweeds to alkaline solution was 1:15. The moisture of the refined dry seaweeds after alkali treatment was 16.4%. (The yield of refined dry seaweeds after alkali treatment from unrefined seaweeds in commercial style was roughly 40%.)

Cooking of seaweeds and processing of agar The seaweeds were cooked twice for agar extraction, and the first and the second extracts were processed for agar individually.

In the first cooking process, 10 g of refined seaweeds was soaked in acidic water for one hr. The concentration and the total volumes of aqueous solutions of sulfuric acid were selected after the repeating preliminary tests as follows; 300 ml of 0.01% H_2SO_4 for *Gelidium* and 400 ml of 0.025% H_2SO_4 for *Gracilaria*. Then, the individual phosphates were added to them at 1×10^{-3} M concentration levels. The seaweeds with acidic water and phosphates were cooked in one liter flasks under reflux condensers for 2.5 hr. Filtration was performed through gauze under reduced pressure, with the utmost care in order to get a constant volume of filtrate in the same series.

In the second cooking process, the residual seaweeds of the first extraction were cooked for 5 min for *Gelidium* and 2 hr. for *Gracilaria*, respectively. The volumes of cooking water including seaweeds were kept identical to those of the first one. Sulfuric

acid and phosphates were not used for *Gelidium* because of easy cooking result in the first extraction, but were added to *Gracilaria* in the same concentration level as the first one.

Agar in air dry condition was produced through the succeeding process of solidification of the extracts at room temperature, freezing at -20°C , thawing in water, preliminary dehydration by manual pressing, and natural drying helped by the sun and breeze.

Analysis and measurements of agar and agar gel Measurements of pH of hot sol, melting point of gel, gel strength and gel strength value by calculation, and analysis of moisture content of dry agar and agar concentration of gel extract were performed by the methods described by MATSUHASHI⁶⁻⁸⁾.

For the measurement of melting point, 5 ml of agar solution was gelatinized in a glass test tube of 10 mm inside diameter and 100 mm length. After holding at 20°C overnight, glass tubes were overturned and placed in the intermediate depth of a 4 liter water bath, which was heated up to the boiling point at a rate of $0.5-1.0^{\circ}\text{C}$ rise per minute. The temperature at which the gel in glass tube suddenly dropped was recorded as the melting point of gel at that concentration level.⁹⁾

Gel strength which is the maximum cracking load (g/cm^2), after 20 sec duration, on the top surface of the testing gel in a 200 ml mold of about 38 mm depth, was measured by use of the Japanese agar gel strength tester (Kiya-seisakusho Co., Tokyo) which is equipped with a cylindrical plunger having a flat bottom area of 1 cm^2 . In the commercial evaluation of agar quality, gel strength of 1.5% agar gel which is defined by the Japan Agricultural Standard has been widely used. In the present experimental work, however, apparent gel strength at 0.80% concentration level was measured for *Gelidium* agar, and apparent gel strength of *Gracilaria* agar was converted from the measured value of the raw gel to the value at 0.60% concentration level. Because, the linear relationship between apparent gel strength at any concentration level and agar concentration has been confirmed for many kinds of agar of various sources^{7,8)}.

Measurement of pH of hot sol was performed by use of pH test papers (Toyo-roshi Co., Tokyo).

For the comparative investigation of extractability of agar, total yield of agar (in absolutely dried condition) from the first and the second extracts was mostly used, which was determined in the following manner.

Generally, duplicate 5-10 g pieces of gel extract were processed to agar in absolutely dried conditions by the same freezing, thawing and drying conditions. The yield of agar was obtained as the multiplication product of the agar concentration (in average of two determinations) and the whole weight of the gelatinized extract. For a few samples, when the concentrations of extracts were too low to keep firm gels, moisture contents of agar processed from the whole amounts of extract gels were determined to obtain the similar values of yield of agar. In these drying procedures, a Quick moisture analyzer

Table 2. Effects of polyphosphates on extractability of agar in the cooking process of *Gelidium pacificum* OKUMURA.

No.	Phosphate	pH of 1st extract sol	Yield of absolutely dried agar			Absolutely dried residual seaweed g	Rate of cooking of residual seaweed %
			1st extract g	2nd extract g	Total g		
0	(Control)	5.8	2.810	0.698	3.508	3.836	(100)
1	Tribasic sodium orthophosphate	6.0	2.047	1.072	3.119	4.552	(119)
2	Dibasic sodium orthophosphate	5.7	2.476	0.938	3.414	4.074	(106)
3	Monobasic sodium orthophosphate	5.7	2.838	0.641	3.479	3.874	(101)
4	Sodium pyrophosphate	5.8	2.894	0.724	3.618	3.769	(98)
5	Sodium tripolyphosphate	5.7	2.713	0.910	3.623	3.867	(101)
6	Sodium hexametaphosphate	5.5	3.100	0.511	3.611	3.483	(91)
7	Sodium metaphosphate	5.6	3.079	0.488	3.567	3.514	(92)
8	Tribasic potassium orthophosphate	6.1	2.400	0.886	3.286	4.179	(109)
9	Monobasic potassium orthophosphate	5.6	2.981	0.494	3.475	3.691	(96)
10	Potassium pyrophosphate	5.6	2.785	0.969	3.754	3.778	(98)

Table 3. Effects of polyphosphates on extractability of agar in the cooking process of alkali-treated *Gracilaria verrucosa* (HUDSON) PAPENFUSS.
*cf. (See Fig. 1)

No.	Phosphate	pH of extract sol		Yield of absolutely dried agar				Rate of cooking of residual seaweed %
		1st	2nd	1st extract g	2nd extract g	Total g	Total %	
0	(Control)	6.1	6.3	1.36	1.04	2.40	(100)	30
1	Tribasic sodium orthophosphate	6.4	...	1.61				...
2	Dibasic sodium orthophosphate	6.3	...	1.83				...
3	Monobasic sodium orthophosphate	6.1	6.1	2.34	2.60	4.94	(206)	80
4	Sodium pyrophosphate	6.4	6.5	1.86	2.32	4.18	(174)	60
5	Sodium tripolyphosphate	6.4	6.5	2.89	2.65	5.54	(231)	95
6	Sodium hexametaphosphate	≤5.8	6.4	4.68	0.95	5.63	(235)	100
7	Sodium metaphosphate	≤5.8	6.4	3.38	1.02	4.40	(183)	50
8	Tribasic potassium orthophosphate	6.3	6.4	1.43	0.92	2.35	(98)	20
9	Monobasic potassium orthophosphate	6.1	6.1	2.15	2.58	4.73	(197)	80
10	Potassium pyrophosphate	6.3	6.5	1.82	2.63	4.45	(185)	70

equipped with infrared lamps (Kett, F-3A; Sanwa-denki Co., Tokyo) was used.

In order to justify the comparison of extractability of agar, the dry weight of *Gelidium* seaweed residue was determined as supplemental data. With *Gracilaria*, the organoleptical rate of cooking of residual seaweed, the standards of which were 100% for the most well cooked seaweeds and 0% for the uncooked ones, was determined. Because, it is usual that well cooked *Gracilaria* does not keep its original form but becomes so digested that a complete collection of such residue is a difficult task.

Results and Discussion

Effects of added phosphates on extractability of agar Tables 2 and 3 show the effects of orthophosphates and polyphosphates on extractability of agar when the additives are used in the cooking process.

In the case of *Gelidium* (Table 2), variations of the yield of agar among 11 test samples were relatively small. However, by the yield of the first extract and the dry weight of the seaweed residue, the action of the phosphates became clearer. Sodium and potassium tribasic orthophosphates (No. 1, 8) delayed dissolution of the agar and required a longer cooking time. On the other hand, the extractability of agar by the monobasic orthophosphates (No. 3, 9), which are the most acidic orthophosphates, were the same as the control. Therefore, it is concluded that uncondensed orthophosphates are not useful in promoting agar extraction of *Gelidium*, even if the acidity is held in the moderate pH range. On the contrary, sodium or potassium pyrophosphate, tripolyphosphate, hexametaphosphate and metaphosphate promoted agar extraction. Among them, softening of seaweed occurred in the shortest cooking period when treated with sodium hexametaphosphate (No. 6), which seemed to be the most useful. The observation was justified by the highest yield of the first extract and the lowest weight of seaweed residue.

In the case of alkali-treated *Gracilaria*, the relationship between variety of phosphate and effects on agar extractability was similar to that of *Gelidium*, and the results were more striking. Sodium and potassium monobasic orthophosphates doubled the yield of agar over the control, and their effects on agar extractability were a little better than those of pyrophosphates. The low pH values of the monobasic acids (for No. 3 and 9) would be one good reason for the superiority of the pyrophosphates (No. 4 and 10). Because, among the samples treated with three kinds of orthophosphates (No. 1, 2 and 3), the yield of the first extract agar was remarkably increased with the increasing acidity of the extract sol.

As for the polyphosphates, all of them showed positive effects on agar extractability, that were even more dramatic than with *Gelidium*. Among them, sodium hexametaphosphate (No. 6) was the most effective. In the first extraction process with hexametapho-

sphate, 3.5 times more agar was obtained than in control, and the seaweeds were softened fairly well, resulting in digestion during the second extraction process to form muddy residues. Metaphosphate (No. 7) was second most effective both in softening seaweeds and in agar extractability in the first cooking.

In the Table 3, the pH values of the first extract sol treated with sodium-orthophosphate (No. 1), -pyrophosphate (No. 4) and -tripolyphosphate (No. 5) were the same, however, the rank order of the yield of the first extract agar was as follows; No. 5 > No. 4 > No. 1. It appears that condensation degree of phosphate closely relates with effectiveness of agar extraction.

Fig. 1 shows the wet and dry residues of cooked *Gracilaria* after the second extraction process. The controls (No. 0-1 and 0-2) and the sample treated with potassium orthophosphate (No. 8) kept the original seaweed structure and were very bulky, however, the sample treated with hexametaphosphate (No. 6) was digested to a muddy residue, and reduced in weight.

Thus, it is possible that the high condensation degrees of hexametaphosphate and metaphosphate, or the complexity of their chemical structures which have molecular sizes not smaller than tripolyphosphate, and their strong acidity may have caused the highest extractability in the first extraction processes both of *Gracilaria* agar and *Gelidium* agar. In the total yield of *Gracilaria* agar, tripolyphosphate was more effective than metaphosphate, which was the reverse of the rank order of extractability in the first extraction process. These things are not conflict with the results for *Gelidium* agar.

The alkali-treated *Gracilaria* which was harvested at the same location in the same year had exhibited a characteristic of difficulty in cooking. For this reason, the common cooking method with sulfuric acid brought a very poor yield of agar even in a prolonged cooking period of 4.5 hr at atomospheric pressure. Therefore, the actions of these polyphosphates were quite significant. The different effectiveness of any polyphosphates for agar extraction from *Gracilaria* or from *Gelidium* may be attributed to the difference of their plant structures.¹¹⁾

As a conclusion, use of polyphosphates with both *Gelidium* and *Gracilaria*, increases the extractability of agar from these seaweeds, and such action is generally greater with increased degree of condensation of phosphate.

Effects of added phosphates on physical properties of agar gel Table 4 shows the physical properties of gels prepared with the processed agar. Generally, it was observed that polyphosphates had little effect on the physical properties of gels of *Gelidium* agar. However, they reduced melting points of gels of *Gracilaria*, and the results were most remarkable when hexametaphosphate and metaphosphate were used. The relatively low values of gel strength of the control sample and No. 1 and 2, which were treated with orthophosphates, are attributed to the incomplete extraction of agar.

Fig. 1. Wet (A) and dry (B) residues of cooked *Gracilaria*. The numbers in the pictures correspond to those in Table 3.

Table 4. Physical properties of gels (sols) processed from the first extracts.

No.*	0.80% <i>Gelidium</i> agar gel			0.60% <i>Gracilaria</i> agar gel		
	pH	m.p.(°C)	GS	pH	m.p.(°C)	GS**
0	7.2	96.1	425	6.7	94.8	280
1	7.7	95.0	400	7.0	95.1	310
2	7.5	96.4	470	7.0	92.7	340
3	7.3	95.7	450	6.6	90.4	360
4	7.5	95.8	460	6.6	≥91.9	340
5	7.8	97.0	490	6.8	≥91.1	400
6	7.5	96.4	460	6.6	≥87.1	350
7	7.3	94.9	400	6.7	≥87.8	390
8	7.6	95.3	370	7.2	94.7	290
9	7.2	96.3	490	6.7	90.3	340
10	7.3	93.2	420	6.8	≥94.2	330

* No. is corresponding to each of Table 1, 2 and 3.

** Calculated gel strength (GS) values, which were based on apparent gel strength values of raw gels.

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