THE FORMATION OF MAGNESIUM-AMMONIUM-PHOSPHATE CRYSTALS IN CANNED SEA FOODS

VI. Influence of the contained substances upon the growth of the formed $MgNH_4PO_4 \cdot 6H_2O$ crystals in test tubes

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It was stated in the previous paper[1] that the growth of the struvite in the canned sea foods will be influenced by the various substances contained therein. If the juice of the canned food is an approximate true solution such as pickle in the canned crab, numerous crystals will be rapidly formed in the can and will grow to larger ones. But, if juice in the canned food is a colloidal solution such as the juice in canned saury in which the skin of the fish is changed to gelatine by the processing, fewer crystals having smaller shape will grow in proportion to the viscosity of the juice.

The authors have studied how the crystallizing and the growth of the struvite in test tubes will be influenced by the contained substances.

1. Influence of the addition of gelatine

1) Experimental method

Two-one hundredth Mol magnesia mixture and 0.02 Mol sodium phosphate solutions were prepared as well as in the previous paper[2]. After 7.5 *cc* of sodium phosphate solution (0.02 Mol) was poured into each test tube, various amounts of gelatine were added and heated to dissolve in a water bath kept at 60 °C. Seven and five-tenths cc of magnesia mixture solution of 0.02 Mol were added to the above stated sodium phosphate solution, and thus solutions containing gelatine in the ratio of 5, 3.3, 1.7, 1.0 and 0.67% respectively were prepared. Those solutions were heated at 100 °C for 20 min. The solutions were cooled to room temperature. The formed crystals were observed under a microscope or by naked eye.

2) Experimental results

Results obtained are shown in Table 1.

As seen in Table 1, the larger the amount of gelatine contained in the solution, the more remarkably are influenced the velocity of crystallization and the velocity of growth of the crystals. In the mixed solution of the chemical constituents which was heated and stored for 50 minutes, when the solution contains 5~1.7% of gelatine, it becomes gel, and when the solution contains 1% of gelatine, many crystals were already formed. The size of the formed crystals in the solution containing gelatine is generally smaller than that of those formed in the solution without gelatine. When if gelatine was plentifully added to the mixture, after 50 days the crystals having no regular faces grew to

Table 1. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of gelatine

Leaving time \ Concentration of gelatine (%)		5	3.3	1.7	1.0	0.67
50 mins.	form	E	E	E	E	E
	size (μ)	—	—	—	—	—
14 days	form	—	—	C	C	C
	size (μ)	—	—	800×300	1000×300	200×60
50 days	form	C E	C E	D E	D	C
	size (μ)	51×5	25×20	380×1700	123～510 × 410～1800	31×130～300

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crystal, D=columnar crystal separated, E=amorphous

become pretty large crystals. But, there were a few that were visible, and almost all of them were microscopic. This fact agreed with the earlier finding[1] that the canned fish meat containing fish skin such as mackerel, salmon and saury have microscopic crystals. After storing of the solution at 37 °C for 10～15 days, the visible crystals were formed in the solutions containing from 1.0 to 0.67% of the gelatine. At concentrations of the gelatine above that upper mentioned, smaller crystals having irregular shape appeared. Accordingly, it may be said that the smaller the amount of gelatine the solution contains, the more regular are the shapes of the crystals which are formed. However, when those crystals formed from the solution containing gelatine are compared with the crystals formed from the solution containing no gelatine, the shape of the former is seen to be more irregular than the latter. A few crystals formed from the latter are separated; many of them gathered to make large crystals.

2. Influence of the addition of glycerol

Glycerol is colorless and odorless, and it is miscible with water in any proportions. The authors have tried to employ glycerol to prepare solutions having high viscosity like colloidal solution and to estimate the influence upon the shape and size of the formed crystal in the solutions to which it was added.

1) *Experimental method*

Ten *cc* portions of 0.02 Mol sodium phosphate solution to which 5 and 1 *cc* of glycerol respectively were added, are poured into separate Petri-dishes and then 10 *cc* of 0.02 Mol magnesia mixture solution was added. Those solutions are stored in a thermostat kept at 37 °C, and the shape and size of the crystals formed were observed at definite time intervals.

2) *Experimental results*

Table 2 shows the results.

Table 2. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of glycerol

Leaving time (hrs.) \ Concentration of glycerol (%)		20	4.8	0
0	form	D	D	C
	size (μ)	76×17	87×19	48×6.4
20	form	D	D	D
	size (μ)	305×77	120×53	96×34
50	form	D	D	D
	size (μ)	312×116	308×98	270×104
	number (per *cc*)	5000	367	112

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crysral, D=columnar crystal separated

As seen in Table 2, immediately after the solutions are mixed, the size of the formed crystals is not remarkably influenced by the solution having various concentrations of the glycerol.

However, after being stored for a long time, the influence of the concentration of glycerol upon the size and the shape of the crystal appeared remarkably. That is to say, a few crystals of large size having regular surface were generated in the higher concentration of the solution. On the other hand, comparatively more crystals of small size having irregular surface appeared in the more dilute solution of glycerol. Those results may be explained by stating that when two solutions containing no glycerol are mixed, the components of the two solutions react immediately and form the crystals.

However, when the solutions containing glycerol are mixed, the reaction between the components in the two solutions will not occur completely, but only partially, and ionic components which have not reacted yet in the solution are gathered to react around the nucleus of the crystal formed at first; resultant larger crystals in smaller number are formed. In this case, the stabilization of the supersaturated solution in the colloidal solutions will also be sufficiently considered in the case of the formation of nuclear crystals.

3. Influence of the addition of agar

The authors have observed the formation of the crystal when agar was added to the mixed solutions of various concentrations.

1) Experimental method

Each 10 cc portions of sodium phosphate solution of 0.02 Mol were poured separately into test tubes. to which 0.3, 0.2, 0.1, 0.04 and 0.02 *g* of agar were respectively added. The test tubes were heated at 70°C.

Ten *cc* portions of magnesia mixture were respectively mixed into the above described test tubes. In those test tubes, agar is contained respectively to the amount of 1.5, 1.0, 0.5, 0.2 and 0.1%. Those test tubes were plugged and stored at 37 °C. The changes in shape and size of the crystals formed in those test tubes were observed at definite intervals of time.

2) *Experimental results*

Results obtained are shown in Table 3.

Table 3. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of agar

Leaving time (days) \ Concentration of agar (%)		1.5	1.0	0.5	0.2	0.1	0
2	form	C	C	C E	C E	C E	C
	size (μ)	467×88	168×34	71×30	71×37	26×19	88×38
4	form	C	C	C	C E	C E	C
	size (μ)	545×152	240×88	220×76	175×82	158×76	218×79
13	form	C	C	C	C E	C E	C
	size (μ)	1200×150	460×88	370×100	220×79	214×75	260×79

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crystal, D=columnar crystal separated, E=amorphous

As seen in Table 3, the condition of growth of crystals formed, the number and the shape of the crystals showed some differences with the concentration of agar added. The higher the concentration of agar was, the less in number and the larger in size the crystals formed. The surface of the crystals was somewhat coarse. Those phenomena will be also explained by the statement made in the previous article of this paper.

4. Influence of the addition of edible oil

As stated above, the formation of the crystals, $MgNH_4PO_4 \cdot 6H_2O$, is considered to occur easily in the so-called true solution which has small quantity of various mixed matters in the juice of the canned foods. However, even in the canned salmon of which the juice has a somewhat larger amount of oil, the formation of the crystals has been observed. Here, the authors have examined the influence upon the formation of the crystals resulting from the addition of edible oil.

1) *Experimental method*

Oil in the canned salmon (silver salmon) was isolated from the juice in a separating funnel. This oil was used as edible oil.

Each 10 *cc* portions of sodium phospahte solution of 0.02 Mol was poured into test tubes and 1, 0.5 and 0.25 *cc* of the salmon oil were respectively added to those test tubes. They were heated at 70 °C. Ten *cc* of 0.02 Mol magnesia mixture were added respectively to those test tubes. Those mixed solutions have respectively contained 5, 2.5, 1.3% of oil. Those solutions were heated at 37 °C. The number, the size and the shape of the crystals formed were observed.

2) *Experimental results*

Results obtained are shown in Table 4.

Table 4. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of oil

Leaving time (days) \ Concentration of oil (%)		5	2.5	1.3
2	size and form (μ)	31 × 19 (C) 3 × 1 (E)	49 × 14 (C) 31 × 19 (E)	360 × 27 (C) 38 × 25 (E)
	appearance	white and muddy	white and muddy	white and muddy
6	size and form (μ)	3000 × 500 (D) 3 × 1 (E)	124 × 26 (C) 31 × 19 (E)	130 × 38 (C) 38 × 25 (E)
	appearance	few crystals were sticked to the wall of the vessel	formed crystals were gathered at the bottom of the vessel	crystals were gathered as the form of branches
13	size and form (μ)	3500 × 1000 (D) 3 × 1 (E)	149 × 27 (C) 31 × 19 (E)	150 × 38 (C) 38 × 25 (E)
	appearance	few crystals were sticked to the wall of the vessel	formed cryatals were gathered	crystals were gathered as the form of branches

Note: Signs which are described in this table show the states of the formed crystals as follows.
C=columnar crystal, D=columnar crystal separated, E=amorphous

As seen in Table 4, in the solutions containing larger amount of oil, the formation of the crystals was slow and the crystals were smaller in size and fewer in number. However, the crystals formed grew gradually to larger shape attaching to the side wall or sinking to the bottom of the test tube.

On the contrary, in the solutions containing smaller amounts of oil, the formation of the crystals was rapid and the crystals were small, but numerous. The crystals formed grew more slowly than in the above mentioned case. The size of the crystal was small.

5. Influence of the addition of materials which decomposed substances from protein

In order to ascertain the influence of the viscosity of the solution upon the formation of the crystals of $MgNH_4PO_4 \cdot 6H_2O$, the authors have added gelatine, glycerol, agar and edible oil to the mixing solution described in the previous article in this paper. Here, the authors have examined the influence of the addition of material which was decomposed substances from protein upon the formation of the crystal, $MgNH_4PO_4 \cdot 6H_2O$. As the decomposed substances from protein, pepton* of various concentrations was used.

1) *Experimental method*

Each 10 *cc* portions of sodium phosphate solution of 0.02 Mol were poured into test tubes to which 1, 0.2, 0.1, 0.04 and 0.02 *g* of pepton were respectively added. Those test tubes were heated at 70 °C and 1.0, 0.2, 0.1, 0.04 and 0.02 *g* of pepton was respectively dissolved in those test tubes. Then, each 10 *cc* of magnesia mixture of 0.02 Mol was mixed to each test tube. The concentrations of pepton in those test tubes were 5, 1, 0.5, 0.2, and 0.1%. The test tubes were plugged and stored at 37 °C. The changes in the size and shape of the crystals formed were observed.

2) *Experimental results*

Results obtained are shown in Table 5.

Table 5. The crystallizing states of $MgNH_4PO_4 \cdot 6H_2O$ by addition of pepton

Leaving time (days)	Concentration of pepton (%)	5.0	1.0	0.5	0.2	0.1
0	size and form (μ)	12× 2(E)	26×12(E)	19×15(C) 4× 3	19×20(C)	19×12(E)
	appearance	white and muddy	white and muddy	white and muddy	white and muddy	white and muddy
2	size and form (μ)	23×38(C) 54×38(E)	11×4.5(C) 50×26 (E)	85×53(C) 38×26(E)	63×67(C)	76×59(C)
6	size and form (μ)	58×38(C) 35×29(E)	30× 15(C) 180×100(E)	190×150(C) —— (D)	260× 79(D) —— (C)	180× 64(C)
	appearance	formed crystals were gathered at the bottom of the vessel	formed crystals were gathered at the bottom of the vessel	few crystals were sticked to the wall of the vessel	few crystals were sticked to the wall of the vessel	few crystals were sticked to the wall of the vessel
13	size and form (μ)	170×100(C) ——	31×19(C) ——	180×110(C) ——	270×70(D) ——	220×93(C)
	appearance	no change	no change	no change	no change	no change

Note : Signs in ( ) are the same as the other tables.

As seen in Table 5, the differences in the size of the crystals formed were observed according to the concentrations of the solution of pepton. In above 1% of the concentration of pepton, the more irregular and smaller crystals generated. The crystals have had no regular surfaces at initial period, but after two days they were observed to become to have crystalline surfaces. In below 0.5% of the concentration of pepton, after the

* Pepton used in this experiment was that customarily employed in bacteriological study, Terauchi pepton.

mixing of solutions, numerous minute crystals having regular surfaces generated immediately.

After the crystallization, the crystals obtained from the solution of the concentration below 0.5 % were observed being attached numerously to the side wall of the test tube. However, the crystals obtained from the solution of the concentration of pepton above 0.5 % showed the powder-like precipitates (this is a mass of gathered crystals which are recognized as microscopical crystals of 10 ~ 20 μ).

In the pepton solution, some gathered crystals were recognized in the course of the growth of crystal after the crystallization, differing from what occurred in the solutions of agar and gelatine. Those states of the crystals were observed in the pepton solution in the various concentrations. Those states were observed earlier in the higher concentration of pepton than in the lower concentration of pepton.

Discussion

From the relation between the viscosities of the juice of marine canned foods and the formation of the crystals of $MgNH_4PO_4 \cdot 6H_2O$, the authors have ascertained that in comparatively larger viscosity of the juice, crystals of smaller size (microscopical size) were found, and in smaller viscosity the crystals of the larger size were found. However, the relation between the juice in the canned food and the formation of the crystals can not be explained by the viscosity of the juice only. There are many factors influencing the formation of the crystals, *e. g.*, the difference of the amount of magnesium, phosphate and ammonium in the juice, the quantities of the juice, the components of the juice (decomposing products of fish meat protein), movement of the juice during the heating and cooling of canned food.

The fact that the size of the crystals is small in canned mackerel, saury and salmon having large viscosity of the juice of the content is considered to be due to large quantity of the juice and to the convection which occurs at the cooling of the canned foods. Thus the stirring of the juice at the time of convection makes the size of the crystals small. This fact agreed with the fact that when a mixture of magnesium, ammonium and phosphate is cooled by stirring, the size of the crystals becomes small as described in the previous paper.[3)]

Summary

Gelatine, glycerol, agar, edible oil and pepton were respectively added in order to ascertain the influence of the viscosity of mixed solution upon the formation of crystals. According to the results obtained, if the viscosity of the solution is large, the crystals will generate slowly, while in a solution of low viscosity, the crystals will generate rapidly. However, the growth of the crystals after their first formation is rapid in the high viscosity solution. After long storing period, there appear few, but large crystals. In the

low viscosity solution, the velocity of the growth is slow; the crystals remain of small size.

These results are the same as the results which ensue upon the addition of edible oil. Apart from the substances such as gelatine glycerol and agar, having colloidal protective characteristics if pepton having lower molecular weight than the gelatine is contained to an amount over 0.5%, the crystals formed are apparently floury, but are actually a gathering of small thick columnar crystals as revealed under microscope. If the pepton is contained to an amount less than 0.5%, many crystals attached to the wall of the vessel. The gathering of the crystals after the formation will occur irrespective of the concentration of the solution. In the solution having high concentration the crystals will be formed more rapidly than in the solution having low concentration. In the solution containing pepton, the crystal is small in case of high concentration, and is large in the low concentration, differing from the cases of the concentration of gelatine, glycerol, agar or edible oil.

Literature cited

1) Tanikawa, E. *et al.* (1956). *Bull. Fac. Fish., Hokkaido Univ.* 7 (3), 247-251.
2) ―――――――― (1957). *Ibid.* 8 (1), 59―64.
3) ―――――――― (1957). *Ibid.* 8 (1), 65―68.