CALCIUM PECTINATE FILMS

Application of the Reversion of the Molecularly Dehydrated Sodium Phosphates in Filming

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The production of a colorless, tasteless, edible film of calcium pectinate was investigated. The initial phase was the study of the rates of reversion of five commercially important molecularly dehydrated sodium phosphates and the effect of calcium pectinate upon these rates. In all the dehydrated phosphates an increase in temperature and a decrease in pH caused an increase in the rate of reversion to orthophosphate. Generally, the presence of calcium pectinate increased the rate of reversion, the exception being tetrasodium pyrophosphate. The metaphosphates were found to have the greatest filming ability. Optimum conditions are at pH 3 and at lower temperatures. Sequestering the calcium ion renders the pectinic acid soluble for coating, dipping, and extrusion, and then ready for drying as a gel or film. Many suggestions have been offered for the use of pectinates as coating agents for certain foods.

INTEREST IN THE USE OF METALLIC PECTINATES (*14, 22*) as coating agents in food and other industries indicates the need for better filming and coating methods. Calcium pectinate is insoluble in hot and cold water and mildly acidified solutions. The addition of molecularly dehydrated sodium phosphates to these solutions will sequester the calcium ions, thus making the pectinic acid soluble for casting, dipping, molding, and extrusion. It may then be dried as either a gel or a film.

In a study of the possibility of producing a colorless, tasteless, edible film of calcium pectinate in this manner, the first step should be to find out how the calcium pectinate affects the rate of reversion of some of the commercially important molecularly dehydrated sodium phosphates. A marked change occurred in the calcium pectinate during the reversion trials, and necessitated a consideration of this phase of the work. Indication of a chemical phosphorylation of pectinic acid was obtained.

Background

The molecularly dehydrated sodium phosphates have long been known because of their importance in corrosion prevention, water softening, and other water treatments. All react with water to form orthophosphate ultimately, by a process called hydration, hydrolysis, or reversion. Bell (*6*) has shown some of the intermediate steps in the reversion to orthophosphate.

Only fragmentary information is available concerning the rates of hydrolysis of the dehydrated sodium phosphates. Generally, analytical difficulties have been the main drawback in making accurate studies; however, recent steps taken to overcome such difficulties (*6, 10, 25*) show that the rate of reversion varies directly with temperature and inversely with pH (*10*) and that the intermediate products vary with temperature and concentration (*6*).

The only quantitative studies of the effect of calcium upon the rate of reversion of the dehydrated phosphates are those reported by Bamann and Nowotny (*4*) and Green (*10*). Little is known about the actual chemical structures of the calcium complexes. There is some evidence that sodium metaphosphate forms calcium complexes more readily than the polyphosphates or the tetrametaphosphate (*18*). The stability of the calcium complex may be affected by temperature (*17, 18*) and pH (*11, 17, 18*).

The filming of metallic salts of pectin compounds has begun to have some importance only recently. Schneider and Bock (*20*) prepared a film using nitropectin and in 1933 a patent (*19*) was obtained by a French company for preparation of a dry pectous film by evaporating the pectous liquid. Maclay and Owens (*14, 22*) in 1947 first mentioned the application of filming pectinates in the food industry. They found that the dried pectinate film withstood temperatures up to 180° F. They also reported (*21*) that the transmission of water vapor was about on the same order as that for plain cellophane, but could be materially reduced by a wax coating on the film.

The only evidence showing the presence of phosphorus in the pectin molecule is the report of Henglein, Krassig, and Steimmig (*13*). They determined the phosphoric acid content of apple and beet pectins and attributed its presence largely to inorganic impurities and to a smaller extent from pectins which are formed by orthophosphoric acid bridging. The phosphate groups are depicted as being attached to the carboxyl groups in a "bridge" effect and calcium or magnesium may be in between the phosphate group and car-

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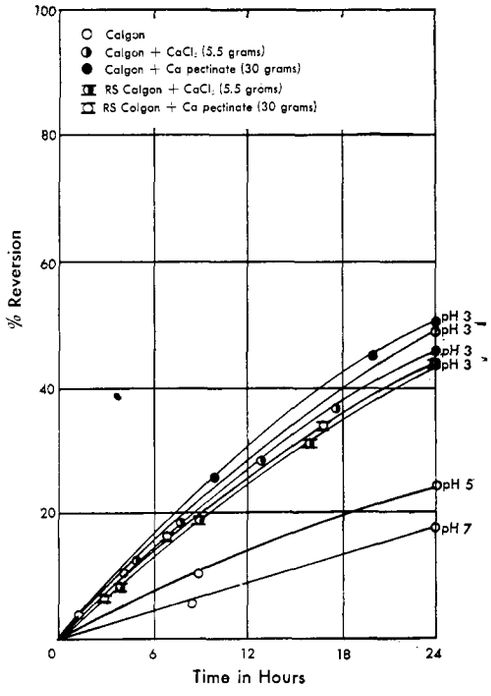

Figure 1. Reversion of sodium hexametaphosphate at 70° C.
0.5% phosphate expressed as PO_4

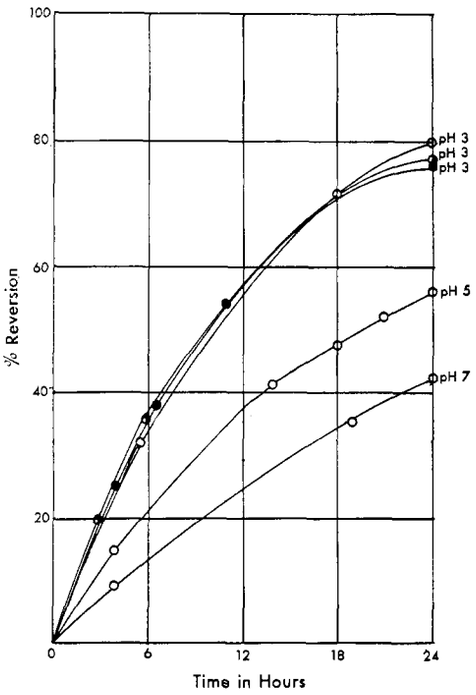

Figure 2. Reversion of sodium hexametaphosphate at 80° C.
0.5% phosphate expressed as PO_4

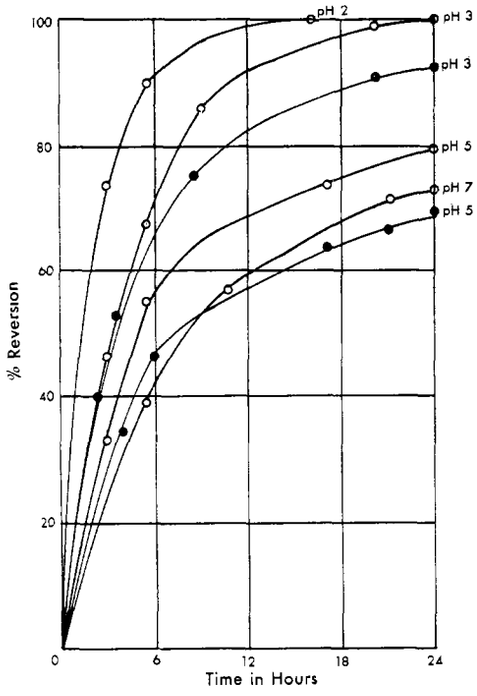

Figure 3. Reversion of sodium hexametaphosphate at 90° C.
0.5% phosphate expressed as PO_4

boxylic acid group. They were able to increase the phosphorus content of pectin only slightly by phosphorylation with phosphoryl chloride. Upon nitration about one half of the phosphorus introduced artificially was present in the nitrated product, while no phosphorus could be found in the nitrated pectins of those not phosphorylated.

It has been known for some time that pectin can be extracted by solubilizing the protopectin and pectinates in the source material with a dehydrated sodium phosphate and recovering the pectinic acid from the filtrate (*3, 16*).

Materials and Methods

The dehydrated phosphates used in this experiment are listed in Table I. Sodium tetraphosphate ($Na_4P_4O_{13}$) was manufactured by the Rumford Chemical Works and had been in this laboratory for several years. The sodium hexametaphosphate [$(NaPO_3)_6$] was manufactured by Calgon, Inc., and is the commercial product Calgon. The RS Calgon is a specially prepared sodium metaphosphate received by this laboratory shortly before this work terminated. It was found by Gilligan and Baker (*9*) that this markedly increased the viscosity of pectin solutions when employed in the extracting solution. Consequently it suggested possibilities in the filming of calcium pectinate. The remaining dehydrated sodium phosphates were donated by the Blockson Chemical Co. and are sodium tripolyphosphate ($Na_5P_3O_{10}$), tetrasodium pyrophosphate ($Na_4P_2O_7$), and sodium polyphos, a commercial mixture of sodium hexametaphosphate and sodium tetrametaphosphate.

Table I. Dehydrated Phosphates Used in Reversion Experiments

Dehydrated Phosphate	Formula	Wt. % Phosphorus Expressed as PO_4		pH of 0.5% Solution of PO_4 Before Adjustment		
		Theoretical	Experimental	Orig. sol.	Ca pectinate added	$CaCl_2$ added
Sodium tripolyphosphate	$Na_5P_3O_{10}$	77.5	74.0	10.2	8.6–8.8	9.6
Tetrasodium pyrophosphate	$Na_4P_2O_{13}$	71.5	70.0	10.5	9.5	9.2
Sodium tetraphosphate	$Na_6P_4O_7$		78.5	8.4	7.4	7.2
Sodium polyphos	$Na_{12}P_{10}O_{31}$	87.8	83.5	7.9	6.5–6.8	6.5
Sodium hexametaphosphate [Calgon]	$(NaPO_3)_6$		89.3	7.9	5.8–6.1	6.1
RS Calgon			91.4	7.9	6.1	6.1

Calcium pectinate was prepared by the simultaneous extraction-demethylation of pectin from apple pomace as devised by Woodmansee and Baker (*26*). As Schultz, Owens, and Maclay (*22*) have shown that low-methoxyl pectinates are best for filming, it was decided to use a pectinate of around 3.5 to 4.0% methoxyl content. To obtain this pectinate the extracting-demethylating mixture is held at pH 1 and 50° C. for 75 hours and the calcium pectinate is precipitated by adding excess calcium chloride to the pectin extract.

Green (*10*) has suggested that the rate of formation of orthophosphate might be taken as a measure of the stability of the dehydrated phosphates. This, of course, is not exact, as intermediate products are formed in the reversion of meta- and polyphosphates to orthophosphate (*6, 24*), and may interfere with the original dehydrated sodium phosphate in performing its function. However, where intermediate products are formed, the formation of orthophosphate does not correspond to the disappearance of an equivalent amount of the original dehydrated phosphate. Therefore reference to "stability" and "reversion" in this paper is limited to the fraction of total phosphorus converted to orthophosphate. The intermediate products do not have the sequestering ability of the parent compounds.

Phosphorus was determined essentially by the colorimetric method of Fiske and Subbarrow (*8*). Sufficient development of color was obtained after 5 minutes, because the determinations

were made in an acid medium and it was necessary to avoid errors due to reversion of the dehydrated phosphates. A known concentration of orthophosphate was added to a known concentration of the dehydrated phosphate and the solution was analyzed as described above. To ascertain that the presence of calcium chloride or calcium pectinate did not interfere with this procedure, similar experiments were carried out in the presence of these compounds (Table II). If calcium pectinate precipitated, it was filtered off through a fast qualitative paper before the final aliquot was taken for analysis. The fairly close results of Table II show that no appreciable error during analysis was introduced either by reversion of the dehydrated phosphates or by the added constituents.

The saponification method of von Fellenberg (*7*) was used for determining the methoxyl content, after the insoluble calcium pectinate had been converted to the soluble pectinic acid. This was done by a modification of the acid-alcohol extraction proposed by Beach (*5*), which lasts for 0.5 hour.

For the preparation of films, a 10-ml. aliquot of the solution was withdrawn, placed in a 5-cm. Petri dish, and allowed to evaporate to dryness at room temperature.

Ash was determined essentially according to the procedure recommended by the Association of Official Agricultural Chemists (*2*) for plant materials. Calcium was determined according to the procedure recommended (*1*) for plant materials.

The reversion experiments were performed in a three-necked, 3-liter round-bottomed flask, a mercury-sealed stirrer being used to agitate the solution throughout the run. The two smaller openings in the flask were closed except for the addition of materials, withdrawal of samples, or a pH check. The flask was immersed in a constant temperature bath and the temperature maintained within ± 0.5° C. of the desired value.

Because previous work (*9*) had shown that Calgon solubilized calcium pectinate in the weight ratio of about 1 to 2, a level of 0.5% (expressed in terms of the orthophosphate ion) of dehydrated phosphate and 1% of calcium pectinate was used. A few determinations of the rates of reversion with calcium chloride at about the same level of calcium as that in the calcium pectinate were made to ascertain if there was any significant difference in the effect of the inorganic and organic calcium salts on the rates of reversion of the dehydrated sodium phosphates.

A Beckman Industrial Model pH meter with a Beckman No. 1170 high temperature glass electrode and a Beckman No. 1170 calomel electrode were used. The pH measuring system was standardized with potassium acid phthalate. Although

Table II. Recovery of Orthophosphate after Allowing 5 Minutes for Color Development

(Orthophosphate expressed as p.p.m.)

Dehydrated Phosphate, 0.5%, Expressed as PO_4	Dehydrated Phasphate			Calcium Pectinate			Calcium Chloride		
	Orig. concn. of PO_4	PO_4 added	PO_4 found	Orig. concn. of PO_4	PO_4 added	PO_4 found	Orig. concn. of PO_4	PO_4 added	PO_4 found
Sodium tripolyphosphate	4.00	2.00	1.99	6.75	4.00	3.97	3.10	2.00	2.00
Tetrasodium pyrophosphate	6.00	2.00	2.01	5.00	4.00	3.98	2.80	4.00	4.05
Sodium tetraphosphate	4.20	4.00	3.95	4.40	4.00	4.02	4.20	4.00	4.05
Sodium hexametaphosphate (Calgon)	4.50	4.00	4.00	5.10	4.00	4.01	6.40	4.00	3.97
Sodium polyphos	5.50	2.00	1.99	4.70	4.00	3.96	1.60	2.00	2.00

Table III. Per Cent Reversion of Molecularly Dehydrated Sodium Phosphates After 24 Hours

Dehydrated Phosphates	70° C.			80° C.			90° C.		
	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7	pH 3	pH 5	pH 7
Sodium tripolyphosphate	62.7	51.6	21.5	96.4	93.4	42.3	100.0	100.0	100.0
+ 1% Ca pectinate	69.2			95.0			98.3		
+ 1.8% $CaCl_2$	61.3			98.0			98.3		
Tetrasodium pyrophosphate	62.5	51.5	23.9	91.5	87.5	75.0	100.0	100.0	97.5
+ 1% Ca pectinate	60.0			95.8			96.3		
+ 1.8% $CaCl_2$	59.0								
Sodium tetraphosphate	56.8	47.8	32.7	83.3	77.3	74.2	100.0	95.0	92.2
+ 1% Ca pectinate	65.			94.5	83.0		94.5		
+ 1.8% $CaCl_2$	61.3								
Sodium polyphos	51.5	33.7	28.2	79.0	60.2	54.2	100.0	82.5	77.9
+ 1% Ca pectinate	58.0			85.0	63.8		93.3		
+ 1.8% $CaCl_2$	54.5			85.8					
Sodium hexametaphosphate [Calgon]	49.1	24.5	18.7	88.8	55.5	42.2	100.0	79.2	73.0
+ 1% Ca pectinate	49.5			76.0			92.5	69.3	
+ 1.8% $CaCl_2$	46.0			76.3					

pH values are certified only to 60° C., reasonably significant values (± 0.05 to 0.10 accuracy) are obtained between 60° and 90° C. (*15*).

Experimental Results

In all these dehydrated phosphates an increase in temperature and a decrease in pH caused an increase in the rate of reversion to orthophosphate. This agrees with the findings of Green (*10*) and Watzel (*24*).

Figures 1, 2, and 3 show primary reversion data at 70°, 80°, and 90° C. for sodium hexametaphosphate (Calgon) at pH 3, 5, and 7. In general, curves of a similar shape were obtained with the other dehydrated phosphates. At 70° and 80° the calcium pectinate does not appreciably affect the rate of reversion. At 90° little difference can be noted as a result of the presence of calcium pectinate during the first 6 hours, but after 24 hours the reversion to orthophosphate has fallen to about 90 to 95%.

Figures 4, 5, and 6 show the effect of pH on the reversion of the molecularly dehydrated sodium phosphates in the absence of added salts. These data were unobtainable in the presence of calcium pectinate, as in most cases sequestering action was not complete at pH 5 and above. The data in Table III indicate that calcium pectinate affects the curves only slightly, the general shape remaining the same.

Sodium tripolyphosphate, the only true polyphosphate used in this experiment, was found unsuitable for filming calcium pectinate between 70° and 90° C. The same is true of tetrasodium pyrophosphate, which is also a linear molecule. The metaphosphates produced films of varying strengths and clearness. Outstanding in this respect were sodium hexametaphosphate and the more recent RS Calgon. Sodium tetraphosphate shows some possibility below 70° C., but not as much as sodium polyphos, which is a mixture of the hexameta- and tetraphosphates.

With all the dehydrated phosphates used, the calcium pectinate would not go into solution in the alkaline range; consequently the pH had to be reduced to at least 5 for the metaphosphates and to 3 for the linear phosphates. In the case of tetrasodium pyrophosphate, the calcium pectinate was never sequestered completely at any pH tried, unless a higher concentration of the pyrophosphate was used. Temperature was found to be im-

portant in the solubility of calcium pectinate in these dehydrated phosphates; with sodium metaphosphate no difficulty was encountered in solubilizing the calcium pectinate at pH 3 at 70° and 80° C. but it was insoluble above this pH. At 90° C., however, it was completely soluble at pH 5.

The calcium pectinate began to precipitate after the reversion experiment had been under way for several hours. Temperature, pH, and the particular dehydrated phosphate affected this rate of precipitation, but no uniformity was observed. With sodium polyphos at 80° C. and pH 3, 7 to 8 hours elapsed before precipitation was noticeable. At pH 5 and the same temperature, calcium pectinate had precipitated after 3 hours, although the rate of reversion of the dehydrated phosphate is much slower. It was typical of most of the dehydrated phosphates used that precipitation of calcium pectinate occurred much more rapidly at pH 5 than at pH 3, although the rate of reversion to orthophosphate was slower. At 70° C. and pH 3 the calcium pectinate remained in solution almost 16 hours and at 90° C. and pH 3 only 2 hours. With sodium hexametaphosphate precipitation of calcium pectinate had occurred after 3 hours at pH 3 and 90° C., but at pH 5 and the same temperature the pectinate remained in solution for 8 hours.

As the metaphosphate appeared to be the most promising of the dehydrated phosphates used, some experiments were carried out at 60° C. At the end of 24 hours at 60° C. and pH 5 approximately 18% of the Calgon had been converted to orthophosphate. The solution was clear for at least 12 hours and only slightly turbid at the end of the run. Another run at 60° C. and pH 3 using RS Calgon showed 20% reversion. The initial viscosity of this run was 9.1 and the film was stronger than any of the others.

When calcium pectinate precipitated during the course of a reversion experiment, the particles were not as turgid as the original calcium pectinate. The liquid was usually brownish at the end of an experiment; the residue was a much darker brown at higher temperatures, but almost white at the low temperatures. As a pectin solution depolymerizes when held for any great length of time over 50° to 60° C., it was decided to recover the calcium pectinate that precipitated in order to determine what changes occurred during the course of a run. Generally this residue was very gummy and extremely difficult to filter, except at the lower temperatures of 60° and 70° C. or at pH 5. The residues obtained at the higher temperatures formed small cubes upon drying overnight at 60° C. and were extremely hard. They could not be dispersed in water after drying.

The calcium pectinate recovered after use in the reversion trials was purified by washing with a 1 to 1 mixture of distilled water and 99% isopropyl alcohol in 3- to 4-liter portions, and filtering through silk. In some samples washings were continued until the filtrate did not show a test for orthophosphate. The residue was then placed in a canvas cloth and subjected to a pressure of 10,000 pounds per square inch for 15 minutes. Two grams of the press cake was placed in 25 ml. of water, allowed to set for 0.5 hour, and tested for orthophosphate. The samples washed free from orthophosphate after the reversion trials (P-10, P-16, P-19, P-20, P-22) showed no orthophosphate present as impurities. The pectinate cakes recovered from the lower temperature runs were very brittle and would crumble in the hand, while those recovered from the higher temperature runs were gummy. The original pomace was fibrous and stringy.

The methoxyl content of the calcium pectinates used in these experiments and the recovered calcium pectinates are presented in Table IV. The per cent methoxyl was of interest in ascertaining if any deesterification of the pectin had occurred during the reversion trials. In most cases no significant differences were observed; the two exceptions were at 80° and 90° C. with Calgon. The samples of recovered pectinates at these higher temperatures seemed to show a type of surface acidity or an occlusion of free base in the pectinate particles—i.e., the end point was reached and in about a minute the solution faded to colorless; this could be repeated numerous times.

The viscosity data could not be measured for the recovered pectinates, since the acid-alcohol treatment did not convert them into a soluble pectinic acid. Only about 15% of each sample could be dissolved. Determination of ash, calcium, and phosphorus on the insoluble fraction and the material in solution showed no significant difference.

Data are shown on the ash, phosphorus, and calcium contents in Table V before and after the acid-alcohol conversion to a soluble pectinic acid. The acid-alcohol wash is intended to remove the larger portion of calcium and other impurities in the pectinate, which lowers the ash content correspondingly. This is accomplished with the original calcium pectinates, but not to as high a degree with the recovered pectinates. However, orthophosphate present as impurities was removed from the recovered pectinates (samples P-1 through P-5, P-15, P-17 in Table V). The acid-alcohol treatment did not wash out much of the phosphorus either in the starting material or in the recovered

Table IV. Methoxyl Content of Original and Recovered Pectinates

Sample No.	Temp., °C.	pH of Reversion	Dehydrated Phosphate	Methoxyl	pH of Converted Pectin
1-M				3.59	3.6
P-1	70	3	Tripoly.	3.56	3.8
2-M				4.44	3.6
P-2	70	3	Polyphos	4.25	3.8
P-3	70	3	Tetraphos.	4.56	3.6
P-4	70	3	Calgon	4.10	3.4
3-M				4.00	3.6
P-5	70	3	Tetraphos.	4.07	3.8
P-7	80	3	Polyphos	4.45	4.4
4-M				3.56	3.6
P-6	80	3	Tripoly.	4.70	4.5
P-8	80	5	Polyphos	3.32	5.8
P-9	80	3	Pyrophos.	3.40	4.6
6-M				3.98	3.6
P-10	80	3	Calgon	3.02	4.0
7-M				3.04	3.6
P-11	80	3	Tetraphos.	2.64	4.2
P-13	90	3	Tripoly.	3.64	4.2
8-M				3.27	4.4
P-14	90	3	Polyphos	3.64	4.3
P-15	90	3	Calgon	3.50	4.4
P-16	90	5	Calgon	2.33	5.7
9-M				3.21	3.9
P-20	60	3	"RS" Calgon	3.50	3.6
P-22	70	3	"RS" Calgon	4.28	3.4
10-M				3.19	4.1
P-17	90	3	Tetraphos.	3.92	4.0
P-18	90	3	Pyrophos.	3.64	4.2
P-19	90	5	Calgon	3.88	3.5

M samples are original calcium pectinates extracted from apple pomace. P samples are recovered pectinates and are inset under M samples to show that reversion experiments were made using that sample.

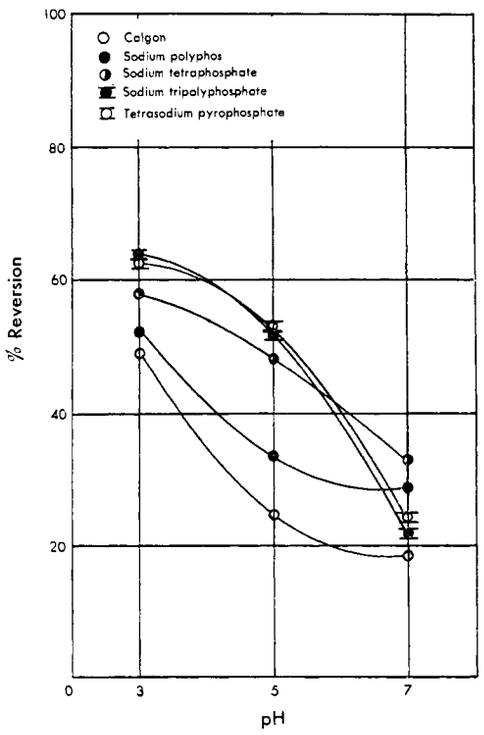

Figure 4. Effect of pH on reversion at 70° C.
0.5% phosphate expressed as PO_4

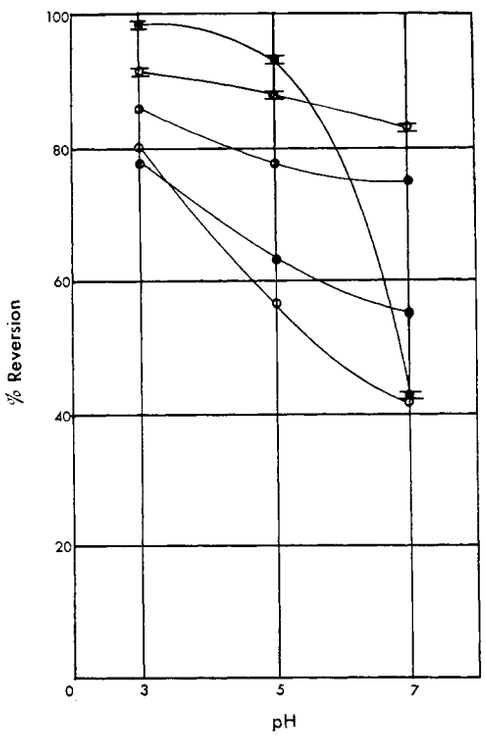

Figure 5. Effect of pH on reversion at 80° C.
0.5% phosphate expressed as PO_4

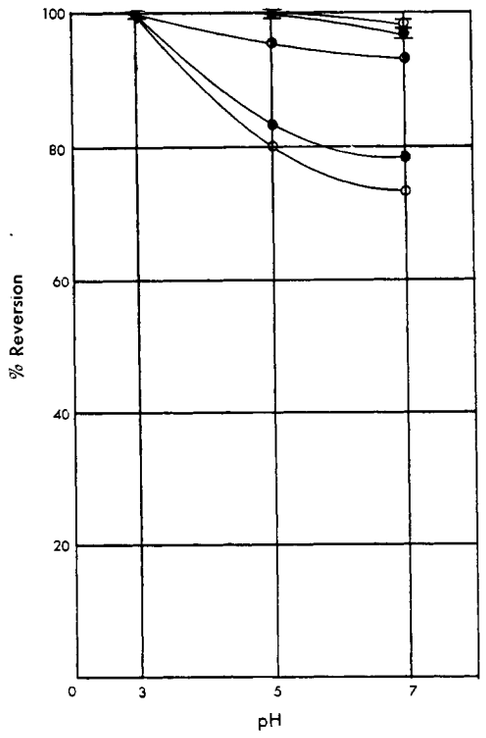

Figure 6. Effect of pH on reversion at 90° C.
0.5% phosphate expressed as PO_4

pectinates that had been washed free of orthophosphate ion after the reversion trials.

Several tests were made to ascertain how tightly the phosphorus was bound to the pectinic acid molecule.

In investigating the stability to acid, 1 gram of sample P-19 (acid-alcohol treated) was allowed to reflux in 1*N* hydrochloric acid; about 13 to 15% of the phosphorus was lost each hour for the first 3 hours. If the color in the phosphorus determination was developed before the insoluble residue was filtered and the latter separated, the filtrate was a very light blue, while the pectin precipitate was a much darker blue. This might indicate either adsorption or that the molybdenun blue reaction takes place with the phosphate ion attached to the pectinic acid molecule.

Several qualitative tests were setup to gather some information on the type and stability of this phosphorylation. Two-tenths gram of calcium pectinate from 6-M and P-10 were placed in a beaker with 25 ml. of water. Standards containing 0.02 and 0.04 mg. of phosphate were treated similarly for each test. Tests were made as follows: One set was heated to boiling and the colorimetric test for phosphorus made, a colorimetric test was made at room temperature, another set was allowed to stand overnight in a water solution before the color was developed, and one set was heated to boiling and then cooled to room temperature before the color was developed. The highest color intensity was in the samples that had been treated hot, the color in the two pectinates being much more intense than the standards. The samples that had been boiled and cooled showed about the same intensity as the ones at room temperature. The pectinate samples allowed to stand overnight showed a greater intensity than the ones at room temperature, but less than when the color was developed in hot solutions. If all the solutions were allowed to stand overnight after development of the color, the intensity of the pectinate solutions increased much more in proportion than did that of the standards.

At the end of the 24-hour hydrolysis period about 50% of the calcium pectinate of samples P-21 and P-23 was recovered in the usual manner and to the remaining half an excess of calcium chloride was added. A gel formed and when washed free of phosphate ions showed an increase in calcium concentration of 1 to 1.5%. This gel was similar to that of the original calcium pectinate, but not as turgid or in such large particles. When the isopropyl alcohol was added to aid in the recovery, a milky-white filtrate resulted which disappeared after the third washing. Although unaccounted for, phosphorus content in one case (P-21) dropped about 1%, whereas it was increased about 1% in the other (P-23).

Discussion

Some of the intermediate steps in the reversion of the dehydrated sodium phosphates to orthophosphate have been shown (*6*). Results from the different precipitating rates of calcium pectinate in the presence of the same dehydrated

Table V. Ash, Calcium, and Phosphorus Content of Original and Recovered Pectinates Before and After Conversion to Soluble Pectinic Acid

Sample No.	Temp., °C.	pH	Dehydrated Phosphate	% Ash		% Phosphorus		% Calcium	
				Before conversion	After conversion	Before conversion	After conversion	Before conversion	After conversion
Original calcium pectinate (average)				10.80	3.53	0.071	0.064	5.37	1.59
P-10[a]	80	3	Calgon	10.04	7.70	0.52	0.40	4.58	3.84
P-16[a]	90	5	Calgon	12.50	10.60	0.32	0.32	5.15	4.99
P-19[a]	60	5	Calgon	13.28	9.66	2.30	2.25	3.24	2.24
P-20[a]	60	3	RS Calgon	12.39	8.15	2.65	2.45	3.36	2.24
P-21[b]				11.86	..	1.67	..	4.59	..
P-22[a]	70	3	RS Calgon	10.37	5.95	1.31	1.24	3.79	1.92
P-23[b]				12.38	..	2.20		5.35	
P-1	70	3	Tripoly.	16.25	6.80	2.85	1.35	4.23	2.24
P-2	70	3	Tetraphos.	14.57	6.72	2.43	1.20	3.12	1.70
P-3	70	3	Polyphos	15.88	6.35	1.88	0.65	3.32	1.55
P-4	70	3	Pyrophos.	10.62	5.73	1.30	0.88	2.91	1.57
P-5	70	3	Calgon	11.64	4.14	1.20	0.28	3.25	1.24
P-15	90	3	Calgon		11.59		1.29		3.32
P-17	90	3	Tetraphos.	..	7.80	..	0.063	..	4.41

[a] Washed free of excess PO_4 immediately after reversion experiment.
[b] Additional $CaCl_2$ added at end of run and then washed free of phosphates.

phosphate would indicate that the intermediate forms may differ, depending on pH, temperature, and concentration of calcium ion. This precipitation of calcium pectinate will, of course, be of importance in the choosing of a dehydrated phosphate to be used as a filming agent and the temperature to be used for filming. Certainly at the concentration and temperatures used in this work the metaphosphates were far superior to the polyphosphates on both accounts. They should be even more satisfactory at temperatures below 60° C., as they revert to orthophosphate more slowly than the polyphosphates.

The metaphosphates, found to be the better filming agents, may form calcium complexes more readily or render the calcium ion soluble more readily than the polyphosphates; this agrees with an earlier report (*18*). The stability of the calcium complex undoubtedly is affected by pH and temperature, which also supports earlier findings (*11, 17, 18*). It may also be affected by the level of calcium present. Complex ion formation in the dehydrated phosphates was not defined in terms of a chemical formula until recently (*23*) and it is still not known whether the metaphosphates form a complex. As the films were obtained by evaporation, they were somewhat salty and not too palatable.

The data in Table V indicate a tie-in between phosphorus and pectinic acid. There appears to be no definite relation of the increase in phosphorus content of the recovered pectinates to either temperature or pH. In general, the pectinic acid picked up more phosphorus with sodium hexametaphosphate than with the other dehydrated phosphates used. There was less phosphorus at 90° C. than at any other temperature.

It is not understood how this reaction between phosphorus and pectinic acid occurs or what influences it. It is highly probable that more than one reaction occurs. The increase in phosphorus in the recovered pectinates might be accounted for as follows:

Chemical phosphorylation of pectinic acid, producing $\overset{O}{\overset{\|}{C}}-O-P$ and or $C-O-P$ linkages.

Phosphate ion bound to polyvalent metal cation (calcium in this case), which may be interlocked with the carboxyl groups of the pectin.

Of the two end products of chemical phosphorylation thus indicated, the preferable reaction would occur between the phosphate anion and the terminal glucosidic bond, resulting in the formation of galactose-1-phosphate. This is a low-energy (3000-cal.) bond, whereas the $\overset{O}{\overset{\|}{C}}-O-P$ is a high-energy bond (10,000-cal.). The intermediate step in this reaction could be the formation of an unstable carbonium ion by the depolymerization of pectinic acid.

The fact that the recovered pectinate was fairly stable to hydrolysis in 1.N hydrochloric acid would indicate a low-energy linkage. Tests for phosphorus in the calcium pectinate recovered from the reversion experiments indicate that the phosphorylation might be reversible. Some support might be derived from the knowledge that the enzymatic phosphorylation of amylose (*12*) occurs with little change in energy (4800 cal.). This would support a low-energy bond formation.

The linking of orthophosphate tetrahedra and calcium ions may cause the calcium in the recovered pectinates to be retained in the acid-alcohol wash. However, there appears to be no definite relation between the calcium and phosphorus contents, which tends to indicate that this is not a simple reaction.

Although additional calcium was picked up by the recovered pectinates (samples P-21 and P-23, Table IV), it did not exceed the value of the starting material. Theoretically, calcium ions could combine with all of the free carboxylic acid groups of a pectinic acid molecule. Actually this would be impossible because of chain branching and the presence of mixed polymerizates such as sugars, cellulose, araban, and galactan impurities. Henglein's (*13*) formula for protopectin follows this line of thought, as he presents a disordered arrangement of polygalacturonide chains. Part of the calcium ions sequestered by the dehydrated phosphate had not been rendered insoluble because of the fractional hydrolysis of these dehydrated phosphates. This would allow a redistribution of the calcium ions between the pectinic acid chains.

The calcium pectinate samples recovered at 60°, 70°, and 80° C. at pH 5 were almost white. As the calcium pectinate as extracted from apple pomace was brown, the araban and galactan impurities could have been removed during the reversion experiment.

The strength of pectinate films would be expected to increase with an increase in molecular weight because of the opportunity for more cross linkages through carboxyl groups of adjacent chain molecules to occur. The lower the methoxyl content the greater the number of these free carboxyl groups to enter into ionic cross linking, effecting an increase in tensile strength for a resulting film or fiber. Obviously, a method of extracting the high molecular weight pectinic acid at room temperature from plant material would be highly desirable for use in filming. To prevent depolymerization during filming, near room temperatures would have to be used.

Considerable more information must be obtained on the changes that both pectin and the dehydrated phosphates undergo in the acid hydrolysis medium. More specific studies are needed on the nature of the calcium complexes of the dehydrated sodium phosphates and the effect of pH, temperature, concentration, and pectin upon their stability.

Summary

In all the dehydrated sodium phosphates the rate of reversion to orthophosphate varies directly with temperature and inversely with pH. The presence of calcium pectinate increased the rate of reversion in every instance, except with tetrasodium pyrophosphate. In all cases the rate of reversion to orthophosphate was slightly greater with calcium pectinate than with calcium chloride, but not greater than experimental error.

There is evidence of a chemical tie-in between phosphorus and pectin by reaction with the orthophosphate ion split off from the dehydrated sodium phosphates. These results point to both chemical phosphorylation of pectinic acid and the interlocking of orthophosphate tetrahedra to calcium ions attached to the carboxyl groups of the pectinic acid chain.

The metaphosphates (Calgon, sodium polyphos, sodium tetraphosphate) were found to have the greatest filming ability. Calgon and particularly RS Calgon were the most outstanding. Better films are formed at lower temperatures than employed in this study. Optimum pH is around 3. There is some question as to whether the measure of orthophosphate can be correctly applied in determining "stability" to hydrolysis of the dehydrated sodium phosphates, particularly at the concentration used here.

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Penicillin, Polymyxin, Thiolutin Control Infection in Beer Fermentation

CONTAMINATION INHIBITION

Antibiotics as Inhibitors of Microbiological Contamination in Beer

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The purpose of this investigation was to study the application of antibiotics to the control of bacterial and secondary yeast contamination in the brewery. Polymyxin was outstanding in its ability to control the Gram-negative bacterial infection of brewer's yeast and beer fermentation. The addition of bactericidal concentrations of polymyxin and other effective antibiotics to the fermenting beer results in a stimulation of the fermentation. Of the antibiotics studied, penicillin is the most effective in the control of the Gram-positive lactic acid bacteria in finished beer. Used in combination with thiolutin for the control of secondary yeast growth, it gives complete protection against microbiological growth in finished beer.

DANGER OF INFECTION with other microorganisms is one of the problems that are common to all microbiological processes, in which pure cultures are used. The fermentation of beer in a brewery is no exception and at times the bacterial infection of the fermentation presents a serious problem.

Brewer's yeast infected with bacteria to a dangerous extent is often treated with such weak bactericides as tartaric or phosphoric acid. This treatment, when carried out properly, kills a large percentage of the contaminating bacteria without affecting the yeast adversely. However, when such a treated yeast is used in a subsequent fermentation, the surviving bacteria again begin to multiply and the original level of infection is reached within a relatively short time.

The use of antibiotics in the control of bacterial infections of brewer's yeast was reported on by Gray and Kazin as early as 1946 (*1*). These investigators found that the antibiotic tyrothricin compared favorably with the commonly used bactericidal substances, but only when its concentration was raised to 500 p.p.m. Other investigators (*2, 3*) have reported that the growth of the lactic acid bacteria in finished beer can be controlled by the addition of penicillin.

Strandskov, Brescia, and Bockelmann (*5*) found that the antibiotic polymyxin inhibits the growth of the Gram-negative rods, Gram-positive lactic acid cocci, and Gram-positive lactic acid rods that commonly contaminate brewer's yeast and beer, at concentration levels that are not inhibitory to brewery yeast. These inhibitory studies were carried out on a yeast extract–dextrose medium.

These findings suggested that polymyxin as well as other antibiotics should be useful bacteriostatic and bactericidal agents in the brewery fermentation, during storage, and possibly in the finished product.

The effect of polymyxin on the Gram-negative bacterial infection during fermentation was studied using as fermenters 3-liter glass cylinders, 3 inches in diameter and 36 inches tall. It was demonstrated in a preliminary experiment that fermentation in such a cylinder proceeds at the same rate as in a plant fermenter. To test the effect on a fermentation, the desired quantity of an antibiotic, in 10 ml. of distilled water, was added to each cylinder except the control, to which 10 ml. of distilled water