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Influence of Phosphate Compounds on Certain Fungi and Their Preservative Effects on Fresh Cherry Fruit (*Prunus cerasus*, L.)

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Studies were conducted to ascertain the retarding effects of four phosphate compounds (sodium hexametaphosphate, sodium tripolyphosphate, sodium tetraphosphate, and tetrasodium pyrophosphate) on molding of fresh cherries (*Prunus cerasus*, L.). In vitro studies on their antimycotic effects against the most common fungal spoilers, *Penicillium expansum*, *Rhizopus nigricans*, and *Botrytis* sp., were also carried out. Sodium tetraphosphate appeared to be the most effective compound in preserving cherries and also had the greatest antimycotic effects in the in vitro studies. A 10% concentration, when applied as a dip, inhibited fungal growth on fresh cherries for up to 30 days of storage at 1.1 C (34 F) and a relative humidity of 94%, whereas untreated controls showed fungal growth at 14 days. Following in order of effectiveness were sodium hexametaphosphate, sodium tripolyphosphate, and tetrasodium pyrophosphate.

To a great extent, the economic survival of the fresh-produce industry depends on intelligent use of chemicals, packaging, handling, and storage procedures that will extend shelf-life and maintain quality. Wider use of pre- and post-harvest chemical treatments that slow respiration, delay senescence, and, therefore, control fungal growth seems inevitable, despite increasing legislative limitations on the use of food additives (9).

Polyphosphates are commonly used in industry as water softeners because of their ability to form stable complexes with metallic ions. These compounds (10) have a high degree of surface activity (especially hexametaphosphate) and concentrate at surfaces when dissolved in water. This may include the cell-liquid interface when microorganisms are present. Polyphosphates bind metallic ions of cobalt, copper, and iron very strongly, and they bind calcium and magnesium less strongly. Apparently, the polyphosphates bind all of these metabolically essential ions more strongly than does any other known organic or inorganic chelating agent except ethylenediaminetetraacetic acid (EDTA; 4). The bound ions are unavailable for metabolic purposes. The chain polyphosphates chelate most strongly and form the most stable complexes, the ring phosphates are less effective, and orthophosphate is not a binding compound (11).

A number of studies have suggested that microbial cell membranes are composed in part of organic chelators, and it has been postulated that these chelators move essential metallic ions across the membrane into the cell. Metal chelating agents may inhibit or promote growth by reducing the available concentration of essential or inhibitory metallic ions, respectively (5). Sodium hexametaphosphate, a long-chain phosphate-glass water softener has been shown (8) to be antibacterial. It apparently inhibits magnesium metabolism and causes a failure in cell division or loss of cell wall integrity. Other commercial polyphosphates have been shown (2) to inhibit the growth of pseudomonads and, as a result, to extend the shelf-life of poultry. Hexametaphosphate and the other polyphosphates may compete with the cell membrane-chelators for available ions and may selectively remove calcium and magnesium or other ions from essential structural elements of the cell wall, membranes, or cytoplasm. This may account for the observed lysis of gram-negative bacteria and the inhibition of growth of gram-positive bacteria.

The inhibition of bacterial growth by polyphosphates (2, 4, 8) and the report of fungal inhibition (1) by other types of chelators suggested that polyphosphates (approved for use in food by the Food and Drug Administration) might be useful in retarding spoilage of fresh fruits. This investigation was undertaken to determine whether polyphosphate compounds

could inhibit the growth or development of fungi and retard the spoilage of fresh cherries.

MATERIALS AND METHODS

Phosphates. Sodium hexametaphosphate (HEX) and sodium tripolyphosphate (TPP) were furnished by Calgon Corp., Pittsburgh, Pa.; sodium tetraphosphate (TP), by Rumford Chemical Works, Rumford, R.I.; and tetrasodium pyrophosphate (TSPP), by Monsanto Chemical Co., St. Louis, Mo. All four phosphate compounds have been approved by the Food and Drug Administration as inert ingredients which are exempt from tolerance requirements when used in good agricultural practice on raw products after harvest (6). Standard concentrations of 1, 5, 10% were used in the studies.

In vitro studies. The growth of three common cherry organisms, *Penicillium expansum*, *Rhizopus nigricans*, and *Botrytis* sp., was observed in the presence of the phosphate compounds by use of a disc diffusion method. Potato Dextrose Agar (Difco) at *p*H 5.6 was employed throughout. A spore suspension was obtained by washing 5- to 7-day-old Potato Dextrose Agar slant cultures grown at 24 C with a small amount of sterile distilled water. The suspension was then adjusted to 10^5 spores per ml with a Levy and Levy-Hauser improved Neubauer Ruling counting chamber. A 1-ml amount of the spore suspension was placed in a petri dish, 15 ml of Potato Dextrose Agar was added, and the spores were distributed by rotating the dish.

Filter-paper discs (modified Muller method; 7) 1.3 cm in diameter were heat-sterilized, saturated with the appropriate phosphate concentration, drained briefly, and spotted on a previously inoculated plate. Discs of the three concentrations were spotted on a single plate. Distilled water-saturated control discs were placed on separate plates. Plates were incubated for 5 days at 24 C and 85% humidity. The diameter of the zone of inhibition was used as the measure of effectiveness.

In vivo studies. Sour cherries (*Prunus cerasus*, variety Montmorency) were obtained from the Howell Experimental Orchard, Utah State University, at the red, firm-ripe stage of maturity. Immediately after harvesting (approximately 50% with pedicels and 50% without pedicels), they were stored in refrigerators at 1.1 C (34 F). After 4 hr of storage, the cherries were transported in a refrigerated trailer to the University laboratories, where they were held overnight at a temperature of 1.1 C and 94% relative humidity. The following day they were treated with the phosphate compounds. Individual lots of 2.5 lb of cherries were immersed for 2 min in solutions of 1, 5, or 10% concentrations of the phosphate compounds. The excess solution was then allowed to drain from the cherries before they were placed in compartmentalized new wooden boxes. Treated and untreated control cherries were then stored at 1.1 C.

Portions of the phosphate-treated and control cherries were inoculated after 5 days of storage at 1.1 C by immersion for 2 min in a mixed spore suspension composed of equal parts of *Botrytis*, *Penicillium*, and *Rhizopus* spores at 10^5 spores per ml. The cherries were then allowed to drain, placed in 1-quart glass containers, and stored at 1.1 C and 94% relative humidity. Four replications for each treatment were used.

All fruit was inspected daily during the first 3 weeks of storage and then once a week, for a total of 2 months, to determine the amount of fungal growth.

RESULTS

In vitro studies. The diameters of the fungal inhibitory zones produced by the various concentrations of the phosphates after 5 days of incubation are presented in Table 1. All four compounds were ineffective at concentrations of 1%. The 5% concentrations produced a small zone of inhibition and the 10% concentration produced the most inhibition. There were slight differences in effects of the 10% concentration on the three organisms. *P. expansum* was most resistant to HEX, *Botrytis* sp. was most resistant to TPP and TP, and the organims were about equally affected by TSPP. In general, TP produced the greatest inhibition, followed by HEX and TPP. The least effective compound was TSPP.

On incubation for an additional week, an opaqueness or cloudiness developed in the pre-

TABLE 1. *Effects of phosphate compounds on growth of Botrytis sp., Penicillium expansum, and Rhizopus nigricans on Potato Dextrose Agar (pH 5.6) at 24 C and 85% relative humidity after 5 days*

Compound	Concn (%)	Zone of inhibition (cm)[a]		
		Botrytis	*Penicillium*	*Rhizopus*
Sodium hexametaphosphate	1	—	—	—
	5	2.0	1.8	2.0
	10	2.8	2.4	2.8
Sodium tripolyphosphate	1	—	—	—
	5	1.9	1.8	2.0
	10	2.2	2.8	2.8
Sodium tetraphosphate	1	—	—	—
	5	2.0	1.9	2.0
	10	2.7	2.9	2.9
Tetrasodium pyrophosphate	1	—	—	—
	5	—	1.7	1.6
	10	1.8	1.9	1.8
Control (untreated)	—	—	—	—

[a] The zone measurement is the average of four replications and includes the diameter of the paper disc (1.3 cm). Dashes indicate that no inhibition occurred.

TABLE 2. *Effects of phosphate compounds on fungal growth of fresh Montmorency cherries stored at 1.1 C and 94% relative humidity*

Treatment	Concn (%)	Days in storage[a]					
		7	14	21	30	45	60
Sodium hexametaphosphate	1	−	−	+	+	++	++
	5	−	−	±	±	++	++
	10	−	−	±	±		
Control (untreated)		−	+	++	+++	+++	++++
Sodium tripolyphosphate	1	−	−	+	+	++	+++
	5	−	−	±	±	++	+++
	10	−	−	±	+	++	+++
Control (untreated)		−	+	++	+++	+++	++++
Sodium tetraphosphate	1	−	−	±	+	+	++
	5	−	−	−	+	+	++
	10	−	−	−	±	±	+
Control (untreated)		−	−	++	+++	+++	++++
Tetrasodium pyrophosphate	1	−	±	+	++	+++	+++
	5	−	±	+	++	+++	+++
	10	−	−	+	++	+++	++++
Control (untreated)		−	+	++	+++	+++	++++

[a] Symbols, − = no growth; ± = very slight growth; + = slight growth; ++ = sparse growth; +++ = moderate growth; ++++ = profuse growth.

TABLE 3. *Influence of combined spore suspensions of Botrytis sp., Penicillium expansum, and Rhizopus nigricans on phosphate-treated lots of cherries stored at 1.1 C and 94% relative humidity*

Treatment	Concn (%)	Days of storage[a]								
		5	10	15	20	25	30	40	50	60
Sodium hexametaphosphate	1	−	−	−	−	+	+	++	++	++
	5	−	−	−	−	−	+	++	++	+++
	10	−	−	−	−	−	+	+	++	+++
Control (untreated)		−	−	+	+	++	+++			
Sodium tripolyphosphate	1	−	−	−	−	+	+	++	+++	
	5	−	−	−	−	−	+	++	++	+++
	10	−	−	−	−	−	+	++	++	+++
Control (untreated)		−	−	+	+	++	+++			
Sodium tetraphosphate	1	−	−	−	−	+	+	++	++	+++
	5	−	−	−	−	−	+	+	++	+++
	10	−	−	−	−	−	−	+	++	++
Control (untreated)		−	−	+	+	++	+++			
Tetrasodium pyrophosphate	1	−	−	−	+	++	++	+++		
	5	−	−	−	+	+	++	+++		
	10	−	−	−	−	+	++	+++		
Control (untreated)		−	−	+	+	++	+++			

[a] Symbols: − = no fungal growth; + = one-fourth or less infected; ++ = about half infected; +++ = three-fourths or more infected.

viously clear inhibitory zone. A second week of incubation resulted in limited growth in this zone, especially with *P. expansum*. The initial activity of the phosphates would appear to inhibit spore germination. A few resistant spores eventually did germinate and mycelial development occurred. Mycelial development may also be somewhat inhibited, as evidenced by the long period required to demonstrate even limited growth.

In vivo studies. No fungal growth occurred on uninoculated cherries during the first week of storage (Table 2). The control lots showed slight fungal growth after 14 days of storage. Among the phosphate-treated lots, the TSPP treatments showed the first slight fungal growth at 14 days. After 21 days of storage, all controls exhibited considerable fungal growth, and the TSPP-treated fruits exhibited definite, but somewhat less, fungal growth. In general, the growth of fungi which might have existed as natural microflora on cherries at the time of harvesting, prior to treatment, was fairly slow. After 30 days of storage, there was a varying degree of fungal growth on all treated lots, with TP-treated lots showing the least growth, followed in order by HEX, TPP, and TSPP. All control lots of cherries showed extensive fungal growth, with the most growth being in the center of the mass. Most of the infected cherries in each lot were those without pedicels. Microscopic observations revealed that the infecting fungi were predominately *Botrytis* and *Penicillium* species. *Penicillium* was observed in all of the control lots and accounted for the greatest amount of fungal mass.

At 45 days of storage, the controls appeared about the same as at 30 days; however, the treated lots showed additional fungal growth. Almost all of the cherries showed gross infection after a storage period of 60 days, with the exception of the TP-treated lots, which were still in fair condition. The cherries retained their natural red color fairly well even after 60 days of storage. As in the in vitro studies, the order of decreasing effectiveness during prolonged storage was found to be TP, HEX, TPP, and TSPP.

The phosphate-treated cherries also withstood infection by the inoculated mixed spore suspension quite well (Table 3). Again, the TSPP-treated lots showed the first signs of growth. The 1 and 5% TSPP-treated lots showed signs of infection after 20 days of storage. Untreated controls were infected after 15 days of storage. Cherries receiving the other treatments all displayed fungal growth after 30 days of storage, with the exception of those receiving 10% TP treatment, which had no fungal growth until 40 days of storage. Cherries receiving this treatment, although showing signs of infection at 40 days of storage, were still considered to be approximately 50% marketable at 60 days of storage. All other treatments exhibited profuse fungal growth after 60 days of storage, and were considered unfit for human consumption. These results corroborated the in vitro work.

DISCUSSION

The sequestrant polyphosphate compounds inhibited the growth of fungi on enrichment media and on stored cherries. Treatment of cherries with a 10% dip solution extended storage life by 1 to several weeks, depending on the specific phosphate. Apparently, germination of the fungal spore is prevented in some manner. Eventually, a few resistant spores germinate and mycelial development commences. The in vitro studies indicated that even mycelial development is somewhat retarded, especially by the more active compounds, but eventually extensive growth occurs. The mechanism of action is unknown but, as is the case with bacteria, most likely involves chelation of metabolically essential metallic ions. Eventual organism growth may involve the degradation of the polyphosphate by resistant cells through the action of phosphatases and the release of the metallic ion. This has been observed (Post, *unpublished data*) in the case of pseudomonad resistance but needs to be tested more carefully.

Fungal growth eventually occurred on all naturally or deliberately contaminated lots of stored cherries. The dominant mold observed under these conditions of storage was *P. expansum*, and infection occurred most readily on fruit without pedicels in which access to the fruit interior was easier. The most effective phosphate was TP, followed by HEX at a concentration of 10%. Concentration refers to that of the solution in which the cherries were dipped, and not necessarily to what resulted on the cherry surface. Undoubtedly, only a small amount of material remained on the fruit surface after draining and drying. There is also some question about the degree of uniformity of residue achieved on the fruit surface. A 10% solution would make the process rather costly; however, refinement of data on amounts of residue, uniformity of residue distribution, and method of application could materially reduce the concentration requirements. More information on the spectrum and mode of action of the chelating phosphates on fungal spore germination and mycelial growth

may also lead to improved methods of application.

LITERATURE CITED

1. ALBERT, A. 1965. Selective toxicity, 3rd ed., p. 222–269. John Wiley and Sons, New York.
2. ELLIOTT, R. D., R. P. STRAKA, AND J. A. GARIBALDI. 1964. Phosphate inhibition of growth of pseudomonads from poultry meat. Appl. Microbiol. **12**:517–522.
3. FURIA, T. E. EDTA in foods: a technical review. 1964. Food Technol. **18**(12):2–10.
4. GRAY, G. W., AND S. G. WILKINSON. 1965. The action of ethylenediaminetetra-acetic acid on *Pseudomonas aeruginosa*. J. Appl. Bacteriol. **28**:153–164.
5. GUNSALUS, I. C., AND R. Y. STANIER [ed.]. 1962. The bacteria, vol. 4, The physiology of growth, p. 37–39. Academic Press, Inc., New York.
6. HAYLEY, P. C. [ed.] 1963. Official FDA clearances. Natl. Agr. Chem. Assoc. News Pesticide Rev. **22**(3):23.
7. MULLER, W. H. 1958. The influence of antibiotics on microorganisms causing fruit and vegetable rots. Am. J. Botany **45**(3):183–190.
8. POST, F. J., G. B. KRISHNAMURTY, AND M. D. FLANNAGAN. 1963. Influence of sodium hexametaphosphate on selected bacteria. Appl. Microbiol. **11**:430–435.
9. SALUNKHE, D. K., G. M. COOPER, A. S. DHALIWAL, A. A. BOE, AND A. L. RIVERS. 1962. On storage of fruits: Effects of pre- and post-harvest treatments. Food Technol. **16**(12):119–123.
10. VAN WASER, J. R. 1958. Phosphorus and its compounds, vol. 2, Technology, biology, functions, and applications. Interscience Publishers, Inc., New York.
11. VAN WASER, J. R., AND C. F. CALLIS. 1958. Metal complexing by phosphates. Chem. Rev. **58**:1011–1046.