Brine Fermentation of Cucumbers Treated with Sodium *o*-Phenylphenate

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Sodium *o*-phenylphenate, used as a fungicidal agent in hydrocooling cucumbers and added at the outset to fermentations of brined cucumbers in concentrations to 100 p.p.m., did not interfere with the natural acid fermentation as tested under laboratory conditions. Cucumber salt-stock quality, as judged by external and internal color, firmness, and bloater formation, was considered unaffected. *o*-Phenylphenol was adsorbed in very high concentrations on the skin of brined cucumbers, whereas the concentration in the brine was reduced to a negligible level. Further investigations are needed on the effectiveness of the chemical as a postharvest agent to prevent microbial spoilage as well as methods to control its high surface residue on the cucumbers.

During the past 20 years, there have been numerous publications on the effectiveness of *o*-phenylphenol and its sodium salt in reducing postharvest microbial spoilage of fruits and vegetables. Smith (1962b) reviewed many of these studies, and reported that *o*-phenylphenol was used in concentrations ranging from 0.1 to 6.0% in water solutions for fruits and vegetables by dipping, spraying, and hydrocooling methods, and was effective in reducing spoilage losses for citrus fruits, peaches, pears, sweet potatoes, papayas, and mangoes. In addition to the antimicrobial properties (Wolf, 1956) of *o*-phenylphenol, it has been reported (Hodge *et al.*, 1952) to have a low order of acute oral toxicity, thus lending itself for use with fruits and vegetables.

Information on the use of *o*-phenylphenol or its sodium salt as a possible antimicrobial inhibitor in postharvest handling of the common cucumber varieties used for commercial pickling has not been published. After a release in 1963 by the U.S. Food and Drug Administration (*Federal Register*, 1963) of a tolerance of 10 p.p.m. of *o*-phenylphenol in or on cucumbers, its use in hydrocooling of cucumbers under commercial conditions was initiated by some pickle manufacturers. The need for improved methods for postharvest handling of cucumbers to control microbial spoilage has been apparent with the increased production of pickling cucumbers grown in the southern states and with the long distances in transit to northern and midwestern packers.

Pickling cucumbers, after being treated with *o*-phenylphenol to prevent spoilage, are either processed into fresh-pack, pasteurized products, or salt-brined in wooden vats ranging in capacity from 200 to 1200 bushels for subsequent natural acid fermentation by *Lactobacillus plantarum* and other species (Etchells *et al.*, 1951). Turney (1964) observed a delayed natural fermentation of brined cucumbers previously treated with the chemical, and this prompted the investigation. Since *o*-phenylphenol is an effective antimicrobial agent (Smith, 1962b; Wolf, 1956), the question was raised as to the effect of a residue of 10 p.p.m., as granted by the Food and Drug Administration (1963), on the acid fermentation and general quality of the salt-stock cucumbers.

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MATERIALS AND METHODS

The source of *o*-phenylphenol (OPP) for these studies was a water-soluble sodium salt (Dow Chemical Co., Midland, Mich., Dowcide A, tetrahydrate of sodium *o*-phenylphenate, 97% pure). The sodium salt is referred to here as SOPP.

Hydrocooled Cucumbers. Model variety cucumbers (size 2A, $1^{3}/_{16}$ to $1^{5}/_{16}$ inches in diameter) were sampled during the hydrocooling operation at a receiving station in eastern North Carolina. The steps in the operation consisted of (1) water-spray washing as the cucumbers traveled along the receiving belt; (2) mechanical grading into different sizes according to diameter and then holding in 20-bushel capacity crates at air-temperature (approximately 30° C.); and (3) hydrocooling with ice water containing approximately 0.1% SOPP. The hydrocooler used was essentially the same type used for peaches and other fruits and vegetables (McClure, 1958; Smith, 1962b). It consisted of an open tank of ice water which was circulated by a pump to a perforated roof which permitted the ice water to flow down fairly evenly over and through the 20-bushel crates of cucumbers as they moved along on a metal track, two crates abreast and about eight crates deep. Water temperature in the recirculating tank was held at about 7° C. by replenishing the ice as needed. The cucumbers were exposed to the ice water containing the SOPP for approximately 15 minutes and were cooled from about 30° C. to 10° to 15° C. (internal cucumber temperature), with the lower temperatures relating to the smaller size cucumbers. Duplicate cucumber samples representative of each treatment were taken from the top 18 to 20 inches of the crates (Table I, Series 1), and were then packed into 1-gallon glass jars (40 to 45 cucumbers per jar). Wood tongue depressors cut to proper length were used to secure the stock just under the shoulder of the jar. The jars were then filled with 40° salometer brine (10.6% NaCl) and closed with 6-lug, 89-mm. diameter Twist-Off caps (The White Cap Co., Chicago, Ill.), previously fitted with $^{5}/_{8}$-inch rubber serum stoppers to provide for repeated withdrawal of brine samples by means of disposable syringes equipped with 22-gage needles. The brined experimental cucumbers were brought to the laboratory and held at 25° C. during fermentation and storage. A slit rubber tubing, closed at one end, was fitted to an 18-gage needle and into the rubber serum stopper of each

Table I. *o*-Phenylphenol, Total Acidity, pH, and Optical Density of the Fermenting Brines from Hydrocooled and SOPP Added Cucumber Treatments

Treatment[a]	Examination of Fermenting Brines for: *o*-Phenylphenol at	Total Acidity as Lactic after				pH after				Absorbance (10×) at	
	6 Days, P.P.M.	2 days, %	6 days, %	13 days, %	27 days, %	2 days	6 days	13 days	27 days	2 days	6 days
Series 1. Hydrocooled cucumbers											
A. Control, before spray	0	0.06	0.26	0.66	0.66	5.1	3.6	3.4	3.4	0.59	5.35
B. Control, after spray	0	0.10	0.26	0.68	0.72	5.4	3.8	3.4	3.4	0.22	4.85
C. Hydrocooled with SOPP	1.1	0.06	0.25	0.62	0.66	6.9	3.6	3.4	3.4	0.10	4.15
D. Same as C + 10 p.p.m. SOPP	2.4	0.05	0.28	0.58	0.62	6.2	3.6	3.4	3.4	0.15	4.05
Series 2. SOPP added to cucumbers and brine to equalize at											
E. Control, none	0	0.06	0.57	0.77	0.80	5.3	3.6	3.4	3.4	0.38	5.30
F. 5 p.p.m. (3.12)[b]	0.6	0.06	0.54	0.74	0.79	5.4	3.6	3.4	3.4	0.22	5.20
G. 10 p.p.m. (6.44)	0.7	0.05	0.54	0.76	0.78	5.4	3.7	3.4	3.4	0.38	5.60
H. 50 p.p.m. (32.2)	11.2	0.06	0.58	0.78	0.78	5.4	3.6	3.4	3.4	0.38	5.30
I. 100 p.p.m. (64.4)	21.5	0.07	0.50	0.76	0.78	5.4	3.7	3.4	3.4	0.42	4.30

[a] Averages of duplicate tests within each treatment. Series 2 was sampled one day earlier, except for 2-day period.
[b] As *o*-phenylphenol (OPP), 1 p.p.m. equals 1.55 p.p.m. of sodium *o*-phenylphenate · $4H_2O$ (SOPP). Cucumber-brine blanks gave 0.1 p.p.m OPP and was subtracted.

lot. This allowed the fermentation gases to escape, and prevented air from being introduced into the head space of the brined material.

Fermentation of Cucumbers with Increasing Concentrations of Sodium *o*-Phenylphenate (SOPP). Model variety cucumbers, size 2A, were obtained on the day of harvest from a receiving station in eastern North Carolina, held overnight at 10° C., then packed in 1-gallon glass jars and covered with 40° salometer brine (10.6% NaCl) as in the previous experiment. Increasing amounts of SOPP were added to duplicate lots of brined cucumbers and calculated to equalize at 0, 5, 10, 50, and 100 p.p.m. of the chemical (Table I, Series 2). The chemical was added immediately following the addition of the brine solution and the jars were sealed and rolled. The jar caps were prepared for brine sampling as described in the previous experiment. For all experimental fermentations, salt (NaCl) was added to the brine in small amounts during the first 12 days, until an equalized holding strength of 6.6 to 6.8% was reached.

Equalization of Sodium *o*-Phenylphenate in Cucumbers and Brine. Three treatments were set up in half-gallon glass containers: cucumbers, brine, and no SOPP; cucumbers, brine, and SOPP calculated to equalize at 100 p.p.m.; and water (to replace cucumbers), brine, and SOPP to equalize at 100 p.p.m. Model variety cucumbers, size 2A, were used and represented about 60% of the weight of the jar contents. Brine samples for SOPP determination were taken at 0, 3, 6, 12, 24, and 36 hours, and 19 days. The cucumbers were also sampled on the 19th day, and SOPP was determined on their skins (outer 2-mm. thickness) and on remaining inside tissue.

Brine Analysis. The nine fermentation treatments (A through I in duplicate) listed in Table I were samples as described by Etchells *et al.* (1964). Samples were taken at the initial period, at daily intervals during the first week, at the end of the 2nd and 4th week, and at the 7-month storage period. Total acidity as lactic, pH, absorbance as a measurement of bacterial growth, and salt (NaCl) content were determined (Etchells *et al.*, 1958). *o*-Phenylphenol (or its sodium salt) was measured on certain selected brine samples by the method of Gottlieb and Marsh (1946) as modified for fruit-dipping solutions by Smith (1962a). Measurements of the color development by the chemical were recorded at 510 mμ using a Beckman DU spectrophotometer and read from an *o*-phenylphenol standard curve.

Cucumber Analysis. Firmness, bloater content (hollow stock), and general quality of the fermented brine stock were determined after a 7-month storage period by procedures previously described (Bell *et al.*, 1955). *o*-Phenylphenol was determined by the method of Gottlieb and Marsh (1946), as modified by the Dow Chemical Co. (1961), on whole cucumber salt-stock and on stock which had been dissected into the skin portion (1- to 2-mm. thickness representing about 18% of the weight) and the inside flesh portion.

RESULTS

Effect of SOPP on Cucumber Fermentations. The treatments shown in Table I, where SOPP was introduced either into the fermentation brines by the hydrocooling operation (Series 1) or by direct addition to the brines in levels up to 100 p.p.m. (Series 2), gave essentially the same results as the control lots (A, B, and E) as shown by the values for total acidity, pH, and absorbance for each sampling period (2, 6, 13, and 27 days). The OPP content of the brines at the 6-day period (Table I) was much lower than expected from the amounts added for each treatment, assuming the chemical had equalized in the container. For example, the hydrocooled cucumber treatment (C) introduced 1.1 p.p.m. into the brine; yet,

an additional 10 p.p.m. gave a concentration in the brine of only 2.4 p.p.m. The same was true for treatments F through I, where 5 to 100 p.p.m. were added. Here, the levels in the brine were about one third the expected amounts. Also, in Table I, four brine-sampling periods for each series are presented as typical of the data for acidity and pH and two periods for absorbance to indicate the rate of fermentation. Total brine acidity, expressed as lactic, developed to above 0.6% with a drop in brine pH to 3.4 within 2 weeks. There was no indication of inhibition of the lactic acid bacteria by the different treatments using SOPP. The increase in absorbance of the brines during the first 6-day period for all treatments indicated a well developed acid fermentation. For each fermentation series, the observed differences between the treatments in acidity, pH, and absorbance of the brine samples for a given period were of such small magnitude as to be attributed to the variability between replicate fermentations. These results indicate that, under laboratory conditions and with a fairly low salting procedure (10.6% NaCl cover brine, held at 6.8%), the chemical, when introduced into brine fermentations through hydrocooling cucumbers or added directly to the brine at the outset in levels to equalize at up to 100 p.p.m., did not inhibit or retard the lactic acid fermentation as compared to natural controls.

Fate of SOPP in Brine-Cucumber Fermentations. As shown in Table I, the OPP content of the brines from the SOPP-treated lots at 6 days was about one third that calculated upon equalization. At 7 months, the OPP content of the brines was much lower, ranging from 0.1 to 1.8 p.p.m., showing a decrease to 1/50 or less from that expected of an equal distribution (Table II). The results of the analysis of the cucumber salt-stock explained, for the most part, the fate of the chemical. The hydrocooled cucumbers with SOPP (treatments C and D) contained 9.6 and 17.2 p.p.m. of OPP, respectively. The skin portions of the cucumbers from these treatments gave values of 35 and 38 p.p.m., and the inside fleshy portions, 9 and 15 p.p.m. This demonstrated an adsorption on the outer layer of the cucumber. Fermentation treatments E through I (Table II) revealed that about two thirds of the chemical was contained as part of the whole cucumbers—e.g., the 100-p.p.m. treatment (I) with the addition of 6.4 p.p.m. as OPP gave 46.8 p.p.m. for whole cucumbers.

To explain the fate of SOPP in cucumber-brine fermentations further, three treatments were followed for SOPP in the brine at close time intervals for 2 days, and then at 19 days the cucumbers and brine were tested for SOPP. The levels of SOPP in the brines for the first 36-hour period (Figure 1) revealed a rapid drop to less than one half the calculated concentration of 100 p.p.m. When the same amount of chemical was added to a second treatment, except that the cucumbers were replaced with water, the brine samples gave the expected calculated level of 100 p.p.m. (Figure 1). At 19 days, the brine from the cucumber treatment contained 25 p.p.m. of SOPP, whole cucumber 94, cucumber skin 219, and cucumber flesh 68. Thus, the amount found in the brine concentration was but one fourth that calculated upon equalization of the brine-cucumber mixture, whereas the cucumber skin contained over twice this amount.

Salt-Stock Quality. At the end of a 7-month storage period, the cucumber salt-stock from the two series listed in Table I was evaluated for external and internal color, per cent cure, texture, bloaters, and general characteristics such as typical fermentation odor. In the hydrocooling experiment (Series 1) with the four treatments in duplicate, no differences were found for the tests and observations listed above. The external color was rated fair to good, internal color good to excellent, and the cure was approximately 75%. The pressure tests for firmness were about 14 pounds, which indicates a rating of "Firm" and is considered acceptable texture. Bloater content was high for the control lots as well as the SOPP-treated lots, which frequently occurs with a natural fermentation. In the second series, with increasing levels of SOPP, the five treatments in duplicate (Table I) were essentially the same for salt-stock quality measurements as the above experi-

Table II. *o*-Phenylphenol Content of Skin, Flesh, and Whole Cucumbers and the Brine after 7 Months' Fermentation

Treatment[a]	Brine, P.P.M.	Whole, P.P.M.	Skin, P.P.M.	Flesh, P.P.M.
Series 1. Hydrocooled cucumbers				
A. Control, before spray	0	0	0	0
B. Control, after spray	0	0	0	0
C. Hydrocooled with SOPP	0.4	9.6	35	9
D. Same as C + 10 p.p.m. SOPP	0.6	17.2	38	15
Series 2. SOPP added to cucumbers and brine to equalize at				
E. Control, none	0	0	...	...
F. 5 p.p.m. (3.22)[b]	0	1.9	...	...
G. 10 p.p.m. (6.44)	0.1	4.8	...	...
H. 50 p.p.m. (32.2)	0.7	20.3	...	...
I. 100 p.p.m. (64.4)	1.8	46.8	...	...

[a] Averages of duplicate tests within each treatment.
[b] As p.p.m. *o*-phenylphenol.

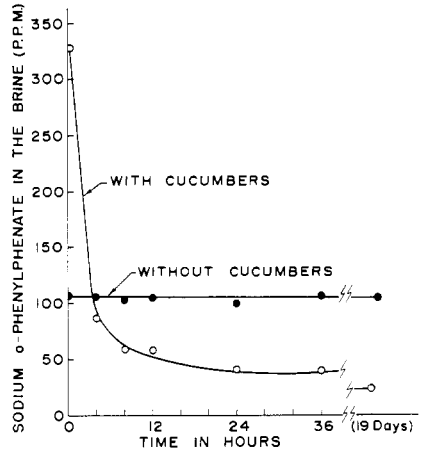

Figure 1. Effect of cucumbers on the equalization of sodium *o*-phenylphenate in 10.6% NaCl brine

ment. The external color of the stock was fair, internal color good, and the cure estimated to be 100%. The firmness ratings were 13 pounds (slightly below "Firm" category), and the stock was free of bloaters. All of the fermentation treatments in both series were typical of a good, clean, lactic acid–type cucumber brine and free of off odors. The higher levels of SOPP (treatment H and I) gave a slight bitter taste to the cucumber salt-stock, and this was understandable considering the amount of the chemical found on the skin portion (Table II).

DISCUSSION

Because of the numerous reports (Hayward and Grierson, 1960; McClure, 1958; Smith, 1962b) indicating the effectiveness of *o*-phenylphenol and its sodium salt as a post-harvest fungicide for fruits and vegetables, it is natural for the cucumber pickle industry to turn to this chemical to assist in solving shipping problems. Even though the U. S. Food and Drug Administration (1963) has released OPP for use on cucumbers at a tolerance level of 10 p.p.m., a number of problems connected with its use should be given attention. This study showed that the chemical did not, under laboratory conditions, interfere with the lactic acid fermentation. However, the study revealed a very high adsorption on the skin portion of the cucumber and some difficulty could be foreseen in controlling the Food and Drug tolerance level of 10 p.p.m. Experiments with oranges (Hayward and Grierson, 1960) have shown the importance of very strict control of SOPP concentration, pH, temperature, and time of exposure of the chemical solution in controlling the residue on the fruit. For example, a 0.1% SOPP solution at 4° C. for 30 minutes would leave a residue on oranges ca. six times higher at pH 10 than if the solution had been at pH 12. This factor could be especially critical, as the pH of the hydrocooler water would tend to decrease because of the carbon dioxide absorption from the air and cucumbers during the circulation of the solution, and would most likely give a much higher residue. In addition to the residue problems, the effectiveness of the chemicals to prevent spoilage of the cucumbers in transit and storage should be determined.

ACKNOWLEDGMENT

The authors express appreciation to the Tree Pickle Co., Inc., Cheektowaga, N.Y., and the Mt. Olive Pickle Co., Mount Olive, N.C., for their cooperation and the use of their facilities. The authors are also grateful to Lawrence J. Turney, H. W. Madison Co., Medina, Ohio, for his suggestions and for bringing the problem to our attention.

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Received for review May 1, 1967. Accepted June 28, 1967. The work was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, Ill. The U.S. Food Fermentation Laboratory is a laboratory of the Southern Utilization Research and Development Division, Agricultural Research Service, U.S.D.A. Approved for publication by the Director of the North Carolina Agricultural Experiment Station as paper 2358 of the Journal Series. Reference to a company or product name does not imply approval or recommendation of the product by the U S.D.A. to the exclusion of others that might be suitable.