

A Modified Gibbs Method for the Determination of 1 P.P.M. or Less of *o*-Phenylphenol in Fruits

L. R. LEINBACH and J. E. BREKKE
Western Regional Research Laboratory, Albany 10, Calif.

Interfering substances in fruits make the determination of trace amounts of *o*-phenylphenol residues difficult. The distillation apparatus reported here is more efficient than older designs, and by distillation from calcium carbonate buffered solutions, suitable recoveries can be obtained when 1 p.p.m. of *o*-phenylphenol or less is present. The method was found satisfactory for all six kinds of fruit included in the study.

THE FUNGICIDAL ACTIVITY of *o*-phenylphenol has been known for 25 years or more (8) and has been used commercially to inhibit growth of decay organisms on fruits (6, 10). Treatment of berry hallocks or boxes (made of thin wood shook) to suppress mold growth (2) led to an examination of analytical methods for detection of *o*-phenylphenol in berries held in treated hallocks. The Gottlieb (4) and Gibbs methods (3) led to somewhat erroneous results in the range 0.1 to 1.0 p.p.m. of *o*-phenylphenol, which was that encountered in the samples of berries from treated hallocks. Beshgetoor *et al.* (7) describe a procedure for detection of small amounts of phenols by the Gibbs method in a range as low as 0.01 to 0.2 p.p.m. of *o*-phenylphenol in industrial waste and river water. In this procedure a second distillation in the presence of CaCO_3 is recommended to adjust the pH to retain salicylic acid, where this may be present. Tomkins and Isherwood (9) determined *o*-phenylphenol in orange peel by a method useful in the range of about 10 to 20 p.p.m. Schiffman (7) has studied a method involving steam distillation and a color reaction with 4-aminoantipyrine, and concluded that lack of specificity and accuracy rendered the method of doubtful value. Harvey and Penketh (5) steam-distilled 500 to 1000 ml. from citrus pulp, extracted with cyclohexane and alkali solution, and determined *o*-phenylphenol by ultraviolet absorption measurements.

It is more difficult to determine *o*-phenylphenol in fruits than in water solutions, probably because of interfering materials. The procedure reported here involves steam distillation and colorimetric assay by the Gibbs indophenol method where recoveries of 85% or better can be obtained at the 1-p.p.m. level of *o*-phenylphenol added to fruits. Proper adjustment of the pH of the distillation mixture and the use of a more efficient steam still are innovations which represent improvements over previous methods. This procedure is

faster and simpler than methods described earlier.

Apparatus and Reagents

Volatile acid distillation apparatus, modified; electric blenders; photoelectric colorimeter; 2,6-dibromoquinone chloroimide.

The solutions for development of color are prepared as follows:

Gibbs Indicator Solution. Dissolve 40 mg. of the chloroimide dye in 250 ml. of 95% ethyl alcohol. Keep refrigerated when not in use. The solution keeps about 1 week. The powder, held refrigerated, keeps indefinitely.

Buffer Solution. Dissolve 15 grams of powdered anhydrous sodium tetraborate (28.4 grams of the decahydrate) in 900 ml. of distilled water at about 200° F.; to avoid bumping stir vigorously while the powder is being added. Add 3.3 to 3.6 grams of NaOH in the form of a strong solution (20 to 40%). Transfer to a 1-liter volumetric flask and make to volume. Five milliliters of the buffer solution when made to 100 ml. with distilled water should produce a pH of 9.6. If the pH varies more than 0.10, the buffer should be adjusted.

Copper Sulfate Solution. Dissolve 50 mg. of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 50 ml. of water, transfer to a 1-liter volumetric flask, and make to volume.

Figure 1 shows the apparatus used for steam distillation. A volatile acid distillation setup, Cash modification, with borosilicate glass (Braun-Knecht-Heiman Co.) was changed by adding part A, Figure 1. The large diameter spherical joint 55/30 provides access to the sample chamber so that chunks of fruit or thick purées can be easily introduced. A Nichrome heating coil is inserted into the outer jacket with the electrical connection through a rubber stopper in the bottom of the jacket, Figure 1. Water can be readily introduced through the same stopper. The heat input to the coil can be regulated by a rheostat.

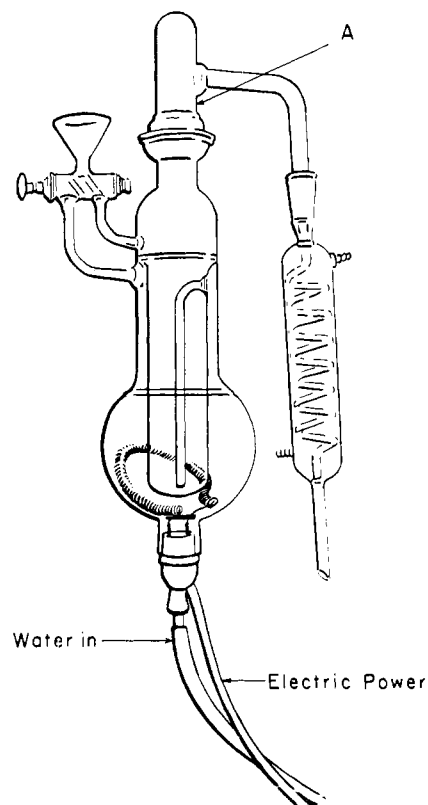


Figure 1. Modified steam distillation assembly

A. Part of standard apparatus rebuilt to allow for introducing piece samples or thick purées

Procedure

Homogenize the fruit in a blender, weigh out 100 grams (± 0.5 gram), and then stir in about 5 grams of powdered CaCO_3 . Transfer the mixture to the distillation apparatus, close with the spherical joint, and clamp. Introduce water from the bottom of the water jacket until the outside jacket of the still is almost full. Place at the condenser outlet a 100-ml. mixing cylinder or equivalent volumetric container. Ad-

Table I. Recovery of 1 P.P.M. of *o*-Phenylphenol Added to Various Fruits

(85-ml. distillate collected from 100-gram sample)

Fruit	% Recovery		
	Natural pH	Acid	CaCO ₃
Red raspberry purée	60	13	71
	75	42	78
	77	42	79
Strawberry purée	0	0	81
	0	0	76
	10	0	82
Prune purée	57	47	62
	74	35	79
	78	41	81
Boysenberry purée	48	9	62
	64	26	75
Fig purée	69	64	70
	74	65	81
Orange juice	85	28	85
	87	31	84

Table II. Effect of Volume of Distillate on Recovery of *o*-Phenylphenol from Strawberry and Raspberry Treated with CaCO₃

(4 samples of each tested)

	Mean % Recovery		
	85 ml.	170 ml.	340 ml.
Strawberry	65.8	79.0	89.8
Range	60-76	72-90	85-100
Raspberry	77.7	93.7	...
Range	77-79	91-96	...

just the rheostat to permit the rapid rise of temperature of the water to a vigorous boil. Close the stopcock on the side-arm and adjust rheostat for a steady but not too vigorous boil in the jacket water. Some materials froth at the start of the steam distillation; the power input can be controlled to minimize this, or a glass rod in the sample tube may serve as an antifoam device. Continue the distillation until 85 ml. of distillate has been collected.

Successive 85-ml. portions can be taken in a series of receivers. About 20 minutes is required for the distillation of 85 ml.

To the distillate in the 100-ml. volumetric receiver, add 5 ml. of the dye, 1 ml. of copper sulfate solution with some mixing, followed by 10 ml. of the buffer solution. The volume is made up with distilled water and the contents of the flask thoroughly mixed. The flask is now kept in the dark for at least 1/2 hour. A portion is placed in a colorimeter tube and the absorbance of the blue solution is measured, using a 600- μ filter. A reagent blank using distilled water in place of distillate is set at zero absorbance.

Standard Curve

A stock solution of *o*-phenylphenol is

prepared by dissolving 50 mg. of pure *o*-phenylphenol in 1 liter of distilled water. This solution is stable for a year or more. A working standard solution (10 μ g. per ml.) is prepared by diluting 200 ml. of the stock solution to 1 liter. Aliquot portions of the working standard solution (1 to 15 ml.) are added to a series of 100-ml. volumetric flasks containing 50 ml. of water to each flask. Add 5 ml. of the dye solution, 1 ml. of CuSO₄ solution, and 10 ml. of buffer as indicated in the procedure above. Dilute to volume (100 ml.) with distilled water. Place the flasks in the dark for 1/2 hour, during which time blue color develops. The absorbance of the blue solution at 600 μ is measured; a reagent blank is used for setting the colorimeter or photometer at zero absorbance. The absorbance measurements follow Beer's law up to 1.5 p.p.m. of *o*-phenylphenol. The blue color develops properly if the pH of the reaction mixture is in the range 9 to 10.

Results and Discussion

Known amounts of *o*-phenylphenol could not be recovered when acid was added to the fruit sample, as specified in previous descriptions of the method (7, 5, 9). Accordingly, distillation was tried at the natural pH of the fruit. Recoveries on some fruits were still low. Better recoveries were obtained from samples adjusted to pH 5.5 to 6.5. To raise the pH, addition of approximately 5 grams (1 heaping teaspoonful) of CaCO₃ to 100 grams of fruit purée was convenient and effective. For each fruit of the six examined, the *o*-phenylphenol recoveries were determined, respectively, at natural pH, with acid added (15 ml. of 10% H₃PO₄ added to sample), and with 5 grams of CaCO₃ added to 100 grams of fruit purée. Blank values were in the range 0 to 0.1 p.p.m. Portions of the same fruit samples were run after an amount of *o*-phenylphenol equivalent to 1 p.p.m., based on the fruit weight, was added in each case. The blanks were deducted from the values of the test samples to give the net values, and thus the per cent of recovery. Eighty-five milliliters of distillate was collected in a series of samples to observe the effects of pH control. Table I presents the results, which show that distillation from low pH samples is extremely variable and usually low, especially for strawberries.

In only one sample (orange juice) was the recovery higher at natural pH than at the pH of the sample mixed with excess CaCO₃. All other tests showed a consistently higher recovery from the samples treated with CaCO₃ to raise the pH of the material in the still pot. Typical pH values of residues in the still pots after distillation were as follows:

	Acid	Natural	CaCO ₃
Strawberry	1.9	3.4	6.5
Red raspberry	1.8	3.5	6.7
Orange	2.1	3.8	5.6

The recovery values noted in Table I illustrate the effect of pH, but in some cases the recoveries achieved by taking only 85 ml. of distillate are somewhat below what is considered satisfactory for an analytical method. Successive 85-ml. portions of distillate were taken from samples of strawberry and raspberry purées treated with CaCO₃ to establish what volume of distillate would be required to give 85 to 90% recovery of 1 p.p.m. of added *o*-phenylphenol. These data are in Table II. Enough additional *o*-phenylphenol is recovered in the second 85-ml. increment of distillate from raspberries to justify routinely collecting 170 ml. Collection of about 340 ml. of distillate is necessary in the case of strawberries. If a portion larger than 85 ml. is collected, a proportionately larger amount of each reagent (dye, CuSO₄, and buffer) is added, the procedure otherwise being the same.

The distillation apparatus may be readily flushed with water after each run. If small amounts of fruit material accumulate on the walls of the distillation chamber, it can be cleaned out by steam-distilling a solution of alkali or detergent for about 30 minutes, or by brushing after removing the spherical joint at the top.

Acknowledgment

The authors gratefully acknowledge the assistance of Victor H. Ortegren in modifying the glass distillation apparatus.

Literature Cited

- (1) Beshgetoor, A. W., Green, L. M., Stenger, V. A., *Ind. Eng. Chem., Anal. Ed.* **16**, 694 (1944).
- (2) Brekke, J. E., Wolford, E. R., Sacklin, J. A., Johnson, F., *Food. Eng.* **29**, 95 (1957).
- (3) Gibbs, H. D., *J. Biol. Chem.* **72**, 649 (1927).
- (4) Gottlieb, S., Marsh, P. B., *Ind. Eng. Chem., Anal. Ed.* **18**, 16 (1946).
- (5) Harvey, D., Penketh, G. E., *Analyst* **82**, 498 (1957).
- (6) Reynolds, H. J., *Down to Earth* **8** (4), 8 (1953).
- (7) Schiffman, C. D., *J. Assoc. Offic. Agr. Chemists* **41**, 578 (1958).
- (8) Sharma, J. N., South African Patent **1255** (1935).
- (9) Tomkins, R. G., Isherwood, F. A., *Analyst* **70**, 330 (1945).
- (10) Wolf, P. A., *Down to Earth* **12** (2), 16 (1956).

Received for review July 17, 1960. Accepted November 10, 1960. Work done at a laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Mention of a specific brand or supplier does not constitute a recommendation by the U.S.D.A. over others of similar nature.