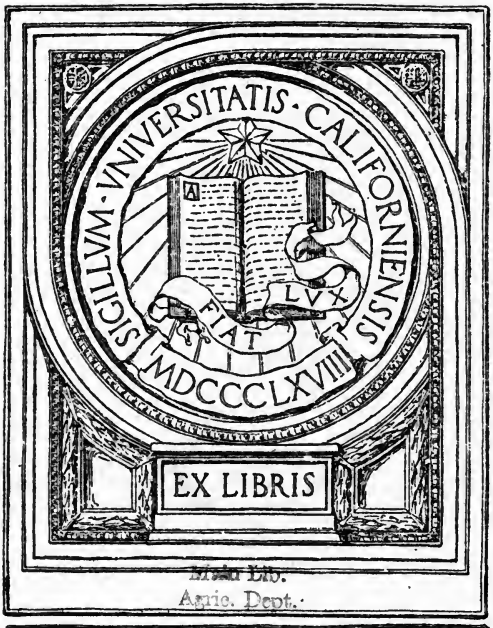

United States Department of Agriculture,

BUREAU OF CHEMISTRY—Circular No. 28.

H. W. WILEY, Chief of Bureau.

PROVISIONAL METHODS FOR THE DETERMINATION OF FOOD PRESERVATIVES AS AUTHORIZED BY THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1905.

INTRODUCTION.

The following revision of the provisional methods for the determination of preservatives has been made in accordance with the action taken by the association at the convention of 1905, when the following motion was passed:

Resolved, That the rearrangement of the provisional methods for the detection of preservatives suggested by the referee be adopted.

In order to carry out these instructions it was necessary to rewrite some sections, and the revision also includes all changes and additions to these methods made since Bulletin No. 65, Provisional Methods for the Analysis of Foods, was approved at the meeting of the association in 1901.

W. D. BIGELOW,
Chief, Division of Foods, and
Referee on Food Adulteration, A. O. A. C., 1901–1905.

Approved.
JAMES WILSON,
Secretary of Agriculture.

WASHINGTON, D. C., *April 9, 1906.*

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CONTENTS.

FOOD PRESERVATIVES.

1. Salicylic Acid.

(a) qualitative detection.

If the material be a solid or semisolid, macerate from 200 to 300 grams in a mortar with about 400 cc of water made slightly alkaline and strain through a cotton bag or separate by means of a centrifuge. Acidify the liquid (or the original sample if it be a liquid) with dilute (1:3) sulphuric acid and extract with ether or chloroform. If an emulsion is formed it may be broken up by the addition of more ether or by whirling in a centrifuge. The ether or chloroform should be washed twice with about one-tenth its volume of water to remove the least trace of sulphuric acid, transferred to a small porcelain dish, and allowed to evaporate at a low temperature. The residue is taken up with a small volume of hot water. It may be divided into several portions, some of which may be used for the detection of other preservatives. A small amount of salicylic acid occurs naturally in many fruits and the portion of the extract used for its detection should represent not more than 50 cc of wine or 50 grams of fruit. A reaction obtained with this amount is due to added salicylic acid.

In testing for salicylic acid two or three drops of a one-half per cent solution of ferric chlorid or of a 2 per cent ferric alum solution are added to the solution of the residue from the ether in a small porcelain dish. In case of the presence of a considerable amount of tannin or other bodies giving a precipitate or color with ferric salts the residue left on the evaporation of the ether when perfectly dry may be extracted with gasoline of low boiling point, the gasoline extract allowed to evaporate, and salicylic acid detected by ferric solution, as indicated above.

In the case of materials containing large amounts of extractive matter and those from which the aqueous solution can not be separated from the solid matter by straining or centrifuging, it may be found necessary to separate the salicylic acid by distillation. This is especially true with foods rich in fat. In such cases acidify the macerated material with phosphoric acid and transfer to a distilling flask with a very short neck and wide mouth. An Erlenmeyer flask with inside diameter of mouth $1\frac{1}{4}$ inches is a good shape. The tube connecting the flask with condenser should be very short, with an inside diameter of not less than three-eighths of an inch.

Conduct steam through a small tube passing through the stopper and dipping deeply into the material in the flask. The distillation of the salicylic acid is facilitated by submerging the distilling flask almost to the stopper in an oil bath and distilling with the temperature of the oil at from 120° to 130° C., or by adding about 20 grams of sodium chlorid to the contents of the flask, for each 100 cc of the substance, to raise the boiling point. Care must be taken not to let the contents of the flask get too low, as the heat will decompose the organic matter.

Distill 500 to 600 cc, make alkaline, evaporate to small volume, acidify with dilute sulphuric acid, extract with about 50 cc of ether, and proceed as directed above. In the presence of a considerable amount of salicylic acid a reaction can usually be obtained by adding a few drops of the ferric solution directly to the first 200 cc of the distillate.

Salicylic acid may often be separated from fat extracted with the ether by washing the ether solution with dilute ammonia. The aqueous liquid is then evaporated almost to dryness and tested with ferric solution, as directed above.

In the case of foods which yield to the gasoline solution of the ether residue a color that obscures the ferric chlorid reaction (e. g., tomatoes) the ether solution may be evaporated, the residue dried in a desiccator or in a current of dry air, sublimed, and collected on a watch glass cooled with ice. The sublimate is then dissolved in hot water and tested with ferric alum, as described above.

The same difficulty may often be avoided, and in fact the extraction with gasoline of the dry residue from the ether extraction may be obviated, by precipitating before extraction

with ferric chlorid or calcium chlorid, making alkaline, and filtering. By this means tannin is entirely separated from the product and other substances whose color masks the salicylic acid reaction are often removed. It is sometimes found convenient in the presence of a considerable amount of fat to wash the ether solution, by shaking in a separatory funnel, with water made alkaline with ammonium hydroxid.[a] Salicylic acid is thus removed completely, while the fat remains dissolved in the ether. If the excess of ammonia be not too great, evaporate the ammonia solution in a dish over the water bath until all free ammonia has disappeared, and test for salicylic acid with ferric solution as directed above.

(b) QUANTITATIVE DETERMINATION.

Extract in a separatory funnel 100 cc of the sample [b] [in the case of solids and semisolids use the aqueous solution prepared from the sample as described under (a)] with 75 cc of sulphuric ether, after the addition of 2 or 3 cc of dilute (1 : 3) sulphuric acid. Separate the clear, aqueous solution, and if any emulsion is present give the separatory funnel a quick, vigorous shake and allow to settle again. If the emulsion is not broken up in this way it may be accomplished by means of a centrifuge or by adding 10 or 15 cc of low boiling point gasoline or petroleum ether and shaking again.

The clear, aqueous portion is then united with the first, and the ether is poured into another separatory funnel, care being taken that none of the aqueous portion be left with the ether. The aqueous portion is returned to the separatory funnel and again extracted with 75 cc of ether, following the same procedure as before. This operation is then repeated twice, making four separate extractions with ether in all.

In case of special difficulty in breaking up the emulsion in any of the extractions a small amount of ether may be allowed to remain with the aqueous portion rather than the reverse, as it is removed in successive extractions. Wash the combined ether extracts by shaking in a separatory funnel with one-tenth their volume of water (using, however, not less than 20 cc of the water at each washing). Care must be taken at each washing to separate the aqueous portion completely from the ether, but none of the ether should be allowed to run into the wash water. In introducing the water into the separatory funnel care should be taken to wash off the stopper and the neck of the funnel to remove any adhering mineral acid.

Distill slowly the greater part of the ether, transfer the remainder to a porcelain dish, and allow to evaporate spontaneously. Thoroughly dry in a vacuum desiccator [c] over sulphuric acid, extract the dry residue with ten portions of 10 or 15 cc each of low boiling point gasoline or petroleum ether, rubbing the contents of the dish with a glass rod or other suitable instrument and transferring the successive portions of solvent to a second porcelain dish. The extracted residue should finally be tested with a drop of ferric alum solution, and if any reaction for salicylic acid be given the residue should be taken up in water, reextracted with ether, and the operation repeated. The gasoline extract is finally allowed to evaporate spontaneously.

Dissolve the residue in a small amount of hot water and dilute to a definite volume. Dilute aliquot portions of the solution and match in Nessler tubes, or with a colorimeter, the color obtained by adding a few drops of dilute ferric chlorid or ferric alum solutions with that of a standard solution of salicylic acid. If a colorimeter be employed, the solution whose color is compared should contain about 1 milligram of salicylic acid in 50 cc. If the color be compared in Nessler tubes without the use of a colorimeter, the solution may advantageously contain about 2 milligrams in 50 cc. To 50 cc of the diluted solution add ferric solution until no further color develops.

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[a] Allen, Commercial Organic Analysis, 2d ed., *4:* 188.

[b] If the qualitative examination has shown a very large amount of salicylic acid to be present, 50 cc or the aqueous extract of 50 grams of sample will be found sufficient and 40 cc of ether may be employed for its extraction.

[c] In examining a substance whose ether extract does not give a color or precipitate with ferric solution, the drying of the residue and its extraction with gasoline may be omitted. The residue may then be transferred by means of warm water directly from the distilling flask to the graduated flask, in which it is made up to a definite volume. Substances interfering with the ferric reaction may often be removed by precipitation with ferric chlorid or lime, as directed at the foot of page 3.

In the case of ferric chlorid a one-half per cent solution should be employed. A 2 per cent solution of ferric alum may be used.[a] In either case, and especially with ferric chlorid, an excess of reagent should be avoided, although an excess of 0.5 cc of 2 per cent ferric alum solution may be added to 50 cc of the solution of salicylic acid without impairing the results.

A correction should finally be made for the salicylic acid removed from the ether by the water with which it is washed. This amounts to about 1.5 per cent of the total salicylic acid present. If the utmost possible accuracy is desired, the method may be repeated on the wash waters, thus practically eliminating the error due to the removal of salicylic acid by the wash water.

Where the nature of the substance is such that extraction by organic solvents is not practicable, as in the case of the presence of a large amount of fat, the salicylic acid may first be separated by distillation, as described, under its qualitative estimation. In such cases it is necessary to collect at least 600 cc of the distillate and continue the distillation until the last 200 cc of the distillate gives no color on the addition of a drop of ferric solution. The distilling apparatus should in all cases be tested with known amounts of salicylic acid in order to determine the amount of distillate necessary to carry over a definite weight of salicylic acid.

It is sometimes practicable to determine the salicylic acid directly in the distillate by the colorimetric method with ferric chlorid given above. If mineral acid used in the distillation be carried over mechanically, however, the accuracy of the method is greatly impaired. Salicylic acid may be recovered from the distillate, after making alkaline and evaporating if desired, by extraction with ether and estimating colorimetrically as directed above.

2. Benzoic Acid.

(a) qualitative detection.

Separate benzoic acid as directed for salicylic acid. If benzoic acid be present in considerable quantity, it will crystallize from the evaporated ether in shining leaflets with characteristic odor on heating. Dissolve the residue in hot water, divide into two portions, and test by the following methods:

(1) *First method.*[b]

Make the residue alkaline with ammonium hydroxid, expel the excess of ammonia by evaporation, take up the residue with water, and add a few drops of a neutral 0.5 per cent solution of ferric chlorid. The presence of benzoic acid will be indicated by the formation of a brownish-colored precipitate of ferric benzoate.

(2) *Second method.*[b]

Evaporate to dryness and treat the residue with 2 or 3 cc of strong sulphuric acid.[c] Heat till white fumes appear; organic matter is charred and benzoic acid is converted into sulphobenzoic acid. A few crystals of potassium nitrate are then added. This causes the formation of metadinitrobenzoic acid. When cool, the acid is diluted with water and ammonia added in excess, followed by a drop or two of ammonium sulphid. The nitrocompound becomes converted into ammonium metadiamidobenzoic acid, which possesses a red color. This reaction takes place immediately, and is seen at the surface of the liquid without stirring.

(b) quantitative estimation.

Evaporate the ether extract obtained as directed under salicylic acid to dryness, thoroughly dry in sulphuric acid desiccator (preferably in vacuum) and sublime under a watch

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[a] This solution should be boiled until a precipitate appears, allowed to settle, and filtered. The acidity of the solution is slightly increased in this manner, but so precipitated it keeps clear for a considerable time, and the turbidity caused by its dilution with water is much less and does not appear for a much longer time than if the unboiled solution be employed. This turbidity is especially objectionable in the quantitative estimation of salicylic acid, as it interferes with the exact matching of the color.

[b] Möller, Bul. soc. chim. Par., 1890 (3), *3:* 414.

[c] If this be the only method employed, the sulphuric acid may be added directly to the residue left on the evaporation of the ether.

glass cooled with a piece of ice, or with a condenser the lower end of which is closed with a piece of rubber dam. Or the ether extract (or its solution in gasoline) may be transferred into the tube *a*, as shown in the accompanying figure, the ether or gasoline removed by a gentle current of air, the tube placed in a vacuum desiccator till its contents are thoroughly dry, and the residue sublimed at the temperature of 250° C., the sublimate being collected in tube *b*. During the sublimation, air is drawn very slowly through the apparatus (a wash bottle is used to gauge the speed of the current) to insure the volatilized benzoic acid passing into tube *b*. The joint between the two tubes is preferably made by means of a cork stopper. The most satisfactory results are obtained by placing the tube *a* inside of an oven

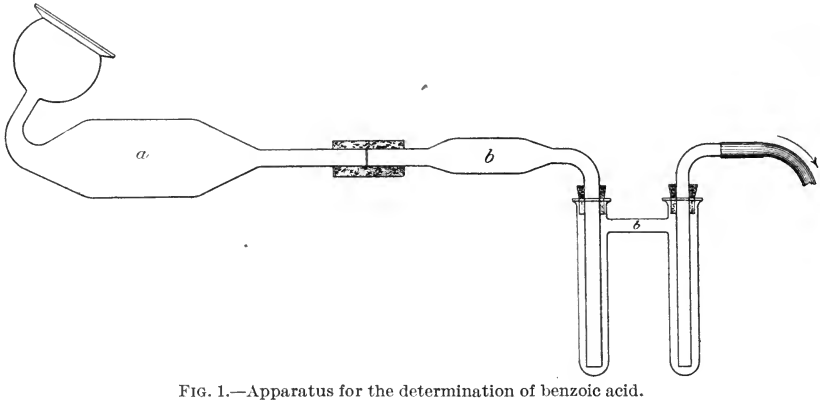

FIG. 1.—Apparatus for the determination of benzoic acid.

the temperature of which is raised gradually until it reaches 250° C. The bulb of the tube *b* should be just outside of the oven, in order that the crystals may form therein. By means of this apparatus considerably higher results were obtained than by subliming on a watch glass, as described above.

The sublimate of benzoic acid collected in tube *b* may be removed by solution in alcohol, and the amount confirmed by titration. Before applying the method to any class of foods, blank experiments should be made to determine whether a sublimate is obtained under the same conditions from the ether extract of that class of foods.

3. SACCHARIN.

(a) QUALITATIVE DETECTION.

Extract with ether (after maceration and exhaustion with water if necessary), as described under salicylic acid. Allow the ether extract to evaporate spontaneously and note the taste of the residue. The presence of saccharin to the amount of 20 milligrams per liter is indicated by an intense sweetness. This may be confirmed by heating with sodium hydroxid, as described below, and detecting the salicylic acid formed thereby. Results by this method indicating the presence of a faint trace of saccharin in wines which did not contain it have been frequently obtained, owing to the presence in wine of so-called "false saccharin."

Acidify 50 cc of a liquid food (or the aqueous extract of 50 grams of a solid or semisolid, prepared as directed on page 3) and extract with ether. Test the extracted matter in the usual way for salicylic acid, return the gasoline extract to the dish containing the residue, dilute the whole to about 10 cc volume, and add 2 cc of sulphuric acid (1:3). Bring the solution to the boiling point and add a 5 per cent solution of potassium permanganate, drop by drop, to slight excess; partly cool the solution, dissolve in it a piece of sodium hydroxid, and filter the mixture into a silver dish (silver crucible lids are well adapted to the purpose); evaporate to dryness and heat for twenty minutes at 210° to 215° C. Dissolve the residue in water, acidify and extract with ether, evaporate the ether and test the residue with two

drops of a 2 per cent solution of ferric alum. By this method all the so-called false saccharin and the salicylic acid naturally present (also added salicylic acid when not present in too large amount) are destroyed, while 5 milligrams of saccharin per liter is detected with certainty.

(b) QUANTITATIVE ESTIMATION.[a]

(1) *First method.*

Extract as directed under the quantitative estimation of salicylic acid. Allow the ether extract to evaporate spontaneously, heat with sodium hydroxid as directed above, let cool, dissolve in a small amount of water, acidify and extract with ether, observing the precautions given under salicylic acid. Determine the salicylic acid formed as directed under the quantitative estimation of salicylic acid. The conversion of saccharin into salicylic acid is not complete, and the analyst must determine a correction for his apparatus and conditions with known amounts of saccharin. At best the results obtained by the method are only approximate.

(2) *Second method.*

Extract as directed above, but acidify with hydrochloric or phosphoric acid instead of sulphuric acid, determine the weight of sulphur in the residue, and multiply by 5.712 for the weight of saccharin, expressed in grams. The results obtained by this method are only approximate.

4. BORIC ACID AND BORATES.

(a) QUALITATIVE DETECTION.[b]

Render decidedly alkaline with lime water about 25 grams of the sample and evaporate to dryness on a water bath. Ignite the residue to destroy organic matter. Digest with about 15 cc of water and add hydrochloric acid, drop by drop, to acid reaction, then add about 1 cc of concentrated hydrochloric acid. Moisten a piece of delicate turmeric paper with the solution; if borax or boric acid is present, the paper on drying will acquire a peculiar red color, which is changed by ammonia to a dark blue-green, but is restored by acid.

A preliminary test may be made by immersing a strip of turmeric paper in about 100 cc of liquid foods, to which about 7 cc of concentrated hydrochloric acid has been added. Solid and pasty foods may be heated with enough water to make them thoroughly fluid, hydrochloric acid added in about the proportion of 1 to 15, and tested in the same manner.

(b) QUANTITATIVE ESTIMATION.[c]

Render 100 grams of the sample decidedly alkaline with sodium hydroxid and evaporate to dryness in a platinum dish. Ignite the residue thoroughly, heat with about 20 cc of water, and add hydrochloric acid, drop by drop, until all is dissolved. Transfer to 100-cc flask, the volume not being allowed to exceed 50 to 60 cc. Add 0.5 gram of calcium chlorid and a few drops of phenolphthalein, then a 10 per cent solution of caustic soda until a permanent slight pink color is produced and finally 25 cc of limewater. Make the volume up to 100 cc. Mix well and filter through a dry filter. To 50 cc of the filtrate add normal sulphuric acid till the pink color disappears, then methyl orange, and continue the addition of the acid until the yellow is just changed to pink. Then add fifth-normal caustic soda till the liquid assumes the yellow tinge, excess of soda being avoided. Boil to expel carbon dioxid. Cool the solution, add a little phenolphthalein; and an equal volume of glycerin. Titrate with standardized sodium hydroxid until a permanent pink color is produced.

One cubic centimeter of fifth-normal soda solution is equal to 0.0124 gram crystallized boric acid.

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[a] For the purpose of confirmation valuable data can be secured with some products by weighing the material extracted by ether and also by titrating it with standard alkali, using phenolphthalein as indicator. One cubic centimeter of decinormal alkali is equivalent to 0.0183 gram of saccharin.

[b] U. S. Dept. Agr., Division of Chemistry, Bul. 51, p. 113.

[c] Thomson's method, Sutton's Volumetric Analysis, page 100.

5. Formaldehyde.

(a) Preparation of sample.

If the material be solid or semisolid, macerate from 200 to 300 grams in a mortar with about 100 cc of water until a sufficient degree of fluidity is obtained. Transfer to a short-necked distilling flask of copper or glass of from 500 to 800 cc capacity and make distinctly acid with phosphoric acid. Connect flask with glass condenser and distill from 40 to 50 cc. In the case of liquids acidify with phosphoric acid and distill.

(b) Phenylhydrazin hydrochlorid method.[a]

Mix 5 cc of the distillate as prepared under (a), or of an alcoholic solution or extract from the substance under examination, with 0.03 gram of phenylhydrazin hydrochlorid, and 4 or 5 drops of a 1 per cent solution of ferric chlorid. Add slowly and with agitation in a bath of cold water to prevent the heating of the liquid, from 1 to 2 cc of concentrated sulphuric acid. A precipitate is formed which can be dissolved by the addition of either concentrated sulphuric acid, keeping the mixture cool, or alcohol. With meats and fats the formaldehyde should first be extracted with alcohol and the filtrate tested. In the case of fat it is necessary to heat the mixture above the melting point of the fat to insure thorough extraction. Milk is shaken with an equal volume of strong alcohol and the filtrate employed. Other liquids are shaken with an equal volume of strong alcohol and filtered in case of the formation of any insoluble matter.

In the hands of different analysts this method is found to give reliable reactions for formaldehyde in solutions of formaldehyde varying from 1 part in 50,000 to 1 part in 150,000. Acetic aldehyde and benzaldehyde give no reaction when treated by this method and do not interfere with the reaction given by formaldehyde.

(c) Phenylhydrazin hydrochlorid and ferricyanid method.[b]

This method may be applied directly to liquid foods or to an aqueous or alcoholic extract of solid foods. To from 3 to 5 cc of liquid food or extract of the same add a lump of phenylhydrazin hydrochlorid about the size of a pea, from 2 to 4 drops (not more) of a 5 to 10 per cent solution of potassium ferricyanid, and from 8 to 12 drops of an approximately 12 per cent solution of sodium hydroxid. The method is not applicable to preparations containing blood-coloring matter. In such cases nitroprussid may be used in place of the ferricyanid. Alcoholic extracts from foods must be diluted with water to prevent the precipitation of potassium ferricyanid.

Milk may be examined directly. Meat may be finely comminuted, extracted with 2 volumes of hot water, and the liquid pressed out and employed for the test. Fats are warmed above the melting point with 10 cc of alcohol (from 80 to 95 per cent) thoroughly shaken, cooled, and filtered with a moistened paper, and the filtrate employed.

When formaldehyde is present to the extent of more than 1 part in 70,000 to 80,000 in the solution tested, a distinct green or bluish green reaction is obtained. In more dilute solutions the green tint becomes less marked and a yellow tinge tending toward greenish brown is formed.

With this method acetic aldehyde and benzaldehyde give a color varying from red to brown, according to the strength of the solution. A reaction may, therefore, be obtained with these aldehydes similar to that obtained with formaldehyde in solutions more dilute than 1 part in 70,000. The presence of acetic aldehyde or benzaldehyde together with formaldehyde gives a yellowish or yellowish green tinge. The reaction for formaldehyde may, therefore, be masked by the presence of other aldehydes, but is characteristic when a clear green color is obtained.

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[a] Arnold and Mentzel, Zts. Nahr. Genussm., 1902, *5:* 353.

[b] Arnold and Mentzel, Chem. Ztg., 1902, *26:* 246; Abs. J. Chem. Soc., 1902, (2), *82:* 367; Abs. Chem. Centrbl., 1902, *73*, pt. 1: 1077.

(d) HEHNER'S METHOD.[a]

To the milk to be tested add strong commercial sulphuric acid without mixing, and at the junction of the two liquids a violet or blue color will appear if the milk contains one or more parts of formaldehyde per 10,000. This color is supposed to be given only when there is a trace of ferric chlorid or other oxidizing agent present. As pointed out by Hehner, milk may be treated directly by this method without any other operation, and some other articles of food rich in proteids, e. g., egg albumen, give the reaction in the presence of water without the addition of milk. The distillate described above may be mixed with milk and this test applied.

(e) LEACH'S METHOD.[b]

Add about 5 cc of the distillate obtained under (a) to an equal volume of pure milk in a porcelain casserole and about 10 cc of concentrated hydrochloric acid containing 1 cc of 10 per cent ferric chlorid solution to each 500 cc of acid. Heat to 80° or 90° directly over the gas flame, holding the casserole by the handle and giving it a rotary motion to break up the curd. A violet coloration indicates formaldehyde.

(f) RIMINI'S METHOD.[c]

Treat 15 cc of milk or other liquid food under examination or of the distillate prepared as directed under (a) with 1 cc of dilute solution of phenylhydrazin hydrochlorid; then with a few drops of dilute ferric-chlorid solution; and finally, with concentrated hydrochloric acid. The presence of formaldehyde is indicated by the formation of a red color, which changes after some time to orange yellow.

This method is suitable for the examination of milk without previous treatment, but more sensitive results may be obtained from the distillate from milk or from milk serum. The reaction is not interfered with by acetic aldehyde or benzaldehyde.

(g) PHLOROGLUCIN METHOD.[d]

Prepare the reagent by dissolving 1 gram of phloroglucin and 20 grams of sodium hydroxid in sufficient water to make 100 cc. To 10 cc of milk or other liquid food under examination in a test tube add, by means of a pipette, 2 cc of this reagent, placing the end of the pipette on the bottom of the tube in such a manner that the reagent will form a separate layer.

A bright red coloration (not purple) is formed at the zone of contact if formaldehyde be present. This solution gives a yellow color in the presence of some other aldehydes, and if it be used for the detection of aldehyde formed by the oxidation of methyl alcohol after the destruction of ethyl aldehyde with hydrogen peroxid an orange-yellow color will slowly appear when an insufficient amount of hydrogen peroxid has been employed. On the other hand, if the excess of hydrogen peroxid be not fully destroyed before the use of this reagent a purple color will slowly form. The clear, red color given by the use of this reagent forms quickly, and in the presence of but a small amount of formaldehyde soon fades.

(h) RESORCIN METHOD.

This method has been suggested for the detection of formaldehyde formed by the oxidation of methyl alcohol in flavoring extracts. Add to the liquid, prepared as directed under flavoring extracts in Bulletin 65, 1 drop of a solution containing 1 part of resorcin in 200 parts of water, and pour the mixture cautiously into a test tube containing 3 cc of concentrated sulphuric acid, holding the tube in an inclined position in such a manner that the two liquids shall not mix. Allow it to stand three minutes, then sway the tube slowly from side to side in such a manner as to produce a gentle rotary motion of the two

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[a] Analyst, 1895, *20:* 155.

[b] Leach, Twenty-ninth Ann. Rept. Mass. Board of Health, 1897, p. 558.

[c] Ann. di Farmacol., 1898, 97; Abs. Chem. Centrbl., 1898, *69*, pt. 1: 1152; Abs. J. Soc. Chem. Ind., 1898, *17:* 697.

[d] Jorissen, Service de Surveillance des Aliments en Belgique, through Bul. soc. chim. Belg., 1897–98, *11:* 12, 211; Abs. Analyst, 1897, *22:* 282.

layers. Persist in this operation, if necessary, for a minute or more, using a piece of white paper for a background and producing only a very gradual and partial mixing of the acid and water. Nearly half of the acid should remain as a distinct unmixed layer at the end. If methyl alcohol be present, in the original sample, the shaking causes the separation of more or less voluminous flocks of a very characteristic rose-red color. The appearance of colored zones or flocks of other hues, even when tinged with red or of a rose-red solution without flocks, should never be considered proof of the presence of methyl alcohol. However, if the flocks are reddish brown, or if the upper layer has a pronounced red, it is often well to repeat the test. By this method for the removal of acetaldehyde 10 per cent of methyl alcohol may be readily detected, and an experienced operator may detect with certainty a smaller amount.[a]

6. Fluorids.

(a) Modified method of Blarez.[b]

Thoroughly mix the sample and heat 150 cc (in the case of solid foods the filtrate prepared as directed under salicylic acid may be employed) to boiling. Add to the boiling liquor 5 cc of a 10 per cent solution of potassium sulphate and 10 cc of a 10 per cent solution of barium acetate. Collect the precipitate in a compact mass (a centrifuge may be used advantageously) and wash upon a small filter. Transfer to a platinum crucible and ash.

Prepare a glass plate (preferably of the thin variety commonly used for lantern slide covers) as follows: First thoroughly clean and polish and coat on one side by carefully dipping while hot in a mixture of equal parts of Canauba wax and paraffin. Near the middle of the plate make a distinctive mark through the wax with a sharp instrument, such as a pointed piece of wood or ivory, which will remove the wax and expose the glass without scratching the latter.

Add a few drops of concentrated sulphuric acid to the residue in the crucible and cover the crucible with the waxed plate, having the mark nearly over the center and making sure that the crucible is firmly embedded in the wax. Place in close contact with the top or unwaxed surface of the plate a cooling device, consisting of a glass tube considerably larger in diameter than the crucible, the bottom of the tube being covered tightly with a thin sheet of pure rubber. A constant stream of cold water is passed continually through the tube. Heat the crucible for an hour at as high a temperature as practicable without melting the wax (an electric stove gives the most satisfactory form of heat).

Remove the glass plate and indicate the location of the distinguishing mark on the unwaxed surface of the plate by means of gummed strips of paper, then melt off the wax by heat or a jet of steam, and thoroughly clean the glass with a soft cloth. A distinct etching will be apparent on the glass where it was exposed if fluorin be present.

(b) Second method.

Dry 100 grams of sample thoroughly after making alkaline with lime water. In case the sample be a solid it must first be taken up with a little water in order to be sure that every portion of it is rendered alkaline. Incinerate the sample and volatilize the fluorin with sulphuric acid in a platinum crucible, detecting the presence of fluorin by means of an etched glass plate, as described above.

This method should only be used with substances low in ash and in which but a small amount of carbonates results from incineration. Carbonates may be advantageously decomposed by treating the ash with acetic acid and evaporating to dryness.

(c) Third method.

If it is desired, the preceding method may be varied by mixing a small amount of precipitated silica with the precipitated calcium fluorid and applying the method given below for the detection of fluosilicates.

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[a] In the examination of other alcoholic liquids the substances interfering with the resorcin test, together with methods for their removal, may be found by consulting the original article—Amer. Chem. J., 1899, *21:* 266.

[b] Chem. News, 1905, *91:* 39; Ann. Rept. Mass. State Board of Health, 1905.

This method is of value in the presence of foods whose ash contains a considerable amount of silica, which unites with fluorin with the formation of fluosilicates. The sulphuric acid then liberates hydrofluosilicic acid, which would escape detection by methods 1 and 2.

7. Fluoborates and Fluosilicates

Make about 200 grams of the sample alkaline with limewater, evaporate to dryness, and incinerate. Extract the crude ash first obtained with water, to which sufficient acetic acid has been added to decompose carbonates, filter, burn the insoluble portion, extract with dilute acetic acid, and again filter. The insoluble portion now contains calcium silicate and fluorid, while the filtrate will contain all the boric acid present.

(a) first method.[a]

Incinerate the filter containing the insoluble portion, mix with a little precipitated silica, and place, with the addition of 1 or 2 cc of concentrated sulphuric acid, in a short test tube, which is attached to a small U-tube containing a few drops of water. The test tube is now placed in a beaker of water, which is kept hot on the steam bath for from thirty to forty minutes. If any fluorid be present the silicon fluorid generated will be decomposed by the water in the U-tube and will form a gelatinous deposit on the walls of the tube.

The filtrate is now tested, as directed under boric acid. If both hydrofluoric and boric acids be present, it is probable that they were combined as borofluorid. If, however, silicon fluorid be detected and not boric acid, the operation is repeated without the introduction of the silica, in which case the formation of the silicon skeleton is conclusive of the presence of fluosilicate.[b]

(b) second method.

Incinerate the filter containing the insoluble portion in a platinum crucible, mix with a little precipitated silica, and add 1 cc of concentrated sulphuric acid. Cover the crucible with a watch glass to whose underside a drop of water is suspended, and heat an hour at the temperature of 70° or 80° C.[c] The silicon fluorid which is formed is decomposed by the water, leaving a gelatinous deposit of silica, and etching a ring at the periphery of the drop of water. Test the filtrate for boric acid as described under (a).

8. Sulphurous Acid.

(a) qualitative detection.[d]

To about 25 grams of the sample (with the addition of water, if necessary) placed in a 200-cc Erlenmeyer flask, add some pure zinc and several cubic centimeters of hydrochloric acid. In the presence of sulphites, hydrogen sulphid will be generated and may be tested for with lead paper. Traces of metallic sulphids are occasionally present in vegetables, and by the above test will indicate sulphites. Hence positive results obtained by this method should be verified by the distillation method.

It is always advisable to make the quantitative determination of sulphites, owing to the danger that the test may be due to traces of sulphids. A trace is not to be considered sufficient either as a bleaching agent or as a preservative.

(b) determination of total sulphurous acid.

(1) *First method.*

Distill 100 grams of the sample (adding water, if necessary) in a current of carbon dioxid after the addition of about 5 cc of a 20 per cent solution of glacial phosphoric acid until 50 cc have passed over. Collect the distillate in a decinormal iodin solution in a flask closed with

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[a] Nevière and Hubert, Moniteur scientifique, 1895, [4] 9: 324.

[b] It must be remembered that, in an ash containing an appreciable amount of silica, sulphuric acid will liberate silicon fluorid rather than hydrofluoric acid. The presence of a fluosilicate is indicated, therefore, and not the presence of a fluorid.

[c] The watch glass may be kept cool by means of a piece of ice.

[d] U. S. Dept. Agr., Division of Chemistry, Bul. 13, pt. 8, p. 1032.

a stopper perforated with two holes, through one of which the end of the condenser passes and through the other a U-tube containing a portion of the standardized iodin solution. Twenty-five cc of decinormal iodin solution may be employed, diluted with water to give the desired volume. The method and apparatus may be simplified without material loss in accuracy by omitting the current of carbon dioxid, adding 10 cc of phosphoric acid instead of 5 cc, and dropping into the distilling flask a piece of sodium bicarbonate, weighing not more than a gram, immediately before attaching to the condenser. The carbon dioxid liberated is not sufficient to expel the air entirely from the apparatus, but will prevent oxidation to a large extent. The U-tube trap may also be omitted if the end of the condenser tube be made to extend below the surface of the iodin solution, and the distillation conducted with a steady flame. When the distillation is finished, wash the contents of the U-tube into the flask and determine the excess of iodin with standardized thiosulphate solution. On account of its lack of permanence the iodin solution employed should be titrated from time to time with a decinormal thiosulphate solution (containing 24.8 grams $Na_2S_2O_3.5H_2O$ per liter). The number of cubic centimeters of decinormal iodin solution employed, less the number of cubic centimeters of thiosulphate solution required at the end of the determination, is multiplied by 0.0032 to obtain the grams of sulphur dioxid per 100 cc of wine.

(2) *Second method.*

In the examination of wine fairly accurate results may also be obtained by the following method. Care must be taken in applying the method to other products than wine to determine whether iodin is decolorized by any substance that may be naturally present.

Place 25 cc of a solution of potassium hydroxid containing 56 grams per liter in a flask of approximately 200 cc capacity. Introduce 50 cc of the sample by means of a pipette, mix with the potassium hydroxid, and allow the mixture to stand for fifteen minutes with occasional agitation. Add 10 cc of 1: 3 sulphuric acid and a few cubic centimeters of starch solution, and titrate the mixture with a N/50 iodin solution. Introduce the iodin solution as rapidly as possible and continue the addition until the blue color will last for several minutes. One cubic centimeter of N/50 iodin solution is equivalent to 0.00064 gram of sulphur dioxid.

(c) DETERMINATION OF FREE SULPHUROUS ACID.[a]

Treat 50 cc of the sample in a flask having a capacity of approximately 200 cc with about 5 cc of 1: 3 sulphuric acid, add a small piece of sodium carbonate (about 0.5 gram) to expel the air, and titrate the sulphurous acid with N/50 iodin solution, as directed under total sulphurous acid.

One cubic centimeter of N/50 iodin solution is equivalent to 0.00064 gram of sulphur dioxid.

9. BETA-NAPHTHOL.

Extract 200 cc of the sample (or of its aqueous extract prepared as directed on page 3) with 10 cc of chloroform in a separatory funnel, add a few drops of alcoholic potash to the chloroform extract in a test tube, and place in a boiling water bath for two minutes. The presence of beta-naphthol is indicated by the formation of a deep blue color, which changes through green to yellow.

10. ABRASTOL.

(a) SINABALDI'S METHOD.[b]

Make 50 cc of the sample alkaline with a few drops of ammonia and extract with 10 cc of amyl alcohol (ethyl alcohol is added if an emulsion be formed). Decant the amyl alcohol, filter if turbid, and evaporate to dryness. Add to the residue 2 cc of a mixture of equal parts of strong nitric acid and water, heat on the water bath until half of the water is evaporated, and transfer to a test tube with the addition of 1 cc of water. Add about 0.2 gram of ferrous sulphate and an excess of ammonia, drop by drop, with constant shaking. If the resultant

[a] Especially adapted to wine.

[b] Moniteur scientifique, 1893, [4], 7: 842.

precipitate be of a reddish color, dissolve it in a few drops of sulphuric acid, and add ferrous sulphate and ammonia as before. As soon as a dark-colored or greenish precipitate has been obtained, introduce 5 cc of alcohol, dissolve the precipitate in sulphuric acid, and shake the fluid well and filter. In the absence of abrastol this method gives a colorless or light-yellow liquid, while a red color is produced in the presence of 0.01 gram of abrastol.

(b) SANGLÉ-FERRIÈRE'S METHOD. [a]

Boil 200 cc of the sample with 8 cc of concentrated hydrochloric acid for one hour in a flask with a reflux condenser attached. Abrastol is thus converted into beta-naphthol and is detected as directed under "9."

11. SUCROL OR DULCIN.

(a) MORPURGO'S METHOD. [b]

Evaporate about 100 cc of the sample (or of the aqueous extract prepared as directed on page 3) to a sirupy consistency after the addition of about 5 grams of lead carbonate, and extract the residue several times with alcohol of about 90 per cent; evaporate the alcoholic extract to dryness; extract the residue with ether, and allow the ether to evaporate spontaneously in a porcelain dish. Now add 2 or 3 drops each of phenol and concentrated sulphuric acid and heat for about five minutes on the water bath; cool; transfer to a test tube and pour ammonia or sodium hydroxid over the surface with the least possible mixing. The presence of dulcin is indicated by the formation of a blue zone at the plane of contact.

(b) JORISSEN'S METHOD. [c]

Suspend the residue from the ether extract obtained as directed above in about 5 cc of water; add from 2 to 4 cc of an approximately 10 per cent solution of mercuric nitrate, and heat from five to ten minutes on the water bath. In the presence of sucrol a violet-blue color is formed, which is changed to a deep violet by the addition of lead peroxid.

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[a] Comp. rend., 1893, *117:* 93. [b] Zts. anal. Chem., 1896, *35:* 104. [c] Ibid., p. 628.

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