Section on Scientific Papers

Papers Presented at the Sixtieth Annual Convention

OUTLINE OF MICRO-ANALYTICAL METHODS FOR FOOD AND DRUGS LABORATORIES.

ALBERT SCHNEIDER, PHARMACOGNOSIST BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.

(Continued from p. 1338, December, 1912.)

There are a number of chemical tests giving color reactions which can be done conveniently by the micro-analyst, as the boric acid reaction with curcuma, the $H_2 SO_4$ color reaction with some barks, capsicum, guaiac, resin, cubeb, etc.; the $H_2 SO_4$ plus formaldehyde color reaction with morphine; the ferric chloride color reaction with salicylic acid; etc. These tests should be used when, in the judgment of the analyst, they may serve to give better information regarding the identity, purity and quality of the drug.

II. METHODS USEFUL IN THE EXAMINATION OF VEGETABLE FOOD PRODUCTS.

1. *Lagerheim's Test for Benzoic Acid.* Place a drop or two of the suspected liquid or semi-liquid food substance into a deep watch crystal of one inch diameter. Place over it a clean dry slide. Now hold the watch crystal over a flame (alcohol lamp*) until the substance (as wine, vinegar, catsup, jam, jelly, etc.), comes to an active boil. The steam vapor, carrying with it the benzoic acid, is condensed on the slide. Remove the slide and set it aside until the condensed moisture has evaporated; very moderate heat may be used to hasten evaporation. Examine under the microscope, whereupon the benzoic acid crystals may be seen, provided any were present. The test is delicate, very reliable and very few substances interfere with it. It is very pronounced in the presence of 0.01 per cent of benzoic acid or of sodium benzoate.

2. *Salicylic Acid Test.* Made like the benzoic acid test. The crystal formation (plates) is very pronounced in dilutions of 1 : 1000. After having examined the crystals under the microscope, add a drop of weak solution of ferric chloride to the crystals upon the slide, whereupon a blue coloration develops. Boric acid is likewise deposited by sublimation, but the test is not as satisfactory as those for benzoic acid and for salicylic acid.

3. *Curcuma Thread Test for Boric Acid.* Boil 5 grams of powdered curcuma in 10 cc. of alcohol. To the evaporated alcoholic extract add a little soda and several cc. of 50 per cent. alcohol. In this place paper (bast fiber), cotton or linen threads and bring to a brisk boil for a few moments. Remove threads and dry between blotting paper, lay them in a very weak solution of sulphuric acid and rinse in water. When dry the threads should be a golden yellow.

The test for the presence of boric acid (also for borax) is made as follows: Dip the end of a prepared thread in a 10 per cent solution of hydrochloric acid and allow to dry. Lay

*Alcohol lamp is preferred because the flame is small and the heating is quickly done.

the thread on a slide, cover with cover glass and examine. It should be of a reddish brown color. To the edge of cover glass apply a droplet of a 10 to 13 per cent. solution of sodium carbonate, followed by a droplet of the suspected solution. In the presence of boric acid, the thread is colored blue, which coloration remains for a longer or shorter period and then changes to gray and violet. The test is a very delicate one and is not hindered by the presence of sodium chloride, magnesium sulphate and aluminum sulphate. Strong solutions of phosphoric acid, silicic acid, calcium chlorite and magnesium chlorite, interfere with the reaction more or less.

4. *Formaldehyde Test.* Concentrated hydrochloric acid added to weak solutions of formaldehyde (1 : 5000) or substances containing formaldehyde, forms stellate clusters having a somewhat crystalline appearance. The formaldehyde can be deposited on a slide by sublimation (as for benzoic acid) and the acid added. The stellate clusters appear upon evaporation of the hydrochloric acid. The test requires further verification to determine its value.

5. *Sulphurous Acid Test.* Moisten starch paper with a very dilute solution of potassium-iodide iodine solution which colors it blue. In the presence of the merest trace of sulphurous acid the paper is decolorized. Do not use heat in this test.

6. *Iodine Reaction.* The color reaction of starch with N/50 iodine solution is of great importance in the examination of fruit products, such as jams, jellies, catsups, etc., as it shows whether or not ripe or green fruits and juices of unripe fruit were used, and whether or not starch paste may have been added as a filler or thickening agent. As is known, green fruits generally contain more or less starch, whereas ripe fruits are quite free from starch. The reaction may be observed only in the fruit pulp cells, indicating the presence of unripe fruit, or it may be limited to the non-cellular portions of such substances as jams and jellies, indicating the use of fruit juices obtained from unripe fruits.

7. *Microscopical Examination of Bacteria and Metals by Direct Sunlight.* Some recent experiments would indicate that very minute quantities of certain minerals as iron, copper, mercury, and a few others, can be detected in liquids and semiliquids (in the form of metallic hydroxides) when examined (on slide mounts) by means of direct sunlight. All transmissible light must be cut off. The actual value of this mode of testing must be determined by further experimentation.

Direct sunlight can also be used in making bacterial counts in liquids, using the Thoma-Zeiss hemacytometer (Turck ruling). The bacteria are readily recognizable on the dark background, standing out far more clearly than in the usual examination by transmitted light, because of the more pronounced color contrasts.

The possibilities in the use of direct sunlight in microscopical work are very promising and should receive more serious consideration.

There are certain micro-chemical color reactions, other than those already mentioned, which are of great value in determining the presence of impurities or adulterants in liquids and semi-liquids. The methods as perfected by F. Emich depend upon the use of cotton fibers treated with certain chemicals which convert the metallic compounds into the sulphides. The prepared threads can be readily transferred to the several solutions used and the color and precipitation effects can be observed under the microscope. The following are the more important reagents and reactions:

1. *Cotton Threads for Metal Tests.* Dip absorbent cotton threads alternately into 15 per cent solutions of sodium sulphide and zinc sulphate, pressing between blotting paper, and air-dry each time.

The threads thus prepared should assume a deep black color with a 1 per cent solution of silver nitrate. They may be kept for a long time and are used to demonstrate the presence of As, Sb, Au, Pt, Cu, Hg, Pb and Bi, in various chemical compounds.

2. *Ammonium Sulphide Vapor Test.* Place a few fibers of absorbent cotton into a drop of the suspected solution and allow the moisture to evaporate. Suspending the threads in the vapor of ammonium sulphide will indicate the presence of Cd, Hg, Ag, Fe, Co and Ni (dark to black coloration).

The prepared threads are used in the following tests:

a. *Arsenical Test.* Dip a sodium sulphide thread into the suspected solution and allow to dry. In the presence of 0.008 per cent. arsenic there is a distinct yellowish coloration, due to the sulphide of arsenic formed in and upon the threads. The arsenical threads will also show the characteristic reactions with hydrochloric acid, ammonia and ammonium sulphide by bringing a drop of the reagent in contact with the thread upon the slide.

b. *Zinc Test.* Dip cotton fibers into the suspected solution, allow the moisture to evaporate, and then dip the threads into a solution of gold chloride. A violet coloration develops which remains in the presence of acids but vanishes in the presence of chlorine water, indicating the presence of zinc chlorite. The reaction is appreciable in the presence of 0.003 μg of zinc chlorite, whereas in the form of the sulphite, 0.1 μg of zinc are required to show the reaction.

c. *Antimony Test.* Dip a sulphide thread into the solution, allow to evaporate and expose to the vapor of ammonium sulphide. If the solution to be tested contains considerable hydrochloric acid, sulphide of antimony is formed upon evaporation. Inasmuch as zinc oxide is not precipitated upon the sulphide thread, simultaneous tests may be made for arsenic and antimony.

d. *Gold Test.* Gives a brown coloration with the sulphide thread, which disappears upon prolonged exposure to ammonium sulphide, more quickly on exposure to chlorine, bromine and sodium hypochlorite. The threads which have been decolorized with chlorine are colored blue to black with iron chlorite and violet to red with zinc chlorite.

e. *Silver Test.* A neutral or faintly acid silver nitrate solution gives a brown to black coloration with the sulphide thread, the depth of the reaction depending upon the concentration of the solution. The fibers can be decolorized by placing in sodium hypochlorite, and the color can be restored by means of zinc chlorite or an alkaline solution of grape sugar. Sulphuric acid will again decolorize.

f. *Mercury Chloride.* Cotton threads dipped into a solution containing mercuric chloride and exposed to the vapors of ammonium sulphide or ammonia, are colored black. The color is quite permanent in the presence of acids. A sulphide thread is colored yellow in neutral solution of mercuric chloride, changing to black in the ammonium sulphide vapor.

g. *Lead Test.* Neutral lead solutions (lead nitrate) turn the sulphide threads yellow and black on prolonged exposure to ammonium sulphide. In acid solutions the color reaction with the sulphide thread is black. The yellow coloration is promptly changed to black upon exposure to ammonium sulphide, or when placed in weak sulphuric acid (1:15). The latter reaction distinguishes between lead and mercury, as the yellow coloration of the mercury is changed very slowly with dilute sulphuric acid.

h. *Bismuth Test.* Solutions color the sulphide thread reddish-brown. Bromine causes the color to disappear. Potassium dichromate causes a yellow coloration, while alkaline solutions of zinc chlorite produce a black coloration. Lead solutions are not reduced by alkaline solutions of zinc chlorite.

i. *Iron Test.* Ammonium sulphide vapor gives a black precipitate which is soluble in weak solutions of hydrochloric acid. Potassium ferrocyanide gives a blue coloration.

j. *Copper Test.* Solutions of copper sulphate give a brown coloration to the sulphide thread, which color remains in 10 per cent. hydrochloric acid, but disappears on exposure to bromine vapor. The threads which have been bleached with bromine give the copper ferrocyanide reaction when placed in an acidulated solution of potassium ferrocyanide.

The following table from the work by Koenig gives the relative sensitiveness of the tests above described:*

Elements in Combination Valency	Reaction	Limit (mg x 10^6)	Comparative Sensitiveness
Bo'''	Curcuma thread	0.1	1 in 33,000
As'''	Sulphide thread	10	1 in 2,500
Sb'''	Sulphide thread	1	1 in 40,000
Sn''	Violet color with sulphide thread	3	1 in 20,000
Au'''	Sulphide thread—brown, purple	3	1 in 22,000
Pt''''	Sulphide thread	8	1 in 6,000
Cu''	Sulphide thread+ferrocyanides	8	1 in 4,000
Ag'	Sulphide thread+Ag	5	1 in 22,000
Hg'	NH_3 vapor	8	1 in 25,000
Hg''	Sulphide thread	5	1 in 20,000
Pb''	Sulphide $PbCrO_4$	8	1 in 13,000
Bi'''	Sulphide+Chromate+Bi	8	1 in 9,000
Cd''	(NH_3SH) vapor	6	1 in 9,000
Fe''	(NH_3SH)—blue	8	1 in 3,500
Co''	NH_3SH or Nitroso—beta—naphthol	0.3	1 in 100,000
Ni''	NH_3SH	0.3	1 in 100,000

Certain factory food products, as jams, jellies, canned whole fruits, catsups, preserves, etc., generally contain large numbers of yeast cells, bacteria and mould. The examination of fruit products of the kinds named, as prepared by the careful housewife, shows that such organisms need not be present in any considerable numbers. Yeast cells, mould spores and bacteria occur in small numbers upon all sound fruits, but the active growth and development of the organisms does not take place in normal fruit. Decayed and decomposed fruit does contain large numbers of the organisms named, and active yeast fermentation is apt to be initiated in all exposed, non-sterilized and insufficiently sterilized fruit pulps and fruit juices.

Most of the factory samples of fruit products thus far examined, showed the presence of abundant yeast cells, mould and bacteria, indicating the use of fruit, fruit pulp, fruit refuse and fruit juices which were decayed prior to manufacture or which underwent fermentative and other decomposition changes prior to or during manufacture. Swollen cans are quite rare in the actual market, as they are culled at the factory before shipment. However, occasionally a swollen can finds its way into the pure food laboratory.

The organisms named prevail in varying amounts in different products. Bacteria are apt to predominate in catsups and pastes, yeasts in jams and jellies, moulds in such fruit as blackberry, strawberry, plums, rain-split cherries, apple

*The comparative degree of sensitiveness of the different chemical compounds concerned in the color reactions above described and tabulated, is indicated by the number of cubic centimeters in which one gramme of the substance in solution is still appreciable. The actual limit, determined experimentally, is indicated in terms of milligrammes, that is 1/1000 mg., represented by μg. Expressing the comparative sensitiveness (CS) in a formula we have

$$CS = \frac{\mu g \text{ limit}}{\text{amount limit}} \times \frac{\text{molecular weights}}{\text{combination valency}}$$

or to give the example for boron, we have

$$CS = \frac{0.000001}{0.00000006} \times \frac{59}{3} = 33000.$$

refuse and in fruit refuse generally; while smuts and their spores are particularly likely to occur in figs and fig jams. If these organisms (dead) occur in considerable numbers it is conclusive evidence that other than fresh fruits or fruit juices were used. The presence of numerous dead yeast cells (2-50,000,000 per cc.) is evidence that the material was undergoing alcoholic fermentation prior to or at the time of manufacture. Since yeast fermentations are usually accompanied by considerable bacterial activity, the presence of some bacteria may be expected. Occasionally a sample of jam comes to hand showing the presence of abundant living yeast cells, as indicated by the bulging of the can, and when opened, by the vinous odor, presence of bubbles, and as seen from the microscopical appearance of the actively budding cells.

Occasionally factory samples are found which are almost as free from organic contamination as are the products of the careful housewife, which is conclusive evidence that manufacturers can, if they will, put up wholesome fruit preparations. The following methods of making microscopic counts and provisional maximum limits are submitted for consideration:

1. *Yeast Counts.* The Thoma-Zeiss hemacytometer with Turck ruling, used with No. 2 ocular and No. 5 objective, will be found very convenient. It is not necessary to make dilutions. Countings can be made readily up to 50,000,000 per cc. without dilutions. If the yeast cells are so numerous as to require dilution, the article is presumably unfit for consumption. Three or four mounts of each fruit product should be examined, and ten areas (1/25 sq. mm. or 0.04 sq. mm.) should be counted in each mount.

Living yeast cells can be distinguished from the dead cells by their larger size, uncolored cell-walls, presence of vacuoles and evidence of active budding. Dead yeast cells shrink as the result of osmotic outflow of cell-sap, and the cell-walls take up some of the fruit colors, including slight coloration.

2. *Bacterial Counts.* As in yeast counts, the hemacytometer is used. The efforts to determine the presence of bacteria in fruit products and to count them, have thus far given rather unsatisfactory results because of the great variety of organic particles (as proteid and plasmic granules and minute starch granules), and even crystalline bodies, which may be mistaken for cocci or bacilli. The most satisfactory method is to centrifugalize from five to ten grammes of the substance dissolved in distilled water and make stained mounts of the sediment. Dead bacteria stain only slightly, organic non-bacterial fragments stain readily, whereas crystalline particles do not stain at all. The starch granules will, of course, be recognized by the blue coloration with N/50 iodine solution. The skilled microscopist has no difficulty in recognizing the larger bacilli in the various food substances provided they are present in considerable numbers (50,000 per cc. or more). Active motion of the suspected rod-shaped particles is, of course, conclusive evidence of the presence of living bacteria. The bacterial content of some substances, as tomato pastes, may be so high as to necessitate diluting. Usually a dilution of 1-10 is sufficient.

3. *Bacterial Cultures.* The only satisfactory method for determining the number of living bacteria in food products, including spore forms, is by means of the usual dilution plate cultures. For this purpose the necessary laboratory facilities must be provided. The full method should include the determination of certain pathogenic forms as the colon group of bacilli, the typhoid bacillus, and a few other objectionable types of bacilli. The maximum number of living bacilli (including spores) permissible in food substances will depend on the kind, and the limitations in that regard should be as for potable water, including mineral waters, following the methods outlined by the Society of American Bacteriologists. The purely quantitative or numerical limitations of non-pathogenic living bacteria will depend upon the character of the food substance. In jams, jellies, marmalades and similar products, the upper limit should not exceed 1,000,000 per cc., whereas in catsups the maximum number may be fixed at 5,000,000 per cc. This matter, however, requires further careful consideration.

4. *Mould Counts.* Mould in food substances is always objectionable, and the presence of such organisms usually indicates the use of mould-infested fruit, though the product may become further infested in manufacture or even after it is ready for shipment or while in storage. However, as moulds are highly aerobic, there is little growth in well-filled, well-sealed containers, but occasionally there is a very extensive growth on the top of the food product, as jam, jelly, etc., in containers which are not quite filled and which are not well sealed, or when the contents are insufficiently sterilized.

Since all species or kinds of moulds are objectionable, the maximum limits are purely numerical or quantitative. The methods of making counts consist in the use of a stage slide cell containing a definite amount of the well-mixed substance

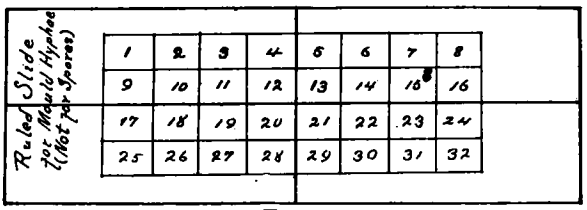

FIG. 1.

Fig. 1. Ruled slide for counting mould hyphae in jams, jellies, canned fruits and similar substances. A small spoonful (capacity of 0.5 or 0.25 cc.) of the thoroughly mixed sample is spread on the slide, within the ruled area, and covered with a rectangular cover glass, or an ordinary slide. The counting is done by means of the low power (No. 2 ocular and No. 3 objective). Because of the amount of material placed on the slide, the dimensions of the areas and the low power used, spore or yeast cell counting is not practicable with this device. The mechanical stage will be very useful in making the counts.

and counting the number of hyphal fragments and hyphal clusters (including the spores) contained therein. The hemacytometer cannot be used. Attempts to use the Rafter counting device proved unsatisfactory, first, because of the depth of the cell (1 mm.), and because of the fact that the use of dilutions is necessary, which is time consuming. Several satisfactory methods may be used. By means of a spoon of 0.5 cc. or 0.25 cc. capacity, take up a well-mixed sample and spread it out on a slide ruled in numbered squares (Fig. 1), and cover with cover glass, and then count all of the hyphae direct, under the low power of the compound

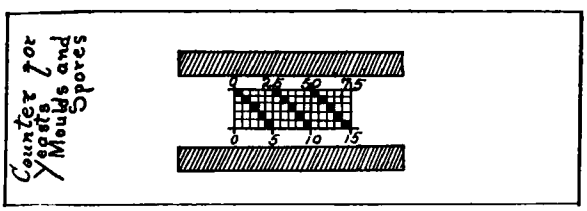

FIG. 2.

Fig. 2. Counting apparatus for mould spores, mould hyphae and yeast cells. The rulings on the slide are 0.04 sq. mm., 1 sq. mm. and 25 sq. mm. On either side of this ruled area are metal slips 0.2 mm. thick, so that the entire capacity of the space within the ruled area is 15 cm. or 0.015 cc. A bit of a thoroughly mixed fruit product, as jam, jelly or catsup, is placed on the slide in the ruled area and covered with a rectangular (No. 2) cover glass. Slight pressure is required to make the cover rest on the metal slips. The counting is done in areas entirely filled (from slide to cover glass) by the substance mounted. The larger areas (25 and 1 sq. mm.) will be most convenient for counting mould hyphae, while the smaller areas (0.04 sq. mm) are used to count mould spores and yeast cells, provided the number present does not exceed 10,000,000 per cc. Should the number of yeast cells or spores exceed 10,000,000 per cc., it will be necessary to make dilutions (1-10). A No. 2 ocular and a No. 5 objective are to be used. If the spore count is to be omitted, only the low power (No. 2 ocular, No. 3 objective) need be used.

microscope. As a rough-and-ready method this is satisfactory, but the magnification used does not make spore counting possible. The mechanical stage is required when making the counts.

Quicker and more accurate countings can be made by means of a ruled slide cell, as shown in Figure 2. This device, furthermore, does not absolutely re-

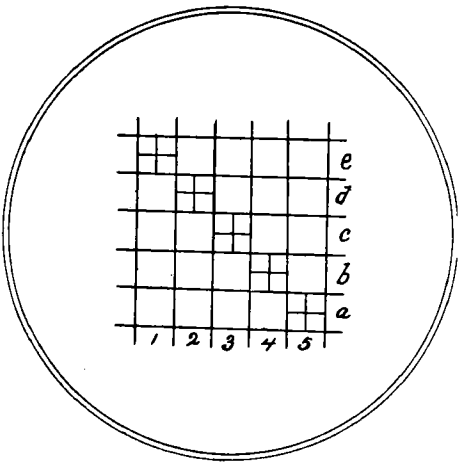

FIG. 3.

Fig. 3. Ocular scale for making yeast, spore and bacterial counts in liquids and semi-solids, as jams, jellies, catsups, preserves, etc. The measuring values of the areas (in microns or micro millimeters) of the squares, for low and high powers, are determined by means of the stage micrometer. Ordinary slide mounts are used, the amount of material being just enough to represent the approximate diameter of the yeast cell, spore cell or bacterial cell. That is, the cover glass must be pressed down sufficiently so as to bring it in capillary proximity to slide. In order to do this the amount of material placed on the slide or cover must be just enough or somewhat less than is required to occupy the capillary space between cover and slide. This precludes, from the mounting, all coarse material and larger solid particles, as sand, larger cells, as some sclerenchyma, etc. With some practice it is possible to make slide mounts in which the variation error does not exceed five per cent. The counts are made in one plane or optical section. The method is sufficiently reliable for all practical purposes and has numerous advantages, as will be found on comparing it with other methods.

The application of the method is as follows:

Let x=the linear value of the ocular rulings in terms of millimeters, and,
n=the number of yeast cells, spores, bacteria, etc., in x^2, then
$n\sqrt{n}$=the number of organisms in x^3, and

$\frac{n\sqrt{n}}{x^3}$ = the number of organisms in one cubic millimeter, and

$\left[\frac{n\sqrt{n}}{x^3}\right] 1000=N$, N, representing the number of organisms in one cubic centimeter.

To give a concrete example we will suppose that with the high power x=0.09 mm., and that based upon the counts of ten or more squares in each of the two or three mounts, n=10; then $10\sqrt{10}=30+$, and

$$\frac{30}{0.000729}=41{,}152, \text{ and } N=41{,}152{,}000$$

quire the use of the mechanical stage, though it will be found convenient. The counting apparatus, shown in Figure 2, may be used with either low or high power (No. 2 ocular with either No. 3 or No. 5 objective). Because of the fact that the ends of the slide cell are open, cleaning is easy, and mounts can be prepared quickly. The use of the ocular scale and the direct slide mounts, as ex-

plained under Figure 3, is much simpler and quicker than the use of the Thoma-Zeiss hemacytometer, and gives results which are perfectly satisfactory for all practical purposes in the examination of food products.

The maximum number of yeast cells, moulds and bacteria permissible within the intent of the pure food and drugs act must be governed by several conditions. All products made from fresh fruits and fresh fruit juices, without the addition of fermentation products as fruit vinegar, wine, cider, etc., should not contain more than 1,000,000 per cc. each of yeast cells or of bacteria, and not more than 50,000 per cc. of mould spores and hyphal fragments and hyphal clusters. The fruit products of the careful housewife contain far less organisms than is indicated by these numbers. Apple butter or apple marmalade is sometimes prepared with fresh cider—that is, with apple cider which is undergoing active fermentation. Such a product may contain many more than 10,000,000 dead yeast cells per cc. If the cider is, however, first strained through several layers of muslin, through cotton or felt paper or cleared by the centrifugal method, before adding it to the apples, the number of yeast cells is in all probability far less than 1,000,000 per cc. In the above case the label should state that apple butter is "prepared with apple cider," as it is otherwise assumed that only unfermented fruit ingredients are used. Fruit products prepared with fruit vinegar, such as catsups, prepared mustards, etc., may contain 5,000,000 acid-forming microbes and yeast cells per cc., but in all probability not more, so that 5,000,000 yeast cells or bacteria per cc. is a reasonable maximum limit. No doubt this limit can be reduced as the canners learn the need of greater care in manufacture. Often the trouble lies in the fact that the decayed fruit is not carefully culled, and again the pulped material is left too long in the pulping or mixing tanks, or the tanks are not properly cleaned and scalded.

It is perhaps impracticable to cull out every decayed or mouldy fruit, but any one who has witnessed the operations in some of the fruit canneries is satisfied that conditions can be and should be improved upon. A careful culling of decomposed fruit would result in a marked reduction in the number of mould hyphae, mould spores, and rotting bacteria present. Manufacturers should be warned, and urged to perfect their methods, no matter what the cost may be. Improved methods no doubt would mean an increase in the price of the manufactured article,nevertheless we should have and we can have a wholesome fruit product.

The methods of micro-analysts whether in private, commercial or government laboratories, should be quite uniform. Much could be done to bring this about if the analysts were to meet for the purpose of comparing methods and results, as has already been suggested. Uniform blank report forms should be adopted and used in the micro-analytical laboratories, somewhat like those used by chemists. It cannot, however, be denied that the efficiency of the work done depends largely upon the ability, judgment and experience of the analyst. The novice is apt to experience only utter confusion, when left to himself, even when examining such simple substances as ground pepper, ginger, flour and starches. The beginner may worry over one article for an entire day, and even then he may not be certain of the findings,. whereas the experienced analyst examines from twenty to

sixty samples in the same time with full confidence in his findings and conclusions.

The reports of the micro-analysts may be made according to the following groups:

I. Drugs of vegetable origin, including dry or solid food products of vegetable origin.

II. Liquid or moist food products of vegetable origin (canned and preserved foods generally).

III. Bacterial examinations of liquids, etc.

There should be a special blank report card for each group of substances, arranged as follows:

FORM No. I.

No.....(I. S., Laboratory or other serial number).
Label .....
.....
Sample received..... Sample examined.....
Condition of wrappings and seals.....
Organoleptic Tests .....
Consistency or Feel.....
Color .....
Odor .....
Taste .....
Adjunct Tests .....
Sand (beaker test).....%
Ash .....%
Acid-insoluble ash .....%
Special tests .....
.....
Microscopical findings.....
.....
.....
.....
Conclusions .....
.....
.....
.....
.....Analyst.

FORM No. II.

(No. label, dates, condition of seal and organoleptic tests, as for form No. I.)

Adjunct Tests.
Sublimation tests for.....
Benzoic acid .....
Salicylic acid .....
Boric acid (curcuma thread).....
Iodine reaction .....
Intracellular .....
Extracellular .....
Special Tests .....
.....

Microscopical findings.
General .....
.....
.....
Cytometric counts.
Dead yeast cells.....per cc.
Living yeast cells.....per cc.
Bacteria .....per cc.
Mould (hyphal fragments and hyphal clusters).....per cc.
Mould spores .....per cc.
Smut spores .....per cc
Conclusions .....
.....
.....
.....Analyst.

FORM No. III.

Bacteriological Examination.

I. Direct Count. (Thoma-Zeiss Hemacytometer with Turck ruling.)
1. Bacilli per cc.....
2. Cocci per cc.....
II. Plate and Tube Cultures. (Lactose-Litmus-Agar).
1. Temperature differential test.
a. (20° C.) colonies per cc.....
b. (38° C.) colonies per cc.....
2. Color differential test.
a. Pink or yellow colonies per cc.....
b. Not pink or yellow colonies per cc.....
3. Gelatine liquefying colonies per cc.....
4. Indol reaction (±).....
5. Neutral red reduction (±).....
6. Gas (hydrogen) formula.....
7. Gram stain behavior (±).....
III. Special Tests .....
.....
.....
IV. Conclusions .....
.....
.....
.....Analyst.

We may give an example of a report as follows:

FORM No. II.

Lab. No. *462.*

Label: *Pure currant jelly. Made by Smith, Janes & Co., Nantucket, Wis.*
Sample received *August 5, 1910.* Sample examined *August 5, 1910.*
Condition of seals: *Good, unbroken sample.*
Organoleptic tests: *Not conclusive.*
Consistency or feel: *Poorly jellied.*
Color: *Normal for Currant jelly.*
Odor: *Faint, somewhat disagreeable.*
Taste: *Not characteristic, bitterish, quite acid.*

Adjunct Tests.
Sublimation tests for
Benzoic acid: *Negative.*
Salicylic acid: *Very marked.*
Boric acid (curcuma thread): *Negative.*
Iodine reaction: *Very marked.*
Intracellular: *Negative.*
Extracellular: *Positive, very marked.*
Special Tests: *Salicylic acid color reaction, with ferric chloride very marked.*
Microscopical Examination.
General. *Some apple tissue (window cells and pulp cells) and currant tissue (sclerenchyma) present. Added wheat starch about 5 per cent.*
Cytometric counts.
Dead yeast cells, *30,000,000*.....per cc.
Living yeast cells, *none*.....per cc.
Bacteria, *6,150,000* .....per cc.
Mould (hyphal fragments and hyphal clusters) *20,000**per cc.
Mould spores, *5,000*.....per cc.
Smut spores, *none*.....per cc.
Conclusions: *Misbranded and adulterated with apple and with wheat starch and made from fermented and decomposed material, preserved with salicylic acid. Not fit for human consumption because of the quantity of yeast, mould and bacteria present.*

JOHN DOE, Analyst.

The great advantage of the micro-analytical work as compared with chemical work lies in the facts that small amounts of the substances are used for analysis, the equipment is comparatively inexpensive and the results are quickly attained. From twenty to forty and even sixty samples of simple spices can be examined in one day, from five to twelve samples of powdered vegetable drugs, cocoas, chocolates, flours, meals, etc., and perhaps an equal number of jams, jellies, etc.

Summarizing Suggestions.

The following concluding suggestions are submitted for consideration and action.

1. That there be a classification of those food and drug products which should be subjected to microscopical examination in food and drug laboratories.

2. That the compound microscopes intended for use in food and drug laboratories be equipped with eye-piece micrometer, polarizer, and four objectives (Nos. 3, 5, 7 and 1/12 in. oil immersion). The No. 5 being necessary for use with the mould counter and the hemacytometer, and the oil immersion objective for occasional bacteriological work.

3. That a mould counter, as described in Fig. 2, be adopted.

*This number of hyphal clusters and hyphal fragments would correspond with hyphal clusters and fragments in about 80% of the smallest areas (0.04 sq. mm.) of the proposed mould counting apparatus shown in Fig. 2. The comparative quantity of mould establishes the article's unfitness for human use to a more marked degree than does the yeast, although there are pathogenic and otherwise objectionable yeasts. Moulds are quite generally objectionable either on account of toxin-like substances formed or because of objectionable gases or flavors developed. Many of the moulds are pathogenic, producing skin and stomach disorders.

4. That a uniform tissue terminology be adopted.

5. That the micro-analysts adopt the following reactions and tests described in this report. Other tests to be added as soon as their value has been proven.

a. The mace test.
b. Conium test.
c. Grahe's cinchona test.
d. Ash determination.
e. Sublimation tests for benzoic acid and for salicylic acid.
f. Curcuma thread test for boric acid.
g. Iodine test for starch.

6. That organoleptic testing be recognized as valuable adjuncts to the microscopical examination.

7. That the micro-analytical laboratories be equipped to do the necessary bacteriological work.

8. That the micro-analytical laboratories be equipped to make official test-tube, beaker and other simple chemical purity and quality tests, recognized and described in the United States Pharmacopoeia, National Formulary and in the official methods of the Agricultural Chemists.

9. That a tentative maximum numerical limit for yeast cells, mould hyphae, mould spores and bacteria in food products and in liquids, be adopted.

10. The fineness of powdered vegetable drugs intended for medicinal use should be stated on the label. Powdered vegetable drugs of which the fineness is not in accord with the pharmacopoeial requirement, should be declared not up to the standard and should be rejected until the dealer reduces them to the required fineness.

11. Official vegetable drugs should be classified or grouped according to the maximum amount of harmless impurities (accidental impurities) which may be permissible, as follows:

Group or Class A. Impurities not to exceed 5 percent.*[2] as ergot, kamala, lycopodium, some resins, gums, seeds, most fruits, ipecac, stem barks, some leaves, flowers, flowering tops, etc.

Group or Class B. Impurities not to exceed 10 percent.,*[3] as some roots, rhizomes, some leaves, most herbs, etc.

12. That official descriptions be prepared of the official vegetable drugs, based upon microscopical characters, as indicated in this report and that these descriptions be supplied to micro-analysts to serve as guides in the critical examination of powdered vegetable drugs and as a means of unifying methods and results.

13. Uniform blank report cards for microscopical and bacteriological work should be adopted and used.

Revised to July 20, 1912.

---

*[2]Inclusive of inert material only as sand, pebbles, dirt, foreign vegetable matter, etc., not intentionally added.

*[3]It is probable that several exceptions must be allowed for this limit, as for example henbane and valerian, in which the impurities generally exceed 10%. However, in the great majority of drugs of this class the impurities need not exceed 10%, provided reasonable care is observed in collecting and garbling.