

THESIS ON GENOLOGY

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THESIS ON OENOLOGY

INTRODUCTION

During the summer of 1901, an outline of the work required for the Degree of Master of Science in Oenology was submitted to the Faculty of the Virginia Polytechnic Institute and was approved by the same. This outline embodied the following requirements: that pure races of yeast should be separated from four sources, preferably from ripe apples, ripe grapes, a finished apple cider, and a finished grape wine; that such malferments that were seen should be separated and determined; that the pure cultures of yeasts and malferments should be carried through Physiological Tests on apple musts and such sugar solutions as would be designated later.

This work has covered a period of two years during which time much parallel work has been done for the Virginia Experiment Station.

This paper is intended to embrace the work done together with the detail of the technique employed in the fulfilment of the requirements given above.

THE SEPARATION OF YEASTS.

Culture Media. Standard Must. Before any work can be done towards separating yeasts or other ferments, a quantity of suitable media of a standard composition must be secured. Since we are concerned chiefly with the ferments on the apple, apple must is the most suitable liquid nutrient media. To prepare apple must for laboratory use,-

grind the apples when thoroughly ripe, so that the sugar content will be at its maximum. Press immediately as cleanly and thoroughly as possible. Place the must or juice in quart champagne bottles, filling them only to the base of the neck, thus allowing for the expansion of the liquor when heated. Cork the bottles as tightly as possible with no. 1 or no. 2 champagne corks. Tie the corks in with strong twine as follows:

Cut the string in two foot lengths, varying with the size of the bottles. Form a loop as in Fig. 1 catching the string a little to the left of its centre. Place over the cork so that it will cross the centre of the cork while b will extend around the neck and under the flange of the bottle. Draw the two ends of the string down by the body of the bottle, and pull until tight. Bring the ends above the cork and tie a single knot. Turn the strings so that they will cross the cork at right angles to a and with the longer end of the string, make a "half hitch" over the centre of the cork. Pull until tight, bring the ends up and tie a tripple knot over the center of the cork.

When the bottles are tied place them in a kettle of cold water. Heat to boiling. Remove immediately and allow to cool. Stand the bottles on end so that the strings and corks may dry. When dry, seal with a good wax by dipping the bottles inverted into the hot wax so that it passes the strings used in tying.

The following formula will give satisfactory results:

One part tallow.	(Boil together till melted and thoroughly mixed. Pour into a bucket of cold water. Remove when cold enough and work by kneading and pulling until bright.) (Can be colored with stains if desired.)
Two parts bees-wax.	
Four parts resin.	

3
(2) continued.

A After sealing, store the bottles in a cold place by laying them on their sides in order to keep corks swollen. Cord up row on row as with pieces of wood.

PREPARATION OF MUST FOR USE IN THE LABORATORIES.

Filter through the ordinary grade of filter paper used in laboratories. The funnel should hold ~~from 800cc.~~ to 1000cc. Agitate the sterilized must thoroughly. Pour contents into the funnel, filling it full. Repeatedly pour filtrate back into the funnel, keeping it full all the time, until it comes through absolutely clear and bright, from which time begin to save. The filter paper is too coarse to clear the must entirely, so by repeatedly pouring back the filtrate the pores of the paper are stopped up with the albuminous matter in the must, and in this way one is able to get a filtrate free from all detritus. Allow the filtrate to run into 500cc. flint glass bottles, which when full, are plugged with cotton, covered with a parchment paper cap and sterilized for 40 minutes in an Arnold's Steam Sterilizer at a moist temperature of 100°C. When through, all the filtrate and the contents of the funnels should be thoroughly sterilized separately in order to prevent any appreciable change in the composition of the standard must. The filtered must is then placed into test tubes, 10cc. in each. - The test tubes used in this work measure 1.7cm. in diameter, and 15.4cm. in length. These tubes are plugged with antiseptic absorbent cotton. The plug is made as follows: tear off a round piece of cotton about five times the diameter of the mouth of the tube and about 1cm. thick. Place in the center of this a small wad of cotton, and with a stout pair of straight round point forceps, pinch up the wad and the center of the first piece of cotton so as to form on the opposite side a distinct suture. Holding the cotton thus, press it back against the forceps by passing it through a ring made with the thumb and forefinger

of the other hand, thereby forming a plug which should be of an equal hardness throughout its length. Insert the plug so made into the mouth of the tube and press it in about 4.5cm'. This plug should be porous enough to allow the free passage of gasses but dense enough to prevent the entrance of extraneous organisms. When about 20 tubes are filled and plugged, tie them into a bundle with a string around the upper and lower ends, so that the bundle will stand alone. Cut a piece of parchment paper sufficiently large to make a cap to go over the end of the whole bundle. Wet this, in order to make it easily pliable, and put it over the plugged ends of the tube, tying it in place. This checks evaporation but its chief object is to preserve the cotton plugs from contact with extraneous organisms. Place this batch of tubes in the sterilizer and sterilize for 20 minutes after boiling. Remove and allow to cool.

Preparation of Gelatin. For solid media gelatin or agar-agar is used. For use in the laboratory, it is prepared as follows: Take 500cc. of filtered must and render it nearly neutral by the addition of potassium-hydrate. It should only faintly redden litmus. Choose a flask large enough so that when the 500cc. of must is added the liquid will reach only to the largest diameter of the flask. Plug loosely with cotton in order to prevent evaporation, and heat in water bath to a temperature ranging from 70 to 80°C. The gelatin is prepared in flakes about 8cm. in width and 20cm. in length. Weigh out a quantity of gelatin equal to 10% of the must, and add it by rolling each flake crosswise and inserting into the flask. The gelatin should be added only as fast as it will go into solution, and to insure this, shake the flask thoroughly after adding each flake of gelatin. When all the gelatin

is added retest for acidity. Remove from water bath and cool to about 40°C. Take one half the white of a fresh egg, beat into a good froth using a little distilled water; pour the same into the center of the liquid surface. Make it spread over the entire surface of the must-gelatin, but do not agitate it. Place flask back into water bath and cook at a slow boil for two to three hours or until the egg is entirely coagulated. The object in using the egg is to clarify the gelatin which it accomplishes by coagulating and enclosing within itself all the solid organic particles or other detritus present. Filter until perfectly clear, using a covered hot water funnel, allowing the filtrate to run into an ordinary 500cc. flask. When through filtering, plug the flask containing the filtrate with cotton, cap with parchment paper and place in sterilizer, heat 212° exactly 15 minutes. To insure complete sterilization, repeat this on three successive days. Never sterilize longer, its power of solidifying will be destroyed. If apple must is used in this preparation, the gelatin is now ready for use, but if grape must is used, the gelatin should stand for two weeks in order to allow the tartaric acid to crystallize, then it is melted and re-filtered. Place the gelatin in tubes (10cc. each) as in case of the liquid. Plug and cap as before. Sterilize 15 minutes. Must-gelatin is the best media during cool weather but its liquifying temperature is below 40°C., therefore it cannot well be used during the Summer. For summer work we usually use agar-agar.

Preparation of Agar-agar. This is a vegetable product derived from sea-weed. It is prepared for laboratory use as follows: Take 500cc. distilled water and add from 1.2 to 1.5% of agar-agar, previously cut into pieces about 1cm. long. Let stand 12 hours in order to swell the tissue; cook three hours, and let stand twelve hours. Take of filtered must about 2% the total quantity, render this nearly neutral as in the case of the gelatin except this may be a trifle more acid and add it to the agar-agar, and cook about three hours in steam sterilizer. If you have time let stand 12 hours give another cooking of 3 hours. Agar-agar is cooked this long in order to coagulate the albumen. Filter as in case of gelatin. Sterilize for 40 minutes from the time that the agar liquifies, on three successive days. Place in tubes and sterilize for 40 minutes after boiling.

Sources of Yeast. Yeast occurs in nature wherever there are saccharine substances, therefore yeasts may be obtained from various sources, but the most common are from the lees of wines or ciders, skins of ripe fruits &c.

Six samples were taken from which the organisms were separated. These are given below.

Sample NO.1. Made cider from mixed apples at the station mill. Placed several hundred cc. of this in a sterile flask and plugged the same with cotton.

Sample No.2. Must from the Hyslop Crab apple.

Sample NO.3. Must from Norton's Virginia grape.

Sample NO.4. Must from the Souldard Crab apple.

Sample NO.5. Lees from Paulding's Pippin Cider.

Sample NO.6. Lees from a French wine, Bois de Terroir.

Preparing Samples for Separation. The above solutions almost invariably contain sufficient yeast to start alcoholic fermentation, however, yeast growth is sometimes suppressed by the presence and growth of large quantities of malferments in which case it is best to pour off the supernatant liquor, agitate the sediment and pour the same into a flask of fresh sterile must. After a few days alcoholic fermentation should be present which is known by the presence of a white deposit and the giving off of Carbon-dioxide upon agitating the flask, this may also be determined with accuracy by making a mount of the liquor and examining with the microscope.

Each of the samples separated showed the presence of a strong yeast growth hence they were sown directly to the Invert Flask, a device used to separate the alcoholic ferments from the other organisms present in the must.

Invert Flask. At this stage we have a liquor containing numerous organisms of two or more classes. The first class, the aerobic are those organisms which grow only in the presence of oxygen; the second class the anaerobic, or the facultative aerobic, are those organisms which are capable of growing without the presence of oxygen. To this second class belong the alcoholic ferments, and the first step towards the purification of these organisms is to separate the aerobic from the anaerobic ferments. This is accomplished by means of the Invert Flask. The Flask, a drawing of which is shown on the next page, is an excellent device when used under the proper conditions. It consists of a 450cc. Erlenmeyer Flask; a vent, a Fig. 2; a draw tube, a bent glass tube with a rubber tube on one end, b Fig. 2; a pinchcock c Fig. 2; and a rubber stopper provided with two perforations. Set the flask up as is shown in the drawing so that the straight tube of the vent will

Attention Patron:

Page 9 omitted from
numbering

reach within one half cm. of the bottom of the flask. Fill the Flask with water and adjust its height by inverting the Flask and drawing off the water through the draw-tube so that its surface will be within one half cm. of the end of the vent-tube. Revert the flask to its original position and mark the height of the water. Pour off the water and fill to the proper height with well filtered must. Replace stopper containing vent and draw-tube. Tie a parchment paper cap over the stopper and mouth of flask so as to completely cover both. Plug the end of the rubber tube with a loose cotton plug, also plug the vent in the same manner. Place in the sterilizer and sterilize about 20 minutes from time boiling begins.

Sow the Flask as follows:- Sterilize the hands thoroughly with 35% alcohol and remove parchment paper cap. Sterilize rubber stopper, and mouth of flask with 35% alcohol and loosen the stopper so that it may be easily removed. Better results are insured if the organisms to be sown are in a fresh active condition. Sterilize the mouth of the vessel containing the organisms, flame and pour off the supernatant liquor without agitating. Replace plug, agitate the deposit thoroughly, flame mouth of vessel and pour the deposit into the invert flask closing the same immediately. Seal with hot paraffin around stopper and glass tubes entering same. Place a pinchcock on the draw-tube. Invert the flask and clamp in position. Pour several cc. of dilute H_2SO_4 into the mouth of the vent in order to prevent the entrance of extraneous organisms. This flask should never be shaken but should be kept absolutely still in order to prevent the falling of undesirable ferments into the neck of the flask. After about two days it will be noticed that a good deposit of yeast rests on the sides of the flask and on the cork, also that fine bubbles of gas will be rising in the

draw-tube. When this latter occurs it is time to sow from invert flask, flame the mouth of a sterile tube of must remove its plugs. Flame cotton plug in the draw-tube of the Flask, remove and allow one or two drops of the liquor in the flask to pass into the tube. Quickly replace plug and re flame the tube. Three tubes are usually sown in this manner and a microscopical mount is made and examined. These three tubes are labeled and set aside for about ten days or until the strong fermentation subsides; at which time the cells should be well broken apart. If we now have tubes containing nothing but alcoholic ferments we are ready to proceed with the next step in their separation. However, if our tubes contain undesirable ferments which may be determined by a microscopical examination we have to resow to the Invert Flask.

In the laboratory here it is impossible to carry the flask for the two days without some agitation, hence it has been necessary to vary the method slightly. In our laboratory sowings from the invert flask contain mycoderma and other aerobic ferments hence instead of allowing the tubes to ferment for ten or twelve days, sow to the petri immediately from the tube sown from the invert. In this way pure cultures can be obtained because the aerobic ferments are in ~~are in~~ great minority hence the majority of the colonies in the petri will be true yeasts.

In separating the yeast from the samples given above it was intended that the first method of handling the cultures should be employed because the cells would be better separated when the cultures were sown to the petri. Sample No. 1. was sown to the Invert Flask three times; Sample No. 11 four times; Sample No. 111 four times; Sample No. V once without success as in each case the cultures contained either

Bacteria or Mycoderma. Hence it was necessary to sow each of the samples to the invert again and as soon as they were sown from the Invert Flask, they were sown to the Petri Dish with complete success.

Petri Dish. We now resort to Koch's plate culture method for the separation of pure cultures of the desired organisms, only we substitute the Petri Dish for the glass plated used by Koch. The Petri Dish is a glass dish ordinarily about 15cm. in diameter with perpendicular sides 10mm. high. This is provided with a glass top of the same shape but large enough to form a cover for the dish. Clean this dish thoroughly and place it in the hot air sterilizer, heat for 30 minutes at a temperature ranging from 130°C. to 150°C. Allow to cool in the sterilizer and remove with sterile hands to a moist chamber, previously sterilized and prepared as follows:- A moist chamber is simply a large Petri Dish, sterilized by washing with Bi-chloride of Mercury, 1 to 1000. Into the bottom of this dish is poured Bi-chloride of the same strength to a depth of .75cm. and on the bottom are placed glass bridges upon which the petris rest. To sow a petri from a culture twelve to fourteen days old, remove a drop from the culture tube with a loop platinum wire to a 10cc. sterile must tube, observing every precaution such as flaming the culture tube before and after opening &c. Thoroughly distribute cells through this liquid by agitation then from this dilute sewing remove some of the organisms by inserting into it a straight/platinum wire to a depth of about 1cm. and then quickly inserting the wire into a sterile gelatin tube previously melted and held at a temperature of 40°C. A higher temperature than the above will kill or weaken the yeast. Replace the plug and roll the gelatin tube thoroughly in order to insure the even distribution of the or-

ganisms throughout the gelatin. Re flame the mouth of the tube, remove plug, and pour the melted gelatin into the petri previously prepared as above. Cause the gelatin to spread over the entire bottom surface of the petri, label and replace into the moist chamber.

When sowing immediately from the Invert, sow a tube from the Invert as described above and barely touch its surface with a straight wire and inoculate the same into the melted gelatin tube. Then proceed to sow Petri as in the first case.

The moist chamber containing the Petri Dishes is place in the incubator, at a temperature ranging from 20 to 25°C. After about three days, the Petri should show colonies, distributed here and there, sufficiently large to be readily seen with the naked eye. When ready to sow from the Petri sterilize the desk with 35% alcohol then place petri on the desk. With a straight wire previously sterilized remove a portion of a colony, and make a microscopical mount. If this colony proves to be yeast, sow it into a sterile must tube. Proceed in this way until about ten tubes are sown from separate and distinct colonies.

From sample I nine tubes were sown from the Petri Dish, sample II, thirteen tubes; sample III, six tubes; sample IV, thirteen tubes; sample V, ten tubes; sample VI, six tubes.

Comparative Notes. These tubes were placed in the incubator and each day notes were taken on them. The notes include the following points: time growth begins; clearness and color of liquor before agitation; amount of resting foam; amount of gas present; whether gas falls or is persistent; amount of deposit; deposit coagulated, flocculant or normal. These notes were continued until fermentation subsided which took from twelve to fourteen days.

Below is given a characteristic note on each tube reserved from the different samples.

Tube I-1 Liquor fairly clear,-slight resting foam,-good compact deposit,-practically nongaseous,-deposit normal.

Tube I-8 Same except liquor beautiful and clear.

Tube II-5 Liquor beautifully clear,-slight resting foam,-trace of gas,-deposit nongaseous.

Tube II-6 Liquor slightly cloudy,-no resting foam,-practically nongaseous,-deposit normal.

Tube II-12 Same as Tube II-5 except deposit more coagulated.

Tube III-3 Liquor absolutely clear and bright,-slight resting foam,-good deposit resting slightly on sides of tube,-extremely gaseous,-deposit coagulated.

Tube IV-4 Liquor beautifully clear and bright,-considerable resting foam,-large deposit,-very gaseous,-deposit,flocculant.

Tube IV-5 Liquor absolutely clear and bright,-no resting foam,-large deposit,-extremely gaseous,-foam coarse,-deposit coagulated.

Tube V-1 Liquor fairly clear,-no resting foam,-good deposit,-nongaseous,-deposit normal.

Tube V-3 Liquor very cloudy otherwise same as above.

Tube VI-1 Liquor very cloudy,-good deposit,-extremely gaseous,-deposit normal.

Tube VI-6 Same as above except liquor very clear and is only moderately gaseous.

Drop Culture. At this time we discard all unpromising tubes keeping only those which show peculiarities or good characters. Each of the tubes reserved is run through the drop culture as perfected by Hansen. The culture tubes from which drop cultures are to be made should be from 10 to 14 days old. With a straight wire touch lightly the surface of the culture tube after agitation and sow into a melted 10cc. tube of gelatin, temperature 40°C. Previously prepare the slides and cover glasses to be used as follows: The slides used have a cell cut into the center of each and the cover glasses are sufficiently large to form a cover to the cell. Cleanse thoroughly with distilled water, wiping them dry so that no lint or other detritus will be present. Place them in absolute alcohol. Remove to a petri and allow the alcohol to evaporate leaving them perfectly clean. Place the cover glasses in a watch glass beside the slides, which are placed cells down in a petri; cover the petri and sterilize in hot air sterilizer as when sterilizing petri dishes. When cold remove to a sterile chamber composed of a bell jar on a ground glass plate or some other simple device. They are now ready for use.

Remove a sterile slide with the fingers, inverting it so that the cell will be up, placing it on a spot previously sterilized with alcohol. Take a fine camel's hair brush and mark around the edge of the cell a narrow line of vaseline. Remove a cover glass with sterile forceps placing it on the end of the slide. Remove from the melted gelatin tube a drop of the gelatin containing the organisms with a sterile loop wire, place it on the center of the cover glass then put the cover glass inverted over the cell of the slide. Press it down so as to seal the cover glass in place, thus forming a hanging drop.

within a sterile cell of a microscopic slide. Place on microscope clamping it into place with a Winkle's mechanical stage, a description of which is too complicated to be entered into here. By means of this stage search with the utmost care the whole drop of gelatin locating and recording the position of each individual cell within the drop.

Search the slide each day watching the growth from an individual cell to a colony large enough to be seen with the naked eye. Sow enough of these slides so that about ten tubes may be obtained from each source. When ready to sow from the drop, each recorded colony is located with a low power lens and marked with ink. Invert the cover glass and pick up each marked colony placing each in a separate tube.

Comparative Notes ^{When} about ten tubes were obtained from each source they were placed in the incubator and comparative notes were taken on them as before. They were then discarded down as before, saving only the best, some of which were run through the Physiological Test.

Physiological Test. Since certain types of yeasts have different powers of fermenting sugar and the resulting alcohol from the same must varies with organisms employed, it is necessary to carry each tube of yeast retained through the Physiological Test.

To prepare this test exactly 400cc. of standard must of a known chemical composition is placed in a thoroughly clean 1100cc. Erlenmeyer flask. This flask is fitted with a cork containing one perforation, through which is passed the stem of a vent as is shown in the illustration. Place over the cork and the mouth of the vent a parchment paper cap, also plug loosely with cotton the outside opening of the vent. Sterilize for thirty minutes in the Arnold's Steam Sterilizer. When cold use every precaution to create a sterile environment, remove

paper cap, and loosen the cork so that it may be easily removed, take a culture of the yeast to be sown ~~about~~ about four or five days old, and with a standard sized wire remove very carefully a drop of this liquor.

Insert it into the flask and wash the yeast off by moving the wire gently backward and forward, turning the wire so that the liquor in the flask will flow through the loop. Then remove the wire; reinsert the cork provided with the vent and seal into place with hot paraffin. Pour dilute sulphuric acid into the exterior opening of the vent until it rises in the chamber high enough to cover the end of the vent tube contained therein. Place the hand on the side of the flask and if the flask is sealed, gas will be given off through the vent due to the expansion of the air caused by the heat of the hand. If no gas passes find the leak and seal it. The utmost care should be exercised in the sowing of these tests as a standard condition should be maintained. At a certain hour on each day, this flask should be weighed; the temperature (incubator) taken in the morning and evening; and notes should be taken covering the points included under the section on comparative notes laying stress upon the aroma. When the weights become constant, send the test to the chemical laboratory and have it analyzed. A convenient form is shown below for the data required and its arrangement.

The chemical constituents of a must in order of their importance are Sugars. There are several forms of sugar in musts, glucose and sucrose being the most important. No organisms can completely ferment out all of the sugar, but true yeasts are capable of fermenting it down from .1% to .25%. The acids are of prime importance. From .40% to .60% of acid, calculated as Sulphuric, is usually present in an unfermented must. In a fermented must the acid is slightly above the original percentage owing to the formation of volatile acids through the action of the yeasts. The volatile acids formed are important from

the fact that they give to the liquor aroma, and also help the flavor. Tannin apparently restrains the yeasts from their complete action, preventing the total destruction of sugar, It seems to mollify the effect of the alcohol upon the human system, It also acts as a clarifier of the liquor.

The glycerol bodies formed by the decomposition of some of the alcohol in an acid solution, contributes to the liquor smoothness and aroma. The percentage of glucerol seems to have a peculiar relation to the percentage of alcohol. If glucerol is absent from a fermented liquor, it indicates that the liquor is a concocted preparation. The Total Solids have but little to do with the must, however about 2.5% is usually present in a fermented must.

Four yeasts were selected and carried through Fermentation Tests on standard apple must, also they were sown to tests on two sugar solutions of the following composition, 10% sucrose, 5% tartaric acid and 1% ammonium chloride; 10% glucose, 5% tartaric acid and 1% ammonium chloride. But growth was obtained on either sugar solution, but the tests on the standard must were carried to dryness. The notes and chemical data are given on the following pages.

Yeast I-10 Separated from MUST FROM MIXED APPLES

Fermentation Test No. ¹³³

5-19-03

Trifle deposit-no gas.

5-20-03

Liquor very cloudy-trifle deposit-slight resting foam-large deposit-extremely gaseous foam-persistent-normal.

5-21-03

Liquor very cloudy-considerable resting foam-large deposit-extremely gaseous-foam coarse-falls immediately-aroma very good.

5-22-03

Liquor cloudy-trifle resting foam-large compact deposit-extremely gaseous-foam falls aroma very good-deposit normal.

5-25-03

No resting foam-liquor cloudy-good deposit-strongly gaseous-foam fine-falls.

5-28-03

Liquor nearly clear-no resting foam-good compact deposit-liquor dark amber in color-considerable gas-aroma yeasty.

YEAST II-5-2 SEPARATED FROM HYSLOP GRAB APPLE

Fermentation Test No. 134

5-18-03

No growth.

5-20-03

Liquor clear and bright-no resting foam-large deposit in suspension and on sides & bottom of the flask. Liquor clouds-trifle foam.

5-21-03

Considerable resting foam. Liquor cloudy-good deposit-spread over bottom-fairly gaseous-deposit fl
floculent-clouds.

5-22-03

Considerable resting foam-Liquor clear and bright-fairly gaseous-aroma good deposit-floculent.

5-25-03

Liquor absolutely clear and bright. Trifle resting foam-aroma very rich-considerably gaseous-deposit coagulated.

5-28-03

Note same liquor light amber in color-aroma especially good-fairly gaseous.

YEAST II-6-9 SEPARATED FROM HYSLOP CRAB APPLE.

Fermentation Test No. 135.

5-19-03

Considerable deposit-enough to cloud liquor-
no gas.

5-20-03

Large pearly white, compact deposit-liquor cloud
cloudy-trifle resting foam-strongly gaseous. Foam
coarse, falls immediately.

5-21-03.

No resting foam-liquor cloudy-deposit large,
compact-strongly gaseous. Foam coarse, falls imme-
diately, aroma good.

5-22-03

Liquor cloudy, no resting foam-large com-
pact deposit. Strongly gaseous, foam falls immediate-
ly-aroma very good.

5-25-03.

No resting foam-liquor very cloudy, strong
deposit-fairly gaseous-aroma rich and characteris-
tic.

5-28-03.

Note same, liquor clearing slightly, trifle

YEAST II-6-9 SEPARATED FROM HYSLOP CRAB APPLE.

Fermentation Test No. 135

gaseous-liquor dark amber in color.

6-1-03.

Liquor clear but not bright.

YEAST III-3-7. FROM NORTON'S VIRGINIA GRAPE.

5 - 18 - 03 Test No. 136 Yeast No. ⁹⁶ III-3-7
 Sowed at 10 AM 400 c. c. standard apple must, No. 6, Erlenmeyer, with
 germs from Tube ~~III-3-7~~ Sown 5 - 11 - 03 190
 Weight at 11,30 AM 630.42 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature			
			9:00 a. m.	4:00 p. m.		
5-19	630.41		22.2	24.0	Total hrs. of Experiment	456
5-20	630.32	.09	23.5	24.1	" " before fermentation	40
5-21	625.79	4.53	24.2	21.5	" " duration of "	416
5-22	622.35	3.44	23.0	24.5	Total loss in grams	24.56
5-23	619.22	3.13	25.5	23.0	Average Temperature	22.7
5-24	616.20	3.02	25.5	23.5	Range of Temperature	6°C.
5-25	614.00	2.20	25.0	23.5	Sent to Chemist	
5-26	612.00	2.00	23.0	22.5	Analysis reported	
5-27	610.60	1.40	21.0	23.0		
5-28	609.30	1.21	22.5	24.0		
5-29	608.57	.22	23.0	25.0		
5-30	607.95	.62	23.0	22.5	Original Must.	1.060
5-31	607.45	.50	22.0	23.0		
6-1	607.12	.33	21.0	22.0	Specific Gravity	1.009
6-2	606.90	.22	20.0	20.5	Alcohol	6.44
6-3	606.73	.17	19.0	21.0	Sugar, Total	0.28
6-4	606.55	.56	20.0	22.0	Sugar, Reducing	0.28
6-5	606.35	.20	21.0	22.5	Sucrose	
6-6	606.20	.15	21.0		Solids, Total	2.06
6-8	605.85	.35			Acid, Total	0.56
					Acid, Volatile	
					Tannin	0.018
					Glycerol	
					Ash	

Analytical Data.

YEAST III-3-7. FROM NORTON'S VIRGINIA GRAPE.

Fermentation Test No. 136

5-19-03

5-19-03

No growth.

5-21-03 deposit-no gas.

5-20-03.

Liquor fairly clear and bright-no resting
foam, small deposit, liquor cloudy-no gas.

5-21-03

Same as Test 134-foam persistent.

5-22-03.

Same as before.

5-25-03.

Same as noted on 134.

5-28-03.

Note same. Test more gaseous than any at
this date.

From the chart showing Fermentation curves of the Yeast, it will be noticed that each of the four yeasts reached its climax on the third day and each decreased in activity regularly except on the fifth day when there was a decided check in the curve due to the higher temperature at that time.

From the chemical data also given on the same page it will be seen that each of the yeasts fermented the sugar to dryness and also the acid was increased slightly.

Spore Cultures. In order to determine absolutely whether an organism is a true Saccharomyces or not, it is necessary to run the culture through the spore culture test.

Mould a round or square block of plaster paris about 6cm. long, 4cm. wide and $2\frac{1}{2}$ cm. thick. Bore into this block two conical holes, 1cm. in diameter and about 1cm. deep. Place this block into a water bath so that it will be completely covered by water and boil for one hour in order to saturate it with water and also to sterilize it. Remove while hot with sterilized forceps to a sterile petri or other convenient dish. Pour into this petri, containing the block, sterile water until its level is about half way up to the plaster block. Take a young culture, four to six days old, and using the usual precautions to prevent impurity, pour into each hole in the block five or six drops of the liquor. Here the distilled water gradually removes by diffusion the food from the liquor containing the yeasts. Hence after a few days these yeast cells begin to form spores within themselves to carry them over this period of adversity. This is quite uniformly the result of the organism being tested as a true Saccharomyces.

This spore formation may be watched throughout the whole time by making microscopical mounts from time to time from the liquor contained within the holes in the block.

Yeasts I-1-10; II-5-2; II-6-9 and III-3-7 were sown to spore cultures and each produced spores as described above.

Giant Colonies. Lindner claims that by inoculating a point on a flat surface of gelatin or agar-agar with a pure culture of yeast and keeping the culture sufficiently long, a more or less characteristic growth will be obtained, and that different races of yeasts produce typically different colonies. This subject has not been thoroughly investigated as yet, but as a means of determining the different races of yeasts it seems to be a field of work which has considerable promise.

Permanent Cultures. Giant Colony Cultures as described above are used to a great extent as Permanent Cultures. It is said that yeasts may be kept in this way for years in an absolute state of purity.

The most permanent method known, the one used at all of the best laboratories, is to sow the organisms into a 10% solution of pure cane sugar (sucrose). Use a small glass bottle, 50cc. capacity, having its mouth ground on the outside. Over this fits a ground glass cap which is formed into a bulb and is then drawn to a small tube open at the upper extremity. A 10% solution of pure cane sugar is made up, 10cc. of which is placed in each bottle. The cap is placed on the bottle and the tube in the end of the cap is plugged with cotton. Sterilize for forty minutes on three successive days. Sow using the uttermost precautions with a loop wire. Replace the cap. Melt some vaseline and

seal the cap into place. Lock the flasks after sowing in a box and place the same where it will not be disturbed. These cultures are said to remain alive for ten or fifteen years however, it is best to resow them once each year. Also carry a set of cultures in must which should be resown every three months. In this way one keeps three sets of pure culture all the time.

MALFERMENTS

THE FOLLOWING PAGES GIVE THE MALFERMENTS SEPARATED WITH
THEIR ORIGIN AND FERMENTATION TEST NOTES.

ASPERCILLUS

SEPARATED FROM TUBE SPOILED APPLE MUST. SOWN TO FERMENTATION TEST

SEE CHART

12-15-1903

Test No. 87

aspergillus
Lab. No. 141
~~FUNGUS~~
~~Yeast No. A-1~~Sowed at 3 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with
germs from Tube A-1, Sown 10-21-1902.

Weight at 3,30 PM 619.00 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-16	618.98			
12-17	618.95	.03		
12-18	618.92	.03		
1-5	618.55	.37		
1-12	618.37	.18		
2-2	617.87	.50		
2-16	617.45	.42		
3-3	616.95	.50		
3-16	616.35	.60		

Total hrs. of Experiment 1278
 " " before fermentation
 " " duration of "
 Total loss in grams 2.65
 Average Temperature
 Range of Temperature
 Sent to Chemist 3-16-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.066	Specific Gravity	1.063
None	Alcohol	None
13.15%	Sugar, Total	11.68
10.36	Sugar, Reducing	11.09
2.65	Sucrose	0.56
14.60	Solids, Total	14.81
0.51	Acid, Total	0.46
None	Acid, Volatile	None
0.018	Tannin	0.014
	Glycerol	
	Ash	

ASPERCILLUS

SEPARATED FROM TUBE SPOILED APPLE MUST. SOWN TO FERMENTATION TEST

SEE CHART

Fermentation Test No. 87.

12-16

No growth.

12.17

Slight growth.

12-18.

Same.

1-5.

Thick mass of mycellium on bottom, -several small patches of sporophores on surface.

1-12.

Same as before except mycellium entirely submerged.

2-2.

Liquor dark amber - only one patch of sporophores on surface.

(29)

ASPERGILLUS

SEPARATED FROM A TUBE OF SPOILED APPLE MUST. SOWN TO FERMENTATION TEST.

SEE CHART

ASPERGILLUS.

SEPARATED FROM SAMPLE NO. II-HYSLOP CRAB-APPLE. SOWN TO FERMENTATION

TEST.

SEE CHART.

Aspergillus 143

Fungus
~~Yeast No. B-2~~

12 - 15 - 1903 Test No. 85
 Sowed at 3 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with
 germs from Tube B-2, Sown 10 - 21 1902
 Weight at 3,30 PM 606.20 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-16	606.20	.15		
12-17	606.05	.15		
12-18	606.05	.00		
1-5	605.87	.18		
1-12	605.76	.11		
2-2	605.18	.58		
2-16	604.72	.46		
3-3	604.23	.49		
3-6	604.10	.13		

Total hrs. of Experiment 1938
 " " before fermentation
 " " duration of
 Total loss in grams 2.10
 Average Temperature 22.0
 Range of Temperature
 Sent to Chemist 3-6-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.066	Specific Gravity	1.064
None	Alcohol	0.28
13.15	Sugar, Total	12.17
10.36	Sugar, Reducing	12.01
2.65	Sucrose	0.15
14.60	Solids, Total	15.01
0.51	Acid, Total Sulfuric	0.36
None	Acid, Volatile	None
0.018	Tannin	0.018
	Glycerol	
	Ash	

ASPERGILLUS.

SEPARATED FROM SAMPLE NO. II-HYSLOP CRAB-APPLE. SOWN TO FERMENTATION

TEST.

SEE CHART.

Fermentation Test No. 85

12-17.

Trifle mycelial growth.

12-18.

Same.

1-5.

Three small fruiting patches on surface.

1-23.

Three large patches light on margin shading to a green in the middle,--raised above liquor, eight or ten small patches are also present,--nearly white.

2-2.

Liquor amber in color.

3-6.

Liquor deep wine color. Practically no mycelium submerged. Entirely covered on surface with a yellow and green veil..

SEPARATED FROM SAMPLE NO. 1-MIXED APPLES SOWN TO FERMENTATION TEST.

SEE CHART

Malferments

Penicillium 144

12 - 13 - 1902

Test No. 84

~~Penicillium~~
Yeast No. 1(F)

Sowed at 3 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube E-1(F) Sown 10 - 10 - 1903

Weight at 3,30 PM 642.95 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-14	642.90		22.0	22.5
12-15	642.88	.02	18.0	23.0
12-16	642.87	.01	22.5	23.0
12-17	642.80	.07	22.5	23.5
12-18	642.79	.01	22.5	
1-5	641.30	1.49		
1-12	640.87	.43		
2-2	639.90	.97		
2-16	639.20	.70		
3-3	638.50	.70		
3-6	638.35	.15		

Total hrs. of Experiment 1952
 " " before fermentation
 " " duration of "
 Total loss in grams 4.55
 Average Temperature 22.5
 Range of Temperature
 Sent to Chemist
 Analysis reported

Analytical Data.

Original Must.	Fermented Liquor.
	Specific Gravity 1.056
	Alcohol 1.46%
	Sugar, Total 11.01
	Sugar, Reducing 11.01
	Sucrose 0.00
	Solids, Total 18.35
	Acid, Total 0.51
	Acid, Volatile None
	Tannin .018
	Glycerol
	Ash

SEPARATED FROM SAMPLE NO. I-MIXED APPLES SOWN TO FERMENTATION TEST.

SEE CHART

~~CHITULLE~~
Fermentation Test No. 84

12-14.

No growth.

12-15.

Considerable mycelia,-growth in liquor.

12-16.

Growing nicely.

12-17.

Dense mass of mycelium floating near surface of liquor.

1-5.

Large mass of mycelium on bottom and several patches of white and blue fruiting bodies on bottom.

1-12.

Same as before.

2-2.

Same.

3-6.

Liquor unchanged, weaker growth than in Penicillium.

SEPARATED FROM SAMPLE NO. IV. SOULARD CRAB-APPLE, SOWN TO FERMENTATION TEST.

SEE CHART.
PENICILLIUM.

curve

Penicillium 145
~~Yeast No. 1~~

12 - 13 - 1902 Test No. 82
Sowed at 3 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube Pen. 1, Sown 10 - 10 1902
Weight at 3,30 PM 619.87 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-14	619.80			
12-15	619.77	.03		
12-16	619.79			
12-17	619.71	.08		
12-18	619.68	.03		
1-5	618.08	1.60		
1-12	617.75	.33		
2-2	616.55	1.20		
2-16	616.10	.45		
3-3	615.63	.47		
3-16	615.33	.30		

Total hrs. of Experiment 2226
 " " before fermentation
 " " duration of "
 Total loss in grams 4.47
 Average Temperature 22.0
 Range of Temperature
 Sent to Chemist 3-16-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.066	Specific Gravity	1.067
None	Alcohol	0.74
13.15	Sugar, Total	11.03
10.36	Sugar, Reducing	10.81
2.65	Sucrose	0.21
14.60	Solids, Total	13.77
0.51	Acid, Total	0.51
None	Acid, Volatile	0.03
0.018	Tannin	0.015
	Glycerol	
	Ash	

SEPARATED FROM SAMPLE NO. IV. SOULARD CRAB-APPLE. SOWN TO FERMENTATION
TEST.

SEE CHART.
PENICILLIUM.

Fermentation Test No. 82

12-16.

Considerable mycelial growth in liquor, -
one small patch on surface apparently ready to
fruit.

12-16.

Very strong spongy growth of mycelium in
liquor and about twelve patches on the surface.

12-17.

Same as before.

1-5.

Surface about $\frac{3}{4}$ covered by a thick mat-
ted fruiting mass, -apparently pure, -greenish and
white in color, -no deposit.

1-12.

Same.

3-6.

Surface entirely covered.

BACTERIUM

SEPARATED FROM SAMPLE NO. IV. SOULARD GRAB SOWN TO FERMENTATION TEST

SEE CHART

Bacterium 146

2 - 10 - 02 Test No. 93 Yeast No. Bac-6

Sowed at 10 AM 400 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube Bac-6 Sown 1 - 23- 1903

Weight at 11 AM 795.17 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
2-11	795.15			
2-12	795.10	.05		
2-16	794.87	.23		
3-3	793.52	1.35		
3-13	792.75	.77		
3-30	791.57	1.23		
4-20	790.40	1.17		

Total hrs. of Experiment 1655
 " " before fermentation
 " " duration of "
 Total loss in grams 4.75
 Average Temperature 22.5
 Range of Temperature
 Sent to Chemist 4-21-03
 Analysis reported

Analytical Data.

Original Must.	Fermented Liquor.
Specific Gravity	1.061
Alcohol	0.65%
Sugar, Total	10.38
Sugar, Reducing	Red 9.19
Sucrose	1.13
Solids, Total	15.34
Acid, Total	.59
Acid, Volatile	.013
Tannin	
Glycerol	
Ash	

BACTERIUM

SEPARATED FROM SAMPLE NO. IV. SOULARD CRAB SOWN TO FERMENTATION TEST

SEE CHART

Fermentation Test No. 95

2-12.

No growth. No liquor.

2-14.

Liquor very cloudy, -trifle veil. Deposit in a loose threadlike mass. No gas.

3-3.

Liquor very cloudy. Decided veil, -larger deposit.

3-13.

Liquor very cloudy, yellowish brown, trifle deposit. Two distinct thin veils on bottom surface covered by a spider-weblike veil.

3-30.

Note same except veil does not fall so readily.

BACTERIUM.

SEPARATED FROM SAMPLE NO. IX-VALLEE'-D'AUGE. SOWN TO FERMENTATION TEST.
SEE CHART.

2 - 10 - 1903

Test No. 94

Bacterium ¹⁵⁷
Yeast No. ~~124~~

Sowed at 10 AM 400 c. c. standard apple must, No. 4, Erlenmeyer, with
germs from Tube ~~IX-715-7~~, Sown 2-2-1903

Weight at 11 AM 796.20 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
2-11	796.19			
2-12	796.17	.02		
2-16	796.00	.17		
3-3	795.25	.75		
3-13	794.68	.57		
3-30	793.85	.83		
4-8	793.50	.35		

Total hrs. of Experiment 1368
 " " before fermentation
 " " duration of "
 Total loss in grams 2.69
 Average Temperature 22.0
 Range of Temperature
 Sent to Chemist 4-8-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.066	Specific Gravity	1.065
None	Alcohol	0.07
13.15	Sugar, Total	9.13
10.36	Sugar, Reducing	8.09
2.65	Sucrose	0.99
14.60	Solids, Total	14.27
0.51	Acid, Total	1.17
None	Acid, Volatile	0.41
0.018	Tannin	0.012
	Glycerol	
	Ash	

BACTERIUM.

SEPARATED FROM SAMPLE NO. IX-VALLEE'-D'AUGE. SOWN TO FERMENTATION TEST.
SEE CHART.

Fermentation Test No. 94

2-12.

No growth.

2-14.

Slight growth.

3-3.

Liquor cloudy, -veil slight-considerable
deposit.

3-13.

Liquor upper half fairly clear straw/color
lower half very cloudy. Slight veil which breaks
and sinks upon the slightest agitation, -small
brownish deposit.

3-30.

Liquor cloudy.

DEMATIUM PULLULANS.

FOUND GROWING ON A SOLUTION OF SUGAR. SEPARATED BY PETRI DISH SOWINGS.
SOWN TO FERMENTATION TEST. SEE CHART.

2-10-1903 Test No. 91 ~~Yeast No. 2~~ ^{Dematium 147}
Sowed at 10 AM 400 c. c. standard apple must, No. 4, Erlenmeyer, with
germs from Tube Dem-2 Sown 2-21 1903
Weight at 11 AM 801.07 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
2-11	801.06			
2-12	801.03	.03		
2-16	800.75	.28		
3-3	799.60	1.15		
3-13	798.75	.85		
3-30	797.12	1.63		
4-8	796.70	.42		

Total hrs. of Experiment 1368
 " " before fermentation
 " " duration of "
 Total loss in grams 4.36
 Average Temperature 22.0
 Range of Temperature
 Sent to Chemist 4-8-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.066	Specific Gravity	1.06
None	Alcohol	0.57
13.15	Sugar, Total	11.0
10.36	Sugar, Reducing	10.0
2.65	Sucrose	1.0
14.60	Solids, Total	14.8
0.51	Acid, Total	0.4
None	Acid, Volatile	0.0
0.018	Tannin	0.01
	Glycerol	
	Ash	

DEMATIUM PULLULANS.

FOUND GROWING ON A SOLUTION OF SUGAR. SEPARATED BY PETRI DISH SOWINGS.
SOWN TO FERMENTATION TEST. SEE CHART.

Fermentation Test No.91

2-12.

No growth.

2-14.

Trifle deposit.

3-3.

Black and white sploched veil on surface
some mycelium in suspension,--strong deposit on sides
and bottom of flask. Liquor slightly cloudy.

3-13.

Liquor clear pale straw color,--surface
covered by a dense white gelatin mass,--deposit in
spots on sides and bottom of flask.

3-30.

Veil thick and fatty as before.

MYCODERMA.

SEPARATED FROM SAMPLE NO. IX-2 VALLEE'D'AUGE. SOWN TO FERMENTATION TEST.

SEE CHART.

12 - 17 - 1902.

Test No. 91-(a)

Mycoderma 153
Yeast No. IX-2

Sowed at 4 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with
germs from Tube IX-2, Sown 9 - 15 - 1902

Weight at 5 PM 630.80 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-18	630.78			
1-5	628.75	2.03		
1-12	627.70	1.05		
2-2	624.55	3.15		
2-16	622.75	1.80		
3-3	621.25	1.50		
3-9	620.69	.56		

Total hrs. of Experiment 1963
 " " before fermentation
 " " duration of "
 Total loss in grams 10.09
 Average Temperature 22.0
 Range of Temperature
 Sent to Chemist 3-9-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.066	Specific Gravity	1.045
None	Alcohol	1.59
13.15	Sugar, Total	8.23
10.36	Sugar, Reducing	7.18
2.65	Sucrose	1.00
14.60	Solids, Total	11.17
0.51	Acid, Total	0.70
None	Acid, Volatile	0.25
0.018	Tannin	0.011
	Glycerol	
	Ash	

MYCODERMA.

SEPARATED FROM SAMPLE NO. IX-2 VALLEE'D'AUGE. SOWN TO FERMENTATION TEST.

SEE CHART.

Fermentation Test No. 91-(a)

12-18.

No apparent growth.

1-5.

Veil strong, thick and markably corrugated
trifle deposit, -liquor clear.

1-2.

Same as before.

2-2.

Same.

3-9.

Same as before extending up sides of flask
as in Test No. 90.

SEPARATED FROM SAMPLE NO. II. HYSLOP CRAB-APPLE. SOWN TO PHYSIOLOGICAL TEST. APICULATUS.

SEE CHART.

corrected

10 - 24 - 1902

Test No. 77

Apiculatus No 100
Yeast No. ~~100~~

Sowed at 4.30 PM 100 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube ~~Apple~~ ^{W70} Sown 10 - 13 - 1902

Weight at 4.50 PM 645.87 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
10-25	645.85		24.0	25.0
10-26	645.37	.48	23.0	23.5
10-27	643.89	1.48	23.0	24.0
10-28	641.84	2.05	23.0	23.0
10-29	639.99	1.85	22.0	22.5
10-30	638.53	1.46	22.0	23.0
10-31	637.20	1.33	21.0	22.3
11-1	635.82	1.32	22.5	23.5
11-2	634.60	1.22	23.0	23.0
11-3	633.56	1.04	22.5	22.3
11-4	632.80	.76	21.2	22.0
11-5	632.18	.62	22.5	24.5
11-6	631.73	.45	21.0	23.5
11-7	631.40	.33	23.0	23.0
11-8	631.25	.15	21.5	22.5
11-9	631.17	.08	20.0	21.0
11-10	631.10	.07	20.0	23.5
11-11	630.93	.17	24.0	24.5
11-12	630.81	.13	25.0	25.0
11-13	630.80	.01	20.0	23.5
11-14	630.72	.08	22.0	24.0
11-15	630.65	.07	23.0	24.0
11-16	630.60	.05	22.5	24.2
11-17	630.55	.05	22.5	

Total hrs. of Experiment 595
 " " before fermentation 27
 " " duration of " 568
 Total loss in grams 15.35
 Average Temperature 22.7°C.
 Range of Temperature 5°C.
 Sent to Chemist 10-17-02
 Analysis reported Dec 12

Analytical Data.

	Original Must.	Fermented Liquor.
Specific Gravity	1.028	1.028
Alcohol	3.25%	3.25%
Sugar, Total	5.26	5.26
Sugar, Reducing	2.73	2.73
Sucrose	2.40	2.40
Solids, Total	7.27%	7.27%
Acid, Total	0.60%	0.60%
Acid, Volatile	0.04	0.04
Tannin	0.01%	0.01%
Glycerol		
Ash		

SEPARATED FROM SAMPLE NO. II. HYSLOP CRAB-APPLE. SOWN TO PHYSIOLOGICAL
TEST. APICULATUS.

SEE CHART.

Fermentation Test No. 77

10-25.

No growth.

10-26.

Liquor very cloudy-no resting foam. Good deposit,-very gaseous.

10-27.

Very gaseous-foam fine-aroma not good.

10-28.

Same as before-aroma acid and earthy.

11-5.

Veil on surface-does not appear to be mycoderma.

11-17.

Still very cloudy.

FORULA.

SEPARATED FROM SAMPLE NO. I DANKFORD APPLE SOWN TO FERMENTATION TEST.

SEE CHART.

2-10-1903

Test No. 92

121
Yeast No. ~~V-6-4~~

Sowed at 10 AM 400 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube ~~V-6-4~~ ¹²¹, Sown 2-7-1903

Weight at 11 AM 811.29 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
2-11	811.29			
2-12	811.20	.09		
2-16	800.00	2.20		
3-5	801.21	7.79		
3-13	797.35	3.86		
3-30	792.25	5.10		
4-6	790.30	1.90		
4-21	789.82	.48		

Total hrs. of Experiment 1679
 " " before fermentation
 " " duration of
 Total loss in grams 21.37
 Average Temperature 22.5
 Range of Temperature
 Sent to Chemist 4-21-03
 Analysis reported

Analytical Data.

Original Must.	Fermented Liquor.
Specific Gravity	1.022
Alcohol	4.15%
Sugar, Total	1.68
Sugar, Reducing	1.69
Sucrose	.01
Solids, Total	7.30
Acid, Total	0.53
Acid, Volatile	
Tannin	.007
Glycerol	
Ash	

FORULA.

SEPARATED FROM SAMPLE NO. 1 DANKFORD APPLE SOWN TO FERMENTATION TEST.

SEE CHART.

Fermentation Test No. 92

2-12.

Considerable growth. Liquor cloudy,-
slight deposit and no gas.

2-14.

Liquor very cloudy,-large deposit,-veil
cloudy covering entire surface,-considerable gas.

3-3.

Heavy,dirty veil over entire surface,-li-
quor entirely clear,-little deposit,-pale straw col-
or,-large deposit.

3-13.

Liquor clear,pale straw color,-good white
deposit,-veil thick,white and foamy.

3-30.

Liquor absolutely clear and bright,-mass-
es of veil falling to bottom,-very large deposit.

MYCODERMA.

SEPARATED FROM SAMPLE IX VALLEE'D'AUGE. SOWN TO FERMENTATION TEST.

SEE CHART.

12 - 15 - 1902

Test No. 90

Mycoderma 135
Yeast No. IX-4

Sowed at 3 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube IX-4, Sown 9 - 15 1902

Weight at 3,30 PM 614.44 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-16	614.43			
12-17	614.42	.01		
12-18	614.41	.01		
1-5	614.05	.36		
1-12	613.86	.19		
2-2	613.25	.61		
2-16	612.82	.43		
3-3	612.34	.48		
3-9	612.18	.16		

Total hrs. of Experiment 2001
 " " before fermentation
 " " duration of "
 Total loss in grams 2.26
 Average Temperature 22.0
 Range of Temperature
 Sent to Chemist 3-9-03
 Analysis reported 5-12-03

Analytical Data.

Original Must.		Fermented Liquor.
1.068	Specific Gravity	1.063
No. %	Alcohol	0.42
13.15	Sugar, Total	12.05
10.36	Sugar, Reducing	10.65
2.65	Sucrose	1.33
14.60	Solids, Total	15.01
0.15	Acid, Total : Sulfur	0.53
None	Acid, Volatile	0.06
0.018	Tannin	0.012
	Glycerol	
	Ash	

MYCODERMA.

SEPARATED FROM SAMPLE IX VALLEE'D'AUGE. SOWN TO FERMENTATION TEST.

SEE CHART.

Fermentation Test No. 90

12-16.

No apparent growth.

12-17.

Slight growth.

12-18.

Fairly large, -distinct veil present.

1-5.

Dense white mottled veil on surface - slightly wrinkled, -considerable deposit, -liquor clear.

1-12.

Liquor lighter, -deposit larger, otherwise same as before.

3-9.

Same as before. Veil crawling up sides of flask in irregular lines.

FORULA.

SEPARATED FROM SAMPLE VIII, APPLE. SOWN TO FERMENTATION TEST.

SEE CHART.

12-15-1902

Test No. 88

170
Yeast No. 110-c-4

Sowed at 3 PM 400 c. c. standard apple must, No. 4, Erlenmeyer, with germs from Tube VIII-0-2 Sown 11-4-1902

Weight at 3:30 PM 602.00 grams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
12-16	602.00		22.5	23.0
12-17	601.92	.08	22.5	23.5
12-18	601.29	.63	22.5	24.0
12-19	600.38	.91	23.0	24.5
12-20	599.27	1.11	23.5	23.5
12-21	598.34	.93	22.5	24.0
12-22	597.46	.88	23.0	24.5
12-23	596.65	.81	21.5	23.5
12-24	596.30	.35	21.2	22.5
12-25	595.45	.75	22.5	22.6
12-26	595.05	.40	22.8	21.4
12-27	594.66	.39	20.0	21.0
12-28	593.80	.86	22.0	23.0
12-29	593.12	.68	22.0	23.5
12-30	592.53	.59	23.0	23.0
12-31	591.91	.62	22.0	24.0
1-1	591.31	.60	22.5	24.5
1-2	590.73	.62	23.0	23.0
1-3	590.27	.46	23.0	24.0
1-4	589.80	.47	23.5	23.5
1-5	589.32	.48	23.0	24.0
1-6	588.85	.47	23.0	23.5
1-7	588.40	.45	23.0	23.5
1-8	587.95	.45	21.5	22.5
1-9	587.60	.35	19.0	20.0
1-10	587.27	.33	19.0	22.5
1-11	586.87	.40	21.0	23.0
1-12	586.60	.27	20.0	20.0
1-13	586.35	.25	19.5	20.0

Total hrs. of Experiment 1368
 " " before fermentation
 " " duration of
 Total loss in grams 20.65
 Average Temperature 22.70
 Range of Temperature
 Sent to Chemist 2-12-03
 Analysis reported

Analytical Data.

	Original Must.	Fermented Liquor.
Specific Gravity		1.020
Alcohol		4.37%
Sugar, Total		2.64
Sugar, Reducing		0.41
Sucrose		2.12
Solids, Total		5.60
Acid, Total		0.44
Acid, Volatile		
Tannin		.012
Glycerol		
Ash		

FORULA.

SEPARATED FROM SAMPLE VIII, APPLE. SOWN TO FERMENTATION TEST.

SEE CHART.

Test No.

Yeast No.

Sowed at 400 c. c. standard apple must, No., Erlenmeyer, with
germs from Tube, Sown 190.....

Weight atgrams.

Date	Wt. at 9:00 a. m.	Loss	Temperature	
			9:00 a. m.	4:00 p. m.
1-14	586.15	.20	20.5	22.0
1-15	585.75	.40	21.5	22.5
1-16	585.40	.35	23.0	24.0
1-17	585.15	.25	22.0	23.0
1-18	584.90	.25	18.0	25.0
1-19	584.64	.26	22.5	23.5
1-20	584.40	.24	22.5	24.0
1-21	584.16	.24	22.0	23.5
1-22	583.95	.21	23.0	25.0
1-23	583.77	.18	22.5	24.5
1-24	583.55	.22	22.0	24.5
1-25	583.37	.18	23.0	23.5
1-26	583.20	.17	21.5	23.0
1-27	583.05	.15	22.0	25.0
1-28	582.92	.13	22.5	24.5
1-29	582.77	.15	21.0	23.0
1-30	582.60	.17	21.0	23.0
1-31	582.45	.15	22.5	24.5
2-1	582.32	.13	23.0	25.0
2-2	582.24	.08	24.0	24.5
2-3	582.11	.13	23.0	25.0
2-4	582.00	.11	25.5	24.0
2-5	581.86	.14	22.5	24.5
2-6	581.72	.14	22.5	23.5
2-7	581.70	.02	20.0	24.0
2-8	581.61	.09	21.0	25.0
2-9	581.53	.08	20.0	23.5
2-10	581.47	.06	21.5	24.0
2-11	581.42	.05	22.5	25.0

Total hrs. of Experiment
 " " before fermentation
 " " duration of "
 Total loss in grams
 Average Temperature
 Range of Temperature
 Sent to Chemist
 Analysis reported

Analytical Data.

Original Must.	Fermented Liquor.
.....	Specific Gravity
.....	Alcohol
.....	Sugar, Total
.....	Sugar, Reducing
.....	Sucrose
.....	Solids, Total
.....	Acid, Total
.....	Acid, Volatile
.....	Tannin
.....	Glycerol
.....	Ash

FORULA.

SEPARATED FROM SAMPLE VIII, APPLE. SOWN TO FERMENTATION TEST.

SEE CHART.

Fermentation Test No. 88

12-16.

No apparent growth.

12-17.

No resting foam-liquor clear- considerable deposit of individual colonies on the bottom of the flask-slightly clouds.

12-18.

Liquor very cloudy-decided veil-foam in clot

12-19.

Considerable resting foam-veil present-liquor very cloudy-fairly large yellowish deposit-
aroma very mild,fruity,good-considerable gas.

12-20.

About $\frac{1}{2}$ inches of very fine foam-aroma very fair.

1-5.

Trifle resting foam-veil thin extending about 1 inch up sides of flask-grayish deposit.