PART V.

REPORT ON THE STUDY OF FERMENTATIONS IN THE MANUFACTURE OF JAMAICA RUMS.

By S. F. ASHBY, B.Sc., *Fermentation Chemist.*

I. Useful Information Regarding Estate Distillery Materials.

Skimmings or Scummings—A mixture of liquor and solid matters skimmed from the surface of juice in clarifiers and coppers (if used) together with wash water from coppers, etc. The solid matter a mixture of pulverised cane fibre (trash), phosphate of lime, pectic and waxy matters, and coagulated albumen. According to the amount of solid matter and of dilution the gravity may vary when quite fresh from that of the juice (15-20 Brix) to under 10 Brix. The reaction to litmus is either neutral, faintly acid or faint alkaline.

Dunder—The liquor left in the still after distillation is completed. A yeast extract. The gravity varies according to materials fermented from under 10 Brix to over 25 Brix, and the same applies to the acidity which varies from about 1 per cent. to over 3 per cent. It is never free from sugar which varies from 0.2 per cent. to over 1 per cent. Sugars other than hexoses (pentoses) and allied bodies may be present which reduce Fehling's solution but are not fermentable by yeast. On an average about 1 per cent. of glycerine has been found in Dunder. It is never free from volatile acid.

Its high density is due to cane and yeast gum and caramel (especially if still is direct fired.)

Molasses—The sweet viscous syrup separated from the crystalised sugar by the centrifugals. It is markedly acid (about 0.5 per cent.) has a specific gravity of about 1.45, contains about 40 to 60 per cent. cane sugar, and 10 to over 20 per cent. glucose. One gallon (imperial) contains 8–10 pounds of fermentable sugar.

Acid—Skimmings, normal cane juice, or rum cane juice, allowed to sour. The production of acetic acid is the object sought. The volatile acidity rarely exceeds 40 per cent. of the total and is usually under one-third the total.

The souring is carried out either with trash cisterns or without the addition of trash. The liquor ferments (yeast) and sours simultaneously.

Lees—The liquor left in the retorts after distillation is completed. It contains a high proportion of volatile acid.

Wash—The liquor (prepared from the mixed materials) which is actually fermented and distilled for rum. The mixing of the materials is called "setting up." When fermenting it is "live" wash, and when fermentation has ceased it is "dead" wash.

Flavour and "Muck Hole"—(See description in first Sugar Experiment Report.)

Rum—The early portion of the alcoholic distillate; (the preliminary runnings if cloudy are rejected) its strength varies from 36 to over 40 proof as determined by the "head." It is water clear (white Rum). Before leaving the estate "Rum store" it is coloured by caramel boiled by the distiller. Each estate has its own standard of colour.

High Wines—The running from the still which follows the rum; collected to a strength of about 20 over proof.

Low Wines—The subsequent runnings collected till all alcohol has distilled over. The strength varies from 40 to 60 under proof.

Retorts—Copper vessels inserted between the still and the coil. The vapours from the still must pass through them. Most estates have one retort which contains the high wines of a preceding distillation. Some estates have both "high wines" and "low wines" retorts, the latter next to the still. The retorts have a capacity of about 1-10 that of the still.

Low Wines Rum—Some estates with one retort (high wines) add the low wines to the wash in the still; other estates, however, distill the low wines independently (they run about one low wines still to 5 or 6 ordinary wash stills) and obtain "low wines rum" a product of inferior quality and price.

Types of Rum.

The two main kinds of Rum are "Common Clean" and "Flavoured or German." The individual estates confine themselves to the manufacture of one of these kinds. Nearly all the "Flavoured" Rum is made in the parish of Trelawny.

Common Clean Rum—may be divided into two kinds depending on the materials used.

1. From washes set up with a mixture of skimmings, dunder, molasses and water. The materials are not allowed to sour. Several estates with up-to-date boiling house plant (vacuum pans, etc.) and a consequent large out put of skimmings and molasses employ this method. The materials must be used rapidly, and fermentation rendered of as short duration as possible. The wash is set up with ¼ skimmings, ¼ dunder, and molasses and water to give an initial gravity of about 16 Brix. The wash attenuates in about 4 days to 3 or 4 Brix. The initial sugar content is about 11-13 per cent. and the attenuation from 11-13 degrees. The rum is light in body and of low ether content, and is mainly consumed locally.

One or two estates which do not make sugar boil their juice and ferment it with dunder. (Appleton).

2. From washes set up from the same materials and also with "acid" prepared either from skimmings, rum cane juice or normal cane juice. The composition of the wash varies:—

Skimmings (fresh about	¼
Dunder ..	½ to ¾
Acid ..	1-10 to ½
Molasses ..	1-15 to 1-10
Water ..	—

The gravity of the setting depends largely on that of the dunder which varies from 10 to 20 Brix. As a rule the setting is not lower than 18 Brix, and may be as high as 24 Brix. The initial sugar content varies from 10 to 14 per cent. and the attenuation corresponds to that. The fermentation period depends on both the acidity of the dunder and on the quantity and acidity (especially the volatile) of the "acid." The wash ferments from 5 to 9 days and is often allowed to lie for a couple of days when dead.

The only acid produced is evidently "acetic" and some of these rums may contain over 1,000 others (Swanswick, Long Pond) where much "acid" is used in the wash.

The yield of proof spirit is from 0.85 to 1.0 per cent. on the sugar fermented and on the attenuation 0.8 to 0.9 per degree. From 5 to 10 per cent. is lost in distillation.

The yield of rum 40 o.p. varies from 60 to 90 gallons per 1,000 gallons wash in still.

The fermenting cisterns (sunk in floor of distillery built of wood and backed by puddled clay) and vats are usually of 1,200 gallons capacity and the still will receive the contents of one cistern. Two stills are usually run per day (daylight). The stills are heated by steam coil or by direct fire. The rums made with 'common clean' materials vary in ether content from under 100 parts to over 1,000. Acetic ether is practically the only one present, and its amount depends entirely on the quantity of acid used in the washes and on the length of time the wash ferments and lies when "dead."

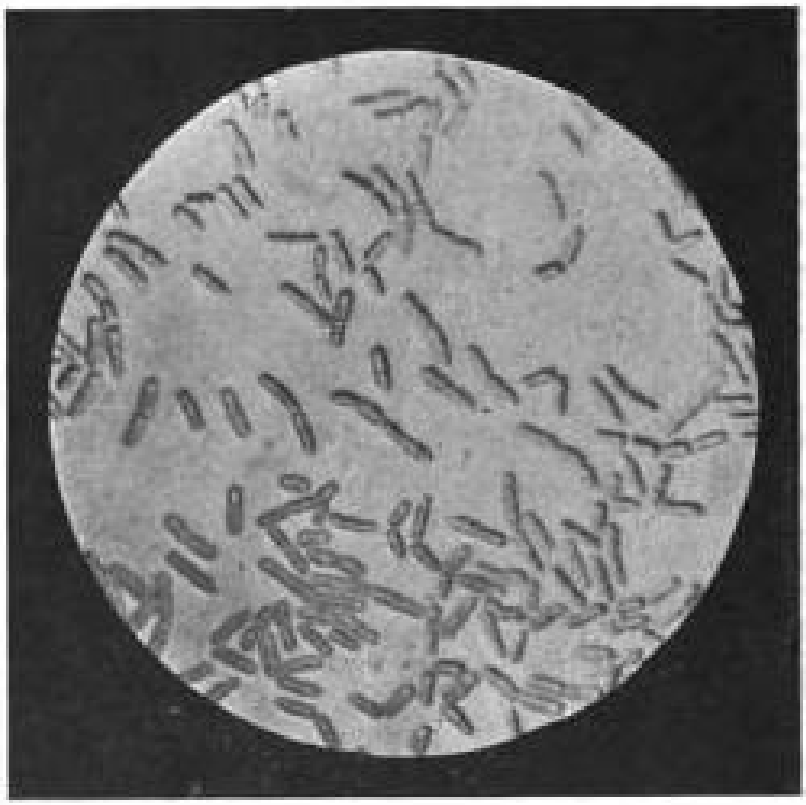
Flavoured or German Rum.—These rums are made on estates having old fashioned boiling house plant where the manufacture of sugar is of secondary importance. The usual common clean materials are employed and in addition "flavour."

"Acid" is prepared from cane juice or skimmings in the usual way in a succession of trash cisterns. A "muck hole" outside the distillery is the receptacle for the thick matter deposited from the dunder, and the wash (dead wash bottom) to which is added cane trash and lees. The matter consists to a large extent of dead yeast and is therefore highly nitrogenous. It undergoes slow fermentation and putrefaction and its acidity is kept low by the addition of marl. When ripe it contains large amounts of butyric and higher fatty acids, both free and combined with lime. It is added to a series of acid cisterns outside the distillery where the butyric and other acids are set free. This complex acid material is the "flavour." The flavour enters the wash after fermentation has begun owing to the presence of acids in it which are injurious to yeast, the fermentation is prolonged and the sugar is never very completely fermented out. Fermentation lasts 9 to 10 days and the dead wash lies for several days longer. An example of the kind of wash follows:—

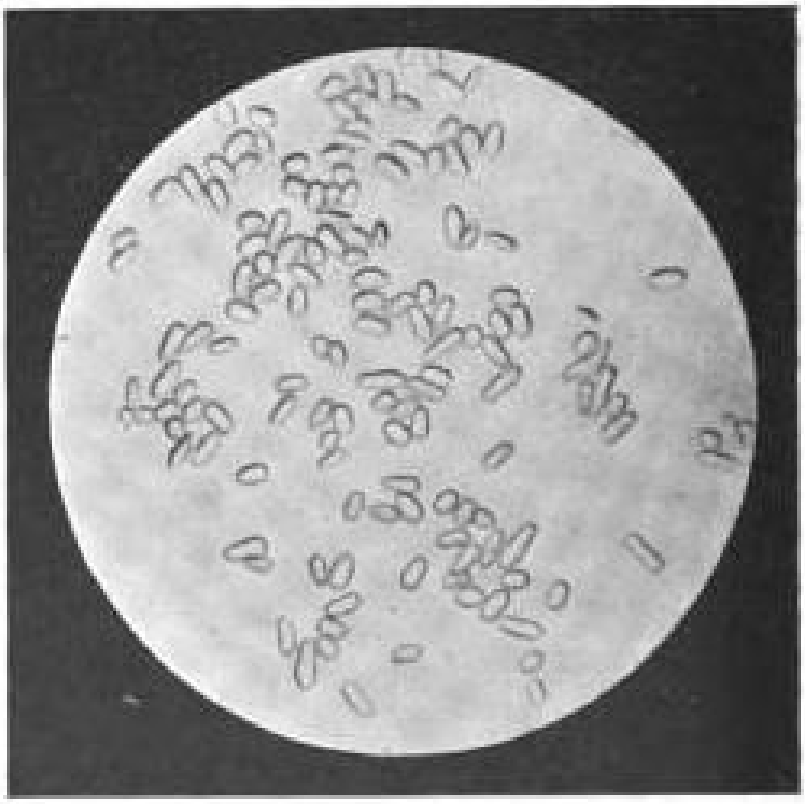
Capacity of fermenting cistern 2,000 gallons.			
Skimmings (fresh	620 galls.	at 12	Brix.
Dunder	760 "	24	"
Acid	220 "	8	"
Molasses	200 "		
Flavour	160 "	8	"
Gravity of fresh wash	25 Brix.		
" " dead wash	12 "		
Attenuation	13		
Temperature when set	80 F.		
Highest temperature attained	89 F.		
Yield of rum	96 galls. at 40 o.p.		

This means a yield of 48 galls. rum per 1,000 galls. wash whereas the attenuation would indicate a yield of about 78 gallons. Only a portion of the high strength distillate is therefore collected as rum of first quality.

These rums show an ether content as a rule from 1,000 to 2,000. While over 95 per cent. of the total ethers is "acetic" there is always present several per cent. of butyric ether and still smaller amounts of esters of higher fatty acids (caprylic, caproic and lauric). Most of these rums find their way to Germany for blending and particularly for "stretching" potato or molasses spirits.



SCHIZOSACCHAROMYCES MELLACEI (JÖRGENS)
TOP TYPE X 300.



SCHIZOSACCHAROMYCES MELLACEI (JÖRGENS)
BOTTOM TYPE X 300.

Photo. by S. F. Ashby.

Yeasts.—Practically three yeasts perform all the conversion of sugar into alcohol in the Jamaica Distillery.

1. Bottom fermenting oval budding yeast.
2. Top fermenting chained fission yeast.
3. Bottom fermenting unchained fission yeast.

Oval budding yeast.—A typical bottom fermenting yeast the cells of which do not form chains. It is oval in shape and often rather pointed at one end. The average dimensions are 7.5-9 m long by 6-7 m. broad. It does not form a film on dead wash but at most a yeast ring. It forms spores on the gypsum block (as a rule four in a cell) in 24 hours at air temperature. It readily inverts and ferments cane sugar. This yeast is present on the rind of the cane and is always found in freshly milled juice. Spontaneous fermentation of juice is therefore always brought about by this yeast. In fresh juice it multiplies quickly and sets up a rapid fermentation. It displaces all other native yeasts in a favourable liquor like juice. The optimum temperature for its multiplication lies above 30 C. but it appears to ferment best at that initial temperature. It will work practically all the sugars out of an undiluted juice if not interfered with by acid-producing bacteria. The fermented liquor has an agreeable odour. In the experimental work at the Sugar Station Distillery where either cane juice or cane juice, molasses, and dunder are usually worked with, this yeast alone sets up and carries through normal fermentation.

On estates where the first type of common clean rum is made (i.e. without "acid") this yeast possesses the wash owing to its properties of quick multiplication and rapid and intense fermentation. Such washes heat up quickly and temperatures as high as 108 F. have been observed. These high temperatures mean injury to the yeast, imperfect attenuation, and marked loss of alcohol by evaporation. Like most bottom fermenting kinds this yeast is markedly susceptible to unfavourable conditions such as poor food supply, excessive temperature and especially high acidity. Volatile acidity injuries it very readily (see experiments in second S.F.S. Report.)

It is injuriously affected by the fixed acids of dunder and works best where the initial acidity of the wash does not exceed 0.5 per cent. In washes with an initial acidity of 1 per cent. and more it gradually gives place to more acid-resistent yeasts. On estates using acid the wash contains both this and fission yeasts, the relative proportion depending on the amount of acid employed. In common clean washes with an acidity exceeding 1.5 per cent. and a volatile acidity of 0.5 per cent., the writerfound it entirely displaced by fission yeasts even quite early in the season.

Top Fermenting Fission Yeast.—A typical top fermenting chained yeast. On washes of high acidity which are not working very intensely this yeast throws up a characteristic light or dark golden yellow thick moist creamy or fatty head which may completely cover the surface of the liquor. The bubbles of gas escaping through the head are cloudy. The head consists mainly of short, rectangular cells in chains of four or more, often in clumps and showing a kind of false branching. When shaken up in a wash the yeast forms into loose flocks which rapidly deposit. There is considerable variation in the size and shape of the cells; the size varies from 6-12 m by 4.5 to 5.5 m., and the chain cells are usually small viz., 6-7 m. long by 4.5 m. broad.

Spores are freely formed in the wash during fermentation. There are four oval spores in a cell and their walls stain blue with iodine (in iodide). The spores are very frequently found in bridge shaped sporangia formed by the reunion after division of two cells or by the union of two neighbouring cells. This yeast has a high optimum for multiplication and fermentation between 34 to 37 C. It endures high acidity (over 3 per cent. total) and is greatly more resistent to volatile acid than the budding yeast. At ordinary temperatures 24 to 27 C. the fermentation is slow but the sugar is efficiently worked out. In pure cultures the attenuation and the yield are as good as from the oval yeast.

In all washes of high total acidity (over 1 per cent.) and especially of high volatile acidity this yeast is generally present and often carries out the entire fermentation. It is the typical yeast of the "Flavoured Rum" washes.

Bottom Fermenting Fission Yeast.—This yeast produces no head in washes, the escaping bubbles being glassy clear. The cells are found single and in pairs, and when the wash is stirred the cells distribute themselves in a fine clay-like suspension, which clears slowly. The cells are variable in shape and size averaging 6-14 m. long by 4-5.5 broad. Spores are formed with the top yeast.

This yeast has a somewhat lower optimum temperature than the top yeast, and like most bottom yeast yields markedly less substance and is more susceptible to external factors than the top yeast. It is often found in acid washes together with the top yeast. It increases more rapidly and ferments more strongly than the top yeast at ordinary temperatures. The attenuation and yield of alcohol in pure cultures are the same as for the top yeast; it appears to leave more unfermented sugar in the dead wash. In a sample of soured cane juice having a total acidity of 2.1 per cent., and a volatile acidity of 0.90 per cent. this yeast alone was found. In the wash set up with this "acid" having an acidity of 1.6 per cent. and over 0.50 volatile, the top yeast was the characteristic worker. It would seem that in highly acid washes the more resistent top yeast gradually increases with the advance of the season. Comparative experiments with these two fission yeasts in Laboratory washes at air temperature indicate that the bottom yeast ferments the wash in one or two days less time.

A bottom yeast with slightly top phenomena has also been isolated. It forms no chains but the cells agglutinate more than the typical bottom yeast. In its properties it comes between the two extremes.

The undermentioned yeasts isolated from distillery materials play no evident part in the actual fermentation of washes.

Fruit Ether Yeast.—Isolated from "foaming" molasses. Forms a dry white friable wrinkled film on material containing sugar. A small oval budding yeast with cells very variable in size. Forms spores on the gypsum block in 18-24 hours at air temperature. The spores are "hat shaped." The yeast is therefore an "anomalus" variety (*Willia anomala*). It inverts and ferments cane sugar and will ferment diluted molasses over 30 Brix.

Produces a very high amount of acetic ether, the distilled wash containing from 12,000 to 40,000 ethers. The fermentation is slow occupying two weeks or more to attenuate 12. In ordinary washes it is easily displaced by more active yeasts. (See second S.E.S. Report, and experimental data in Laboratory records).

Pastorianus Yeast.—Isolated from "foaming" molasses. A top fermenting yeast which forms spores abundantly on the gypsum block in 18 hours. Will ferment diluted molasses of 50 Brix. Inverts and ferments cane sugar but is easily surpassed by the oval budding yeast and fission yeast in appropriate washes. It yields a fermented product of good aroma and has been used successfully in the preparation of orange wine.

Torula from Molasses.—Does not form spores and cannot invert and ferment cane sugar. A small chained oval or spherical yeast which ferments the glucose in molasses of the highest gravity. The cause of "foaming" (see second S.E.S. Report)

Large celled Oval Yeast.—A spore-forming top fermenting yeast which works badly in estates' washes.

Ludwig's Yeast —Found occasionally in small amount in fermenting cane juice and also present in "acid" (Long Pond and Swanswick). Is probably present on the cane. Corresponds in size, division, and spore formation to *Saccharomyces ludwigii.*

Mycoderma Species.—Commonly present as grey and white wrinkled films on dunder, sour skimmings, and dead washes which are allowed to lie two or more days. Produce no fermentation, but oxidize alcohol to carbonic acid and water. No spores formed.

Culture Media.—The medium which had answered well for the cultivation of all yeasts is a cane juice peptone broth. Prepare as follows:—

Fresh cane juice is tempered with milk of lime, heated to the boiling point and filtered. Care must be taken to avoid excess of lime or the liquor will darken excessively. This liquor may be transferred to a Carlsberg Can and boiled for half an hour on two successive days to sterilise it.

The medium has the composition:—

tempered cane juice	100
peptone	0.5
potassium phosphate	0.05-0.1

The cane juice should first be diluted to 13-14 Brix. The broth is heated in the steamer and filtered and then rendered distinctly acid with Hydrochloric acid or Lactic acid. The acidity should be between 0.05 and 0.1 per cent. If not clear the medium is allowed to stand a day and filtered again. It is distributed into Frendenreich flasks (a few c.c. in each) and sterilised by heating ½ hour in the steamer (Koch's) on three successive days.

To prepare a solid medium 1.5 per cent. agar is dissolved in the broth. The medium should have a more or less pale sherry tint and should not be reddish brown.

Media containing dunder are very dark and difficult to clear. The acid of dunder affects the solidifying power of agar. 10 to 15 per cent. of gelatin may be used instead of agar but cultures must then be kept in the cool incubator at 20 to 22 C. The yeasts grow much better in the cane juice medium than in a purely artificial one. The oval budding yeast retains its vitality well in the cane juice broth for over a year. The fission yeasts die out more easily but living cells are present in fair numbers after twelve months.

Fermentation experiments are carried out in flasks containing 1 litre of wash and plugged with cotton wool. Washes with an acidity exceeding .7 per cent. are generally sterile after ½ hour steaming. The progress of fermentation is determined by daily weighing, the loss being taken as carbonic acid.

The following factors are determined in all experiments (Laboratory or distillery).

Fresh Wash—
Gravity
Acidity (total and volatile)
Sugar
Alcohol (if necessary)

Dead Wash—
Gravity
Acidity (total and volatile)
Sugar
Alcohol.

The wash should be quite dead and the yeast well settled. If yeast is suspended the spindle gives a reading 0.15 to 0.4 too high. After 48 hours the reading will be correct. The weight of sugar fermented is very closely double the loss of weight.

On estates the Arnabokli or Jamaica Saccharometer is still frequently employed. It is corrected for 80 F. and gives a reading roughly half as high again as the Brix spindle.

Sending Yeasts to Estates.

At the start of crop the distiller gets a spontaneous fermentation in whatever liquor he can get. This is either skimmings, cane juice of low gravity and purity (rum cane juice) or, if he is fortunate, fresh juice from the first mill. Dunder left from the preceding season is often used to mix with the juice. This dunder often contains matters which inhibit or interfere with the growth of the yeast. It is improved by vigorous boiling with or without the addition of lime. As soon as he has molasses and fresh dunder he can set up a normal wash. If he has to start on skimmings they often contain very little or very feeble yeast, and easily get spoilt by bacteria which render them ropy or viscous. Much difficulty is therefore often experienced in getting a good start to fermentation. In any case the yeast which develops is the oval budding kind. If "acid" is made and used from the outset in quantity the oval yeast frequently works badly and gives place gradually to the more suitable fission yeasts. In the meantime there may be loss by bad attenuation and accumulation of materials.

In sending yeasts to estates at the start of each crop the object of the Laboratory had been to get in suitable yeasts from the outset and curtail the period of uncertainty. For estates not making "acid" the oval budding yeast, and for estates making acid—one or both of the fission yeasts.

Yeasts were sent to a few estates in December 1907, and January 1908, to still more in December 1908 and January 1909, and to over twenty estates in December 1909 and January and February 1910.

The following estates got yeast this crop:—

Catherine Hall	December	oval yeast.
Albion	do.	do.
Sevens	January	oval and bottom fission.
Parnassus	do.	do. do. do.
Cinnamon Hill	do.	top fission.
Running Gut	do.	top fission.
Ironshore	January	top fission.
Appleton	do.	oval and bottom fission.
Bog	do.	oval and bottom fission.

Orange Valley	January	top and bottom fission.
do.	April	do. do. do.
Green Park	February	do. do. do.
Denbigh	do.	oval and bottom fission.
Content	January	top fission
Kent	do.	top fission
Spring	February	oval and bottom fission.
Llandovery	do.	do. do. do.
Swanswick	do.	rop fission.
Gale's Valley	March	top and bottom fission.
Belleisle Estate Co.	April	top fission, and later bottom fission and oval.

Yeasts are required from the middle of December to the middle of February. The estates taking them later had really started weeks earlier. The number of Common Clean Estates willing to test the yeasts could doubtless be doubled for the crop 1910-1911. They would need to be circularised in November.

Preparation of Yeasts for Estates.

The yeast is first grown in the Frendenreich flasks in the cane juice broth. Two or three transfers should be made when fermentation has almost ceased; ½ c.c. suffices after shaking up the liquor. This is done with sterile pipettes in the glass chamber after washing down the latter with 2 per 1,000 mercuric chloride solution,; 5 c.c. are then added to 60 c.c. sterile wash in small Pasteur flasks. After fermenting in these flasks for 3 to 4 days they are shaken up and the whole liquor poured into flasks plugged with cotton wool containing 1 litre sterile wash. When fermentation has nearly ceased these flasks are shaken and the liquor poured into large flasks containing 10 to 12 litres sterile wash. The wash is allowed to die completely and the yeast permitted to settle out (24 hours after wash is dead). The covering liquor is then poured carefully away and only sufficient left to give a thick muddy suspension when the yeast is is shaken up with it. The mixture is poured on to moistened filter paper in Buchner funnels and the moisture drawn out as affectively as possible by means of the Geryk air pump. The yeast and the filter paper are lifted out, wrapped in dry filter paper with a covering of glazed paper, packed in a small tin with cotton wool and mailed by Letter Post to the Post Office nearest the estate without delay. The estate must be advised to get the yeast working on the day of arrival.

The washes in the Pasteur, litre, and large flasks should, for preference, be set up from a mixture of molasses, dunder and water, using ⅓ to ½ dunder (according to its acidity and gravity). The gravity of the wash should be such that it will attenuate 12 if allowed completely to die. The Pasteur and litre should have added to them 0.2 per cent. asparagin, and the wash in the large flasks 0.1 to 0.2 per cent. ammonium citrate or ammonium sulphate. In the absence of molasses muscovado sugar or concentrated cane juice may be used. If neither dunder or molasses are available the wash may be set up with muscovado sugar and citric acid (1 per cent. of a gravity of 12 Brix). To this should be added .2 per cent. asparagin for the Pasteur and litre washes, and .2 per cent. ammonium citrate for the large flasks, .05 per cent. potassium phosphate should also be added.

Before adding the yeast the wash should be warmed to 30° C. The litre and large flasks should be packed round with saw dust or better filter

packing to keep up the temperature and make the fermentation more uniform. The flasks containing the litre washes should be weighed daily to judge if fermentation is vigorous and normal. If a yeast is to go to several estates within a short period a little may be kept back in the large flasks after decanting off the dead liquor and this will serve to start another large flask (or more than one). Before sending away, a little of the yeast should be examined under the microscope to observe if it is true to its type and free from living bacteria.

Directions for Working the Yeasts on the Estates.

Set up ten gallons of fresh wash in a clean keg; the wash to consist of dunder ½, molasses and water, and to be of such a gravity as to give an attenuation of 12-13 Brix (18-19 Arnaboldi) if the wash were allowed to die completely. The temperature should be 86-88 F. Stir in the yeast, cover the keg and allow to stand in a warm place. When this wash has lost 9-10 Brix (14-15 Arnaboldi) by attenuation, stir up properly and pour the entire liquor into 50 gallons fresh wash. When this has attenuated to a like extent stir up and pour the whole into 500 gallons freshly set wash. When this is working well (after 24-36 hours) make up to 1,000-1,200 gallons. A freshly set 1,000 gallon wash can be started again from that by adding to it 50- 75 gallons when the attenuation has fallen 9-10 Brix and after thoroughly stirring up. The yeast can be got through the distillery more rapidly by keeping back 10 gallons of the fermenting 50 gallon wash and using it to start a fresh 50 or 100 gallon wash in the same puncheon (with the head knocked out) which may be poured into 1,000 gallons when it has attenuated 9-10 Brix. Skimmings should not be used in setting up wash except in the last 500 gallons.

When circularising the estates they should be asked if they propose to use "acid" in the coming crop. Content, Kent, Cinnamon Hill, Running Gut, Ironshore, Gale's Valley, and Swanswick have already employed the top fission yeast with success. Catherine Hall, Albion and Parnassus would be best suited with the oval budding yeast. Sevens, Spring, Appleton, Denbigh, Bog and Llandovery, Green Park and probably the Belleisle Estate Co. might get both oval and bottom fission yeasts. Orange Valley should get both fission yeasts. If two yeasts are sent together the distiller should be advised to grow them separately in 10 and 50 gallons and then pour the two 50 gallon washes together into 1,000 gallons of ordinary estate wash. The yeast better adapted to the conditions would then get the upper hand.

Bacteria.

Acetic Bacteria.—Forming a film on liquors containing alcohol oxidising it to acetic acid. They can be isolated from dead washes, "acid," etc., by means of cane juice peptone agar to which 2 per cent. of alcohol has been added. The commonest forms are B. kutzingianum (or an allied species), B. xylinum and B. xylinoides. The first named does not give the blue stain with iodine. It forms a blue to white delicate very friable ascending film and clouds the liquor strongly. (For experiments with this species see second S.E.S. Report and Laboratory records). B. xylinum develops the characteristic tough thick white skin on any nonfermenting liquor containing cane sugar and not less than 10 per cent. proof spirit.

B. xylinoides forms a very similar skin on "acid" and liquor containing over 10 per cent. proof spirit. One or more of these species are always present in fermenting cane juice or estate washes and cause a rise of acidity

by forming gluconic acid from sugar. When the wash is dying and especially when it is dead they produce acetic acid.

Saccharobacillus pastorianus has also been found in fermenting washes and especially in soured skimmings and cane juice. It is present as long narrow rods often covered with small particles of matter deposited on them from the liquor. The liquor is strongly clouded and the bacteria cause the appearance of silky waves. This organism grows freely in the presence of alcohol and therefore increases with the yeast during fermentation. In cane juice broth it gave rise to 0.8 per cent. total acidity of which 30-35 per cent. was volatile (acetic). The fixed acid is lactic acid.

"*Acid.*"—This is prepared either from skimmings or cane juice by allowing them to ferment and sour in special cisterns. As a rule trash is added to the liquor which on some estates is pumped into a succession of cisterns in each of which an increase of acidity occurs. The acidity of the ripe acid rarely exceeds 2.5 per cent. and of this as a rule less than ½ is volatile. The highest volatile acidity hitherto observed was 0.9 per cent. out of a total acidity of 2.1 or about 43 per cent. The liquor is fermented by yeast (oval or bottom fission) and the acid increases rapidly at the same time. This increase frequently stops attenuation when several per cent. of sugar is still present. Hence "acid" shows a most variable gravity according to the relative activities of the yeast and bacteria. The amount of acid formed is the same whether attenuation has been good or bad. (See data in Laboratory records on Swanswick "acid.") The trash not only infects the liquor with bacteria but increases aeration. It also seems to carry on a strong infection when fresh juice is added. No marked film forms on the acid so that the acetic bacteria do not have a chance to unfold their full oxidising activities. B. xylinoides, B. xylinum and Saccharobacillus pastorianus have been isolated from ripe acid.

The acetic bacteria form gluconic and acetic acids. The Saccharobacillus form lactic and acetic acids. The fixed acidity preponderates. (See first and second S.E.S. Reports.)

Jelly and Slime forming Bacteria.—Certain species readily form jelly and slime in weakly acid liquors containing cane sugar. Skimmings and cane juice often undergo a viscous fermentation with the production of gas and the skimmings may frequently be drawn out into long threads (ropy skimmings). This condition interferes with the yeast fermentation which is prolonged and incomplete.

The liquor shows the presence of small cocci single, paired and less frequently in chains. The viscous or ropy condition of the liquor is due to the very diffluent cell walls of the bacteria. The acidity produced in cane juice does not exceed 0.3 per cent. a trace of which is volatile. The growth on cane juice agar is very moist and slimy but no slime is produced on ordinary glucose agar. In glucose broth the chains are very marked so that the organism is a true Streptococcus.

Growth is very rapid in cane juice at 27-40 C. A nonslimy variety has also been isolated. The condition is most marked at the start of crop in both cane juice and skimmings and is due to dirt from the cane and the mill. Owing to its slimy capsule the Streptococcus is not destroyed during tempering in the clarifiers. Thorough cleaning of gutters and skimmings boxes and diluting the hot skimmings with cold water have successfully checked this condition.

Rice Grain.—This occurs not infrequently in washes on estates where no acid is employed. The wash becomes almost filled with gelatinous spherical grains about 1 mm. to 2 m.m. in diameter. The fermentation by the yeast is prolonged and often incomplete. It is caused by a rather thick rodshaped bacterium 1.5-3m by 1 m. Three varieties of this or-

ganism have been isolated (see Laboratory records). It strongly resembles the Bacterium vermiforme of Marshall Ward (ginger beer plant). In cane juice the acidity does not exceed .2 per cent. It grows rapidly at 30 C. and just as well in cane juice with 6 per cent. of alcohol by volume as in cane juice without alcohol.

It produces no change in litmus milk and grows out into long more or less coccoid chains without gelatinous sheath in glucose broth. It probably enters the wash from the skimmings.

This organism evidently does not injure the yeast by means of its chemical products. Its interference is physical as the gelatinous masses attach themselves to the yeast cells and grow over them excluding the yeast from contact with the liquor and preventing the cells from rising and whirling in the wash, a condition necessary for active fermentation. A similar kind of interference must be attributed to the streptococcus of viscous ropy skimmings or juice.

Termo bacteria, B. subtilis and B. mesentericus vulgatus have been found in cane juice. These motile forms have not been closely investigated.

Laboratory Experiments with Yeasts, 1908–1909.

Fruit Ether or Anomalus Yeast:—

In 9 litres of wash, started 23 January, 1908.
Pure cultures in sterile washes.

1. Wash of molasses and water with 0.1 per cent. Ammonium sulphate:

Initial Gravity	14.5 Brix.
Total Sugars	11.0 per cent.
Acidity	0.15 per cent.

Dead Wash—

Gravity	3.0 Brix.
Sugar	0.96 per cent.
Acidity	0.45 per cent.
Attenuation	11.5 degrees.
Sugar fermented	10.04 per cent.

Distilled 12 February 1908, 20 days after setting up. In two portions each of 4,200 c.c.

1st. part for high wines, used in retort for second portion:—
Rum 297 c.c. at 40 o.p.
" 70.7 parts per 1,000 wash.
Ethers 33,400 per 100,000 alcohol.

2. Wash of molasses and water with 0.1 per cent. Sodium nitrate:—

Initial gravity	14.5 Brix.
Total sugars	11.0 per cent.
Acidity	0.15 per cent.

Dead Wash—

Gravity	3.2
Sugar	0.48
Acidity	0.35
Attenuation	10.52
Sugar Fermented	10.52

Distilled 13 February 1908—21 days after setting up.

Rum 256 c.c. at 40 o.p.

" 60.9 parts per 1,000 wash.

Ethers 30,365.

3. Wash of molasses and water with .1 % Sulphuric Acid.

Initial gravity	14.5 Br.	*Dead Wash*	4.2 Br.
Sugars	11.0 %	"	0.82%
Acidity	0.25 %	"	0.54%
		Attenuation	10.3 degrees
		Sugar fermented	10.18

Distilled 14 February 1908, 22 days after setting up.

Rum 224 c.c. at 40 o.p.

" 53.3 parts per 1,000 wash.

Ethers 30.740.

4. Molasses, dunder (1-5th.) and water.

Initial gravity	14.5 Br.	*Dead Wash*	8.0 Br.
Sugars	10.5%	"	2.78%
Acidity	0.3 %	"	0.55%
		Attenuation	6.5 degrees.
		Sugar fermented	7.72.

Rum 144 c.c. at 40 o.p.

". 34.3 parts per 1,000 wash.

Ethers 46,030.

Taking 6 parts of Rum for every 1 degree attenuation per 1,000 of wash as normal distillery yield, the yields should have been:—

1. 69.0	actual	70.7
2. 67.8	"	60.9
3. 61.8	"	53.3
4. 39.0	"	34.3

The ethers varied from 30,653 to 46,030, and consisted almost wholly of acetic ether. As the ether was formed at the expense of alcohol the yields must be regarded as satisfactory particularly that for the molasses wash to which ammonium sulphate was added. The acid of dunder appears to have an injurious effect on this yeast. Chemical esterification in such liquors could not account for the enormous amount of ether produced. It is evident that both alcohol and acetic acid are formed in the yeast cells by the enzymes, zymase, and oxydase (the latter probably the same enzyme as the oxydase of the acetic bacteria) and are at once brought into union by another enzyme, the whole process occurring within the cell. Certain acetic bacteria are known to yield a vinegar containing a marked amount of acetic ether while other species are quite unable to do so. A yeast is also known which oxydises alcohol to acetic acid and some non-fermenting myecodermas are capable of producing acetic ether in alcoholic liquors. The cells of some species contain, therefore, only the oxydase, others both oxydase and ether producing ferment (esterase). Some of the mycodermas and acetic bacteria which form films on dead washes in Jamaica distilleries may esterify in the way indicated although such forms have not as yet, been isolated in the Laboratory.

Experiments with Fission Yeasts in Dunder and Concentrated Cane Juice Wash.

The juice was boiled down to the consistency of thick syrup without any tempering. The dunder was derived from cane juice and dunder washes from which all alcohol had not been distilled out. This dunder, therefore, had undergone some souring, and was rather high in volatile acidity:—

Dunder—

total acidity	1.30
volatile "	0.53

Concentrated Cane Juice—

total acidity	0.09
volatile "	0.014

The yeasts were first grown in a mixture of the cane juice and dunder without added water in sterilised flasks containing 1 litre.

Brix ..	19.9
Sugar ..	12.1
Total acidity	1.03
Volatile acidity ..	0.49

Bottom and top fission yeasts were employed. The wash died in 6 days with bottom yeasts; the wash died in 7 days with top yeasts.

Dead Wash—

Brix ..	6.9
Acidity ..	1.10
Volatile Acidity ..	0.4
Sugar ..	0.4
Proof Spirit ..	11.90
Attenuation ..	13.0 degrees
Sugar fermented	11.7
Yield on attenuation	0.91
Yield on sugar fermented	1.02

The yeast sediment was then added to 10 litres in large flasks:—

Brix ..	20
Sugar ..	11.8
T. Acidity ..	1.02
V. Acidity ..	0.45

The bottom yeast washes were again dead in 6 days, and the top yeast washes in 7 days.

Dead Wash—

Brix ..	7.0
Sugar ..	0.45
Acidity ..	1.12
Proof spirit ..	12.0
Attenuation ..	13.0
Sugar fermented	11.35
Yield on attenuation	0.92
Yield on Sugar fermented	1.0

Distillation.

1	Bottom	yeast	wash	distilled	when	dead	Ethers	971
2.	Bottom	yeast	wash	distilled	when	dead 4 days	Ethers	1185
3.	"	"	"	"	"	6 days	"	1404
4.	Top	"	"	"	"	4 days	"	1186
5.	"	"	"	"	"	5 days	"	1293

In this experiment both bottom and top yeasts gave identical attenuations and yields. In spite of the high volatile acidity (45 per cent. of the total) the washes were rapidly (6 and 7 days) worked down with little residual sugar and with excellent results on attenuation and sugar fermented. The rums were high in ethers. Even when distilled as soon as the wash was dead, the rum contained 971 ethers and when the dead wash was allowed to lie six days they were increased to 1404. In washes high in volatile acidity considerable esterification occurs during actual fermentation, and is again greatly increased when the dead wash is allowed to lie.

Grown in pure culture in sterile wash, the top yeast does not yield a rum of higher ether content than the bottom yeast.

Experiment with Poor Dunder.

Washes in litre flasks were set up with dunder water and muscovado sugar.

Gravity of dunder	6.0 Brix
Acidity of "	0.7
Gravity of wash	15 Brix.
Dunder ⅓ total volume.	

Oval and fission yeasts were used; the same amount of yeast was added to the sterile wash (in 1 litre flasks) in each case. To some flasks 10 c.c. of 10% asparagin solution was added at the outset. The loss in weight day by day was as follows:—

	1st. day	2nd. day.	3rd. day.	4th. day.	5th. day
Oval yeast with asparagin	14.2	21.7	12.1	7.6	
" " without "	0.7	2.7	1.7*	5.1	17.6
Fission yeast with asparagin	0.5	11.8	20.7	16.6	
" " without "	0.4	2.9	3.2*	4.2	15.4

The fermentation was normal and active with both oval and fission yeasts in the presence of asparagin. In the absence of asparagin the yeasts scarcely multiplied and the fermentation was very feeble. When, however, asparagin was added on the third day to the latter cultures multiplication set in, and 48 hours later they were fermenting strongly.

These results show clearly that washes set up with a poor dunder are often deficient in nitrogenous food for the yeast with the result that attenuation is feeble and incomplete. In practice such washes are quickly overrun by bacteria, show rapid increase in acidity and become still more unsuited for a vigorous yeast fermentation. In the experimental distillery washes set up with similar dunder worked and attenuated very feebly; 24 hours after addition of 0.15 per cent. ammonium sulphate (equal to 15 lbs. per 1,000 gallons wash) they began to work vigorously and showed a normal attenuation.

Distillery Experiment.

The following experiment selected from a number of such carried out in the Experimental Distillery in 1908, will show what the Jamaica fission yeast are capable of yielding under well regulated conditions.

The fission yeast was of the bottom fermenting type.

It was first developed in a molasses and dunder sterile wash in a flask containing 12 litres (nearly 3 gallons). The yeast was then added to 10 gallons of similar wash in a keg after sterilising the latter with superheated steam and cooling it to 86 F. When fermentation was almost completed in the keg the contents were stirred up and the liquor added to 50 gallons fresh wash (not sterilised) in a puncheon. When fermentation has started a further 50 gallons wash was added and fermentation allowed to proceed till the wash was quite dead.

Wash in puncheon as set up:—

Dunder ⅓ the volume.	
Gravity	19 Brix.
Acidity	0.7
Total sugar	13.2

Dead Wash—

Gravity ..	5.6 Brix
Acidity ..	0.8%
Sugar ..	0.59%
Alcohol ..	13.2 proof spirit
attenuation ..	13.4
Sugar fermented	12.6
Yield on attenuation	0.93
Yield on sugar fermented	0.99

The wash was dead in from 4 to 5 days.

The wash was divided into two portions; the first was distilled for high and low wines (the retorts also receiving charges of wash). The high and low wines were used in toto to charge the retorts for the second distillation 42 gallons wash being introduced into the still.

Charge of retorts	29.90 lbs.absolute alcohol
Total yield of alcohol	52.81 " " "
Net yield	22.91 " "
do. ..	2.886 gals. " "
do. ..	68.71 " on 1000 wash
do. ..	86.01 gallons of Rum. at 40 o.p. 1000 wash.

Taking 6 gallons of rum for every 1° attenuation per 1000 gallons wash as an ordinary average yield 80.4 gallons rum would have been expected on that basis; the actual yield was however 7 per cent. in excess of that amount and must therefore be regarded as highly satisfactory. The above yields are expressed in imperial gallons. In terms of wine gallons the figures are 1-5th. higher, viz., ordinary average yield 96.5 wine gallons. Actual yield 103.2 wine gallons.

Observations of Estate Materials.

The following determinations made on materials at the estate and on samples at the Laboratory illustrate the composition of dead washes, dunder, and "acids" produced in some "Common Clean" distilleries where rums are made containing 900—1200 parts of ethers per 100,000 alcohol. On such estates no "flavour" is employed so that the ethers in the rums consist wholly or almost wholly of acetic ether.

Distillery A.

	Dead Wash.	Dunder.	"Acid" (ripe)
Brix ..	7.4	14.4	6.1
Total Acidity ..	1.55	1.9	2.1
Volatile acidity	0.50	0.51	0.90
Alcohol as proof spirit	12.30	0.58	8.77
Sugar ..	0.49	0.64	0.64

The acidity of the ripe "acid" varied from 1.9-2.4 per cent. and the gravity from 3.5-10 Brix.

Distillery B.

	Dead Wash.	Dunder.	"Acid."
Brix ..	11.9	19.7	7.1
Total Acidity ..	1.9	2.55	2.5
Volatile acidity	0.52	0.95	0.95
Alcohol as P.S. ..	0.62	1.19	1.11

THE "RICE GRAIN" BACTERIUM.

This organism with remarkably gelatinised cell walls has been already referred to as causing trouble in "common clean" washes particularly in distilleries where only fresh materials are fermented and no "acid" is made.

A sample of dead wash containing the organism was after appropriate dilution plated out in cane juice peptone agar. After some days a variety of colonies including those of yeasts appeared in the me dium. Three different types of more or less gelatinous colonies of bacteria could be distinguished.

1. Of no particular shape, raised into a mass and breaking through the agar by rupturing it. These colonies at the surface were smooth, milky, dull and pasty and easily rubbed into a milky homogeneous suspension in water. Such a suspension showed under the microscope small flat, or irregular spherical gelatinous grains about 18-20 microns in diameter and free from any tendency to coalesce with each other. The flat grains showed a more or less circular outline with alternating deep and shallow depressions as indicated in the figure. Embedded in the jelly near the ends of the arms formed by the main depressions and transverse to the surface were two more highly refracting rods separated by the secondary depressions. The almost spherical grains had a convoluted appearance with a fundamentally similar structure, the rods being also transverse to the surface at the ends of the involved arms. This condition was evidently the final state of development of the grain. The resultant appearance was due to the division of rods with gelatinous cell walls, having the property of gelatinous thickening on one side particularly.

The rods are coloured yellow by aqueous iodine in iodide with darker staining granules; the jelly is hardly tinged. The aniline stains colour the rods intensely but do not affect the jelly. The individual rods vary much in length especially in the spheres where they are elongated into threads exceeding 10 micron. The minimum length is 1.5 microns and the breadth about 1 micron. In cane juice peptone broth the organism increases to a finely granular loose deposit in three days at air temperature but multiplies markedly more rapidly at blood heat. The deposit is easily brought into suspension by shaking whereby the minute flocks (grains) are rendered just visible to the naked eye. The liquor overlying

the deposit is practically clear. The liquid culture shows a similar appearance under the microscope as the agar material.

Perfectly free rods are not to be found, hence the clearness of the overlying liquor. The organism grows as rapidly and abundantly in cane juice broth containing 6 per cent. alcohol by volume as in broth free from alcohol. The increase of acidity in the broth (equal to 0.1 per cent. at the beginning) does not exceed 0.25 per cent. and only a trace of this is volatile. The acid formed is probably lactic. Gas production is absent or doubtful. In litmus milk the organism produces no change in fourteen days. In nutrient broth and in glucose broth (containing 0.5 per cent glucose) growth is slow with formation of a flocky white deposit. Under the microscope long chains of short rods (almost coccoid) are visible without gelatinous envelopes. The chains grow out from the cells embedded in the grains of the inoculation material, the individual cells being 1.2-1.5 microns in diameter.

2. Spheres with rough (faceted) surface, translucent, shining, and gelatinous like the agar. The spheres break through the agar and split it into radiating rents. The masses are at least 1 Millimetre in diameter and as a rule from 1-2 m.m. The whole sphere can be lifted away on the loop of the platinum needle. In water it cannot be reduced to a homogeneous suspension but breaks into fragments of jelly. Under the microscope the fragments of jelly are very irregular. The structure is however, very similar (though greatly more irregular) to that of the grains of No. 1. The rods are either transverse at the ends of gelatinous arms or they may be equally gelatinised on both sides. By the rupture of the jelly the rods often project freely. The length of the rods is very variable, long threads up to 50 microns being frequent. The diameter like No. 1 is almost 1 micron.

In cane juice peptone broth this organism increases by the formation of large irregular masses of jelly or by a gelatinous deposit difficult to raise and then breaking into lumps of jelly. The liquor is distinctly cloudy which is due to the presence of large numbers of free cells with or without gelatinous capsules. The cells are often paired and also form chains of three to ten or more cells. This form also grows equally freely in juice containing 6 per cent. alcohol and behaves quite similarly to No. 1 in litmus milk, nutrient broth and glucose broth. The increase of acidity in cane juice broth is also under .25 per cent. and growth at blood heat likewise very rapid.

Grown in conjunction with a bottom fission yeast both organisms increase freely and the fermentation is 1-2 days more prolonged than in pure yeast culture. The physical interference of the organism with the yeast has been already referred to.

3. Colonies on the surface, transparent, convex, watery (mucoid, not ropy), entire, round and shining. Where colony has broken to the surface a central gelatinous mass, showing under the microscope similar but less marked gelatinous fragments as No. 2 with rods similarly embedded.

The watery part of the colony under the microscope shows rods, single paired and chained with or without an indistinct gelatinous capsule equally developed all round the cells.

In cane juice broth the organism forms an abundant translucent deposit and the liquor is still more cloudy than with No. 2. When shaken up the liquor becomes opaque due to an abundant homogeneous suspension. The dimensions of the rods are the same as for 1 and 2. Its behaviour in litmus milk, nutrient broth and glucose broth is also quite similar. In cane juice broth with 6 per cent. of alcohol the growth was less rapid

than in the broth alone. The increase of acidity in cane juice was also under .25 per cent.

The appearance of the colonies applies also to the cane juice agar slants. On this medium each of the three types shows its characteristic growth. It is evident that the three forms are varieties of the same organism. In No. 1 the development of the grain is much more limited than in No. 2. In No. 3 the jelly is less robust and more diffluent, and may be compared with agar which has lost its property of solidifying by heating in a strongly acid liquor. Reference has already been made to the strong resemblance particularly of variety No. 2 to Bacterium Vermiforme of Marshall Ward.

Orange Wine.

Enquiries from several sources came to the Laboratory in the autumn of 1907 and again in the spring of 1908, as to the best way of making orange wine by direct fermentation of the sweetened juice. No experiments had at that time been carried out in connection with the orange wine making and there appeared to be no literature on the subject. The so-called orange wine on the market appeared to consist of diluted rum flavoured with orange essence (or the essential oil from the rind), and highly sweetened. This was more in the nature of a cordial or liqueur and could not be regarded as in any way a true wine.

A preliminary experiment was therefore started in March and April 1908.

A bottom fermenting fission yeast was selected to carry out the fermentation owing to the known resistent properties of fission yeast in general. In order to acclimatise the yeast to a liquor containing a high proportion of citric acid it was first grown in a mixture of molasses, water, and citric acid; the composition was—

Brix—18.
Acidity—0.75.
Citric Acid—10 grams to 1 litre.
Ammonium phosphate—1 gram to 1 litre.

The yeast attenuated this wash in four days to 2 Brix, and while it was still working 100 c.c. was used to start a fresh wash prepared from orange juice.

The orange juice was obtained by squeezing the juice of ripe oranges with the rind entirely removed, through a linen cloth.

Gravity of juice—13.8 Brix.
Acidity of juice—1.08 per cent.

To 1,500 c.c. of this unsterilised must, in which cane sugar had been dissolved was added (as stated above) 100 c.c. of the fermenting wash containing the fission yeast. The gravity fell in 7 days from 23.5 to 0.5 Brix, and the final acidity was 1.18 per cent.

After allowing the greater part of the yeast to settle out, the still very cloudy wine was decanted off and bottled. The bottles were filled almost to the corks which were sealed with paraffin. The bottles stood at air temperature for 8 months during which the wine had become perfectly clear and of a dark sherry colour.

The wine had a pleasant aroma of orange and an agreeable though rather marked acid taste. The palatability of the dry wine was improved by the addition of 10 per cent. pure white cane sugar. After this addition

the wine was readily appreciated when drunk alone and was also found to be very refreshing beverage when consumed with the addition of two parts of Soda Water. It was pointed out, however, that this wine was not so strongly flavoured as that made by orange growers, and this was attributed to the fact that the oranges had not been squeezed with the rinds still on. The usual practice was to cut the entire oranges into quarters and squeeze out the juice in a wooden press operated by hand. In this way a part of the essential oil contained in the outer rind was set free and entered the juice. To this juice it was customary to add a small proportion of lemon juice (a sample showed a gravity of 10.4 Brix and an acidity of 3.25 per cent.) to improve the flavour and increase the acidity. White albion sugar was then dissolved in the juice until the gravity was raised to 22-24 Brix. To get this must fermenting a "starter" was then added.

This was prepared by mixing muscovado sugar with warm water to a gravity of about 15 Brix and allowing this to set up a spontaneous fermentation occasioned by the cells and spores of yeasts contained in the sugar. As soon as this was working strongly it was poured into the orange must. If a successful fermentation was set up in the must the latter worked for one to two weeks and finally stopped before all the sugar was worked out, or was intentionally stopped by decanting off the liquor from the yeast deposit. It was pointed out that this method of fermenting the must had some disadvantages namely:—

1. The "starter" spoiled the natural flavour of the wine owing to the characteristic taste of the sugar used.
2. Fermentation often failed in the must after the addition of the "starter" or the fermentation rapid at first fall away quickly and left a product containing insufficient alcohol and too much sugar. This cleared badly and often turned sour (vinegar).

A pure culture yeast acclimatised to orange juice at the Laboratory appeared therefore to offer the most promising solution to the problem. The fission yeasts, well suited to acid distillery washes do not give a pleasant aroma to fermented must. On the other hand a pastorianus yeast isolated from molasses was found to yield a product of very agreeable aroma. It has therefore been employed in the experiments detailed below.

Some preliminary work with this yeast indicated that one or more substances contained in the rind of the orange exercised an injurious effect on it when present in the juice from the outset. Juice was accordingly prepared from the fruit after removing the rind. When fermentation was active the liquor obtained by squeezing the rinds separately was added in order to increase the flavour of the finished product.

The yeast was first grown in a wash of molasses, citric acid and ammonium phosphate, then in a mixture of that wash with increasing amounts of orange juice and finally added to the orange juice must. The juice as squeezed from the fruit showed—

Gravity—11.85 Brix.
Acidity—1.12 per cent.

The gravity of this juice was increased to 20 Brix by the addition of white crystal sugar, and 0.1 per cent. ammonium citrate added. To start this must one-tenth its volume of a fermenting juice was added which had been attenuated by the pastorianus yeast from 19 Brix to 8.3 Brix. Two days after fermentation began one-sixth of its volume of liquor squeezed from the rinds alone was added. The gravity fell from 20 Brix to 0.3 Brix in 11 days and the must was then dead. After the bulk of the yeast had settled the wine was bottled and kept at air temperature for 15 months.

An examination of the perfectly clear dark sherry coloured wine after that period yielded the following figures:—

Acidity—0.64 per cent.
Sugar—0.60
Alcohol as proof spirit—21.43 per cent.

The wine had a fine sherry like aroma and was very palatable after the addition of 10 per cent. cane sugar.

Another must fermented by the same yeast a week later was set up from a juice of—

Gravity—12.05 Brix.
Acidity—1.18 per cent.

Sugar was added to raise the gravity to 20.8 Brix. This must underwent a more prolonged fermentation and ceased to work with an appreciable amount of sugar unfermented. The must attenuated from 20.8 Brix to 2.8 Brix in 24 days. It was then bottled and cleared very slowly. Fifteen months later the perfectly clear wine showed:—

Total acidity—1.10 per cent.
Volatile acidity—0.15 per cent.
Alcohol as P.S.—18.57 per cent.

In aroma and taste it scarcely differed from the other wine.

When fresh juice is allowed to ferment spontaneously it works slowly and finally dies before all the sugar is fermented. A white dry film usually forms on the surface consisting of myecoderma or a species of Monilia while Apiculatus yeast is often abundant in the deposit. The Apiculatus yeast can only ferment the invert sugar and leaves the cane sugar untouched. As the juice contains about half the total sugar as cane sugar the attenuation stops half way.

A juice worked for 6 days and attenuated from 11.85 to 6.45 and went no further. In another portion of the juice a little added fission yeast reduced the gravity from 11.70 to 1.75 Brix. owing to the power of inverting the cane sugar.

When used in larger bulks (10-15 gallons) of sweetened orange juice prepared by pressing the oranges with the rind on, the pastorianus yeast has several times failed to yield a satisfactory fermentation, results which raised the question as to whether this yeast is really well adapted for working in sweetened juice as usually set up. Fermentation certainly sets in more rapidly and vigorously if the sugar is previously melted in hot water to a consistency of syrup and then raised to the boiling point after the addition of 5 per cent. citric acid. This causes the inversion of the bulk of the sugar. The first experiment indicates that the fission yeasts work readily in sweetened juice and it will probably be safer to employ such yeasts in spite of the fact that they do not yield such a good flavoured wine.

The data set out in this paper must be regarded as of the pioneering order and should prove useful as a start in the solution of the difficulties connected with the making of genuine orange wine.

Orange Vinegar.

This is a product with which the wine maker has often had involuntary acquaintance. About 2½ gallons of an excellent vinegar have been made at the Laboratory in the following way:—

Juice was extracted from the fruit freed from rind.

The gravity was:—10.6 Brix.

Acidity—0.80 per cent.

To this was added sugar inverted by boiling with 2% citric acid. The gravity of the sweetened must was 16.5 Brix. It was pitched with the pastorianus yeast. After 9 days the liquor was dead and showed a gravity of 0.5 Brix. It was allowed to stand in a large flask with a loosely fitting cotton wool plug. After a few weeks an acetic film developed on the liquor and after a further month this had broken up and the liquor was fairly clear.

The total acidity was—5.35 per cent.

Volatile acidity—4.0 per cent.

equal to nearly 5 per cent. of acetic acid. The vinegar was rendered practically clear by filtration through cellulose (filter or blotting paper pulped in water).

Yeast Cultures in Cane Juice Peptone Broth.

Inoculated 26 May, 1910. Frendenreich flasks.

1. Beer yeast from Jorgensen's Laboratory, Copenhagen maintained in cane juice broth at Hope. Sets up a speedy fermentation after 12 months in the broth. Used in top fermenting breweries in Denmark. Has been employed successfully in Kingston in a wort of brown sugar, hops and water.

2. American whisky yeast—same source—dextrin fermenting power not tested.

3. Saccha. thermantitonum—same source—an oval budding yeast with an alleged high optimum temperature for both growth and fermentation. This strain shows nothing striking in those respects.

4. Bottom fermenting oval budding yeast—the typical yeast of cane juice and washes of relative low acidity. Has been sent to estates in 1908, 09 and 10.

5. Top fission yeast—has been three years in culture. Isolated from a Bluecastle wash. This culture has been used for supplying estates in 1908, 09 and 10.

6. Bottom fission yeast—three years in culture; originally from Mesopotamia wash. A typical bottom yeast.

7. Bottom fission yeast with slight top phenomena from Friendship wash—three years old. Has preserved its power of vigorous fermentation better than No. 6. Has been sent to estates as "bottom yeast" in 1909 and 1910.

8. Bottom fission yeasts—isolated in spring of 1910 from a sample of Swanswick "acid." Its fermenting power not yet tested in 1 litre portions of wash.

9. Bottom fission yeast—from "Long Pond "acid" in Spring 1910. Not yet tested.

10. Top fission yeast—from Long Pond wash, 1910. Not tested.

11. Top fission yeast—from Swanswick wash 1910. Not tested.

12. Saccha. ludwigii—from Long Pond "acid" 1910 apparently top fermenting.

13. Same as 12—from a different plating.

14. Chained budding yeast—from Swanswick "acid". Not investigated.

15. Narrow oval budding yeast—from Long Pond "acid." Not investigated—may be a variety of No. 4.

16. Pastorianus yeast—from molasses—three years in culture. Used in "orange wine" experiments.

17. Budding yeast—from Parnassus and Moneymusk discoloured crystal sugars. Very abundant in sugars. May be a Torula identical with the torula causing foaming of stored molasses. Does not ferment cane sugar.

18. Willia anomalous ("Anomalus" fruit ether yeast) three years in culture from molasses. This culture was used in experiments with the fruit ether yeast.

19. Mycoderma sp.—from Long Pond dead wash.

20. Mycoderma sp. mixed with B xylinoides—from Swanswick "acid" (See remarks on "ester formation").

21. B. xylinoides—from Long Pond "acid" About% 1 alcohol was added to the broth.

22. B. xylinoides—from Swanswick "acid." Alcohol added to broth.

23. Oval yeast and Rice grain variety 2.

24. Large oval yeast—three years in culture.

Literature of "Rum" and "Fermentation."

Rum.—Literature very scanty.

In Library:—

Uber Brauntwein .. Eugene Sell.
Articles by P. Greg (Mesopotamia Estate) in Bulletin Botanical Department, Jamaica.
Vol. 2. pts 3, 8, 9.
Vol. 3. pt. 1.
Sugar Experiment Station Reports 1 and 2.
Bulletin Dept. Agr. (new series) Vol. 1. No. 1.
Bulletin Dept. Agr. (new series) Vol. 1. No. 3.
Regarding "artificial rum" see
Rum Arrack etc., by Gaber.
Report Whisky Commission 1908.

Fermentation —

Betriebskontrolle, etc	..	P. Lindner.
Atlas	..	"
Prakticum	..	Henneberg
Spiritusfabrikation	..	Maercher-Delbruck.
Koch's Jahresbericht	..	1890-1905.
Technische Mykologie	..	Lafar.
Fermentation organisms	..	Jorgensen.
" "	..	Klocker.
Gærungs Essig ..	..	Hassack.
Centralblatt fur Bakteriologie		Abteilung 2.

S. F. Ashby,
Fermentation Chemist.
30 May, 1910.