Ratio of Alcohol to Yeast in Fermentation. By Katherine E. Golden.

Fermentation is, essentially, the breaking up of chemical compounds into simpler and more stable compounds. Some form of fermentation goes on in all living cells, the nature of the fermentation and the resulting products depending on the organism and the body fermented. The results may be simple, as, for example, where a single organism is used, or complex where a number of organisms are working together. Where a single organism is used, the predominating resulting product gives the fermentation its name.

In the alcoholic fermentation, besides the alcohol are formed CO_2, succinic acid, glycerine and a number of by-products, the nature and quantity of which depend on the orgenism, and the conditions under which it is grown. Beers and wines depend mainly on these by-products for their aroma and special character, so that experimenters, using the same kind of grape, have obtained many different wines; the same way for beers, using the same wort, but varying the yeast, different beers are obtained. Even from apple must good wines have been produced, by the use of certain yeast cultures. Again, mixing certain yeasts in the brewing, characteristics are obtained which are impossible with a single form. Large breweries now have competent bacteriologists, who seem to the uninitiated, to be able to manipulate their yeasts, molds and bacteria much as a juggler does his implements.

Yeast is the organism most commonly used to induce the alcoholic fermentation, though it can be induced also by certain bacteria and molds. The yeast which is used in brewing is S. cerevisiæ, there being two well marked varieties, the cerevisiæ, which produces top fermentation, and that which produces bottom fermentation. Top yeast works at a comparatively high temperature, the action is rapid, and the yeast rises to the surface of the liquid; this is used in the brewing of ale and porter. Bottom yeast works at a low temperature, the action is slow, and the yeast is at the bottom of the liquid; the bottom yeast is used in the brewing of lager beer.

Wort, which is the basis of beer, is made in the following manner: First there is the malting of the grain, which consists of the germination; then the stoppage of the germination by heat. The first stages are for the purpose of changing the chemical constitution of the grain; diastase is developed from the albuminoid matter; the diastase then acts on the starch, changing it to maltose and dextrine. When this development has reached the proper point, the germ is killed by drying. The grain is then cleaned and crushed and placed in warm water to allow the diastase to act still further on the starch, the completion of this

process being determined by the iodine test. The solution is then drawn off and boiled, hops being added. The hops give to the beer a bitter taste, besides aiding in its keeping; they also, by means of their tannic acid, facilitate the coagulation of the protein material, which is going on by means of the boiling. The wort is then cooled rapidly, after which it is ready for fermentation.

There are different methods used by manufacturers in the fermenting of the wort, but by whatever method there are always three stages into which the fermentation can be divided: the *main fermentation*, which begins in a short time after the yeast is added, during which time the maltose is decomposed, new yeast cells are formed and a rise in temperature takes place; the *after fermentation* is the next stage; maltose continues to be decomposed, the formation of yeast cells nearly ceases, the yeast settles and the beer clears; the last stage is the *still fermentation*, maltose is still decomposed, dextrine is changed into maltose, but no new yeast cells are formed. The *main fermentation* lasts from four to eight days; the other stages vary in time, and are controlled by changing the conditions.

In the experiments which I made the study was on the main fermentation, and was to determine the ratio between the amount of alcohol and the number of yeast cells formed. Wort, that was ready for fermenting, was obtained from one of the breweries, filtered, then placed in flasks, and sterilized by the fractional sterilization method. Two litres were used in a flask. Pure yeast, which had been separated from a compressed cake by the Hansen orientation method, was used; a colony, which had been grown from a single cell, was placed in 5cc. of wort in a test tube, and allowed to remain there twenty-four hours. This quantity was then added to the wort in the flask. This corresponds to the method employed in breweries, where a quantity of yeast is first grown in a small amount of wort; this quantity, called "pitching yeast," then added to the main quantity that is wanted for beer. After the addition of the pitching yeast, the flask was shaken thoroughly, and 1 cc. taken out with a sterilized pipette, for the purpose of counting the yeast cells. To the 1 cc. was added 1 cc. dilute H_2SO_4 for preventing further growth of yeast, and also for dilution. The wort was kept in a constant temperature oven at 25°c., this being a temperature at which the yeast grows vigorously.

At the end of every twenty-four hours for seven days the flask containing the wort was shaken vigorously for some time, so as to distribute the yeast cells thoroughly, then 1 cc. taken in the manner described, and also 200 cc. for determining the alcohol. The alcohol was estimated by direct distillation; 100 cc. was distilled over, then an accurately tared pycnometer of 50 cc. capacity used for the weighing. When the temperature varied from 15.5°c., Allen's formula for

correcting the density was used. $D = D' + d\ (.00014 + \frac{1-D}{150})$ D = required density; D' = observed density; d = difference in temperature between 15.5°c. and observed temperature.

After the specific gravity of the distillate was obtained, Allen's tables were used for determining the per cent. of absolute alcohol.

The apparatus for counting the yeast cells was made by taking a thin strip of brass, cutting an oblong hole through it, then cementing a strip of glass to one side of it, and using a similar strip for a cover on the other side. This gave a chamber of known dimensions, so that when the yeast liquid was placed in it the thickness of the layer was known. To obtain the other two dimensions, a micrometer having small squares engraved on it was placed in the eye-piece of the microscope, and the value, with a system of lenses then determined. The cell contents of a number of these squares were counted, and the average obtained. To determine the number of squares to be counted, countings and determinations were made until the number obtained had no influence on the average. This number of squares was then used on duplicate samples:

ALCOHOL.

No.	*Hours.*	*Sp. gr. alc.*	*Per cent. abs. alc. by wt.*	*Per cent. inc. alc. per day.*
1.	24	.9915	2.41	
2.	48	.98498	4.60	2.19
3.	72	.984215	4.89	.29
4.	96	.98337	5.22	.33
5.	120	.98297	5.405	.185
6.	144	.98277	5.500	.095
7.	168	.9826	5.575	.075

YEAST.

No.	*Hours.*	*No. cells in .02 c. mm.*	*Increase per day.*
1.	½	1.9	
2.	24	36.5	34.6
3.	48	55.6	19.1
4.	72	74.8	19.2
5.	96	95.6	20.8
6.	120	107.1	11.5
7.	144	112.6	5.5
8.	168	118.2	5.6

The table shows clearly that as the yeast cells increased in number the quantity of alcohol also increased in a nearly corresponding degree, so that, taking the results at the end of twenty-four hours, there is a direct ratio between the two. During the first twelve hours this does not hold good, as during approximately that period there is a large growth of yeast, but no apparent fermentation, as is evidenced by the lack of gas given off. For this reason the time between the "pitching," or inoculation of the wort, and the beginning of active fermentation is called the "incubation" period.

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The Circulation of Protoplasm in the Manubrium of Chara—Chara Fragilis. By D. W. Dennis.

About the middle of May last Mr. Omer Davis, a student in the Biological Laboratory, at Earlham, while studying the fertilization of Chara Fragilis noticed that the nucleus of the manubrium traveled rapidly around the periphery of the cell, with the circulating protoplasm. The phenomenon was subsequently noticed by all the members of a class of eighteen, and the attention of many other persons was called to it, some of whom were familiar with many of the phenomena of moving protoplasm in the leaves of Chara, the stamen hairs of Tradescantia and in other stock illustrations, it astonished all alike. The circuits of the nucleus were timed by Mr. Davis and myself, and found to range from 15, when the phenomenon was first noticed, to 26, something like a half hour later in a minute.

The circuit of this particular cell was not measured, but a measurement of a large number of cells later convinces me that it could not have been less than five-eighteenths of a mm. This gives a rate of 7.2 millimeters in a minute, or more than four times as fast as the fastest rate given in Goodale's Physiological Botany for protoplasm in a closed cell. I reported these facts to Prof. Barnes, who said they were, so far as he could learn, entirely new, and he asked me to prepare the matter for publication in the "Botanical Gazette." Early ni June I began what I hoped to make an exhaustive study of the phenomenon for this purpose, but could not find a single case in which the motion was going forward. Disintegration had taken place in most of the cells, and in all the motion had stopped. The phenomenon seems, therefore, to be one connected with the growth and maturation of the cell in which it occurs. All I can say is that next May we shall permit nothing to interfere with the most exhaustive study we can give to the