been made according to the principles above outlined, but the examples given are sufficient to illustrate the practicability and accuracy of the methods of separation. The work is being continued upon other mixtures, particularly those of the simpler reducing sugars with the higher saccharides maltose, lactose, and raffinose. It is needless to remark that in working with unknown mixtures the character of the different constituents must be determined by careful qualitative tests before beginning the work of analysis.

The application of some of the methods described to various problems of the sugar-cane industry, will form the subject of another paper.

[CONTRIBUTION FROM THE SUGAR EXPERIMENT STATION OF THE LOUISIANA STATE UNIVERSITY.]

THE FERMENTATION OF SUGAR-CANE PRODUCTS.[1]

BY C. A. BROWNE, JR.

Received January 1, 1906.

THAT the juice of the sugar-cane and the various products prepared therefrom are exceedingly susceptible to fermentative changes, has been known since the first beginnings of the sugar industry, for we find that the oldest writers upon the subject all make mention of the various phenomena of fermentation. The views upon the subject of fermentation, prior to the epoch-making discoveries of Pasteur, were necessarily very inexact; they are, however, exceedingly interesting in an historical way and one or two extracts from various authorities will bear quoting. The following passage from Porter's classical work upon the sugar-cane is especially characteristic: "If the fresh juice of canes is left to itself, the feculent parts are soonest decomposed. Of the first kind of feculencies one portion sinks to the bottom and the other rises to the surface, while the acid thus produced acts upon the second sort of impurities by diffusing them through the whole fluid mass. The acid which is generated at the very commencement of spontaneous decomposition holds the feculencies in most intimate combination with the fluid part. When the fermentation is well established it is continued for several weeks,

[1] Read before the New Orleans meeting of the American Chemical Society, January 1, 1906.

gradually decomposing the essential salt." The passage cited, with its obscure phraseology, sounds as if it might be taken from some ancient work on alchemy, yet the book in which it occurs was published in 1843. In discussing the practical bearings of fermentation upon the sugar industry, however, our authority treads on firmer ground, for he goes on to say: "The longer the fermentation goes forward the more difficult becomes the separation (of impurities) by heat and alkalies. If any feculencies are retained in the syrup, they very much impede the crystallization of the sugar." Wray, another well-known authority, whose book was published five years later than that of Porter, also states that "the glutinous fecula or ferment contained in the juice decomposes the sugar, converting it into alcohol." This old idea that fermentation was due to some principle inherent in the juice itself was held even beyond the time of Pasteur's investigations and faint echoes of this belief are heard even at the present time.

In the light of recent investigations upon the vegetable enzymes, we may say that this ancient conception of fermentation is in a certain measure correct, for there are certain "glutinous ferments contained in the juice" of all plants which produce spontaneously chemical changes of an exceedingly interesting character.

I. THE ACTION OF ENZYMES IN THE SUGAR-CANE.

If the green tops of a sugar-cane be well macerated, the juice expressed, and treated with an antiseptic agent, such as chloroform or thymol, it will be found that the sucrose content of the juice undergoes a gradual diminution, though no traces of microorganic life are evident, and that simultaneously with this decrease in sucrose the content of reducing sugars increases. We have here a well marked instance of the activity of the enzyme invertase which occurs almost universally throughout the vegetable kingdom, especially in the green or growing parts of plants. This presence of invertase has a very practical bearing outside of its physiological importance. The gradual falling off in sucrose content of sugar-cane which has been windrowed for any length of time is due very largely to spontaneous inversion. If the green tops of the cane are removed at the time of cutting, the diffusion of inverting enzymes into the stalk is prevented and the loss of sucrose will be much less evident.

A very marked peculiarity of sugar-cane juice, as of all vegetable juices, is the rapid darkening in color which takes place immediately after expression. This darkening is much more evident within the body of the cane, especially in the region of the eyes and growing parts, when its tissues are laid open to the air. We have here the evidence of another enzyme belonging to the class of oxydases. The intense blue coloration which the tissues and juice of plants take on with tincture of guaiac is ascribed to an oxydase; the decomposing action which plant extracts exercise upon hydrogen peroxide, discovered by Schoenbein, has been similarly explained, though Loew[1] attributes the latter phenomenon to a special enzyme, catalase, and Pozzi-Escot[2] to a new class of ferments called reductases. The juice from all parts of the sugar-cane reacts readily with both guaiac[3] and hydrogen peroxide; juices from sterilized canes, however, exhibit none of the reactions named. The catalytic action of cane juice upon hydrogen peroxide diminishes very rapidly on standing, especially in warm weather, as may be seen from the following diagram.

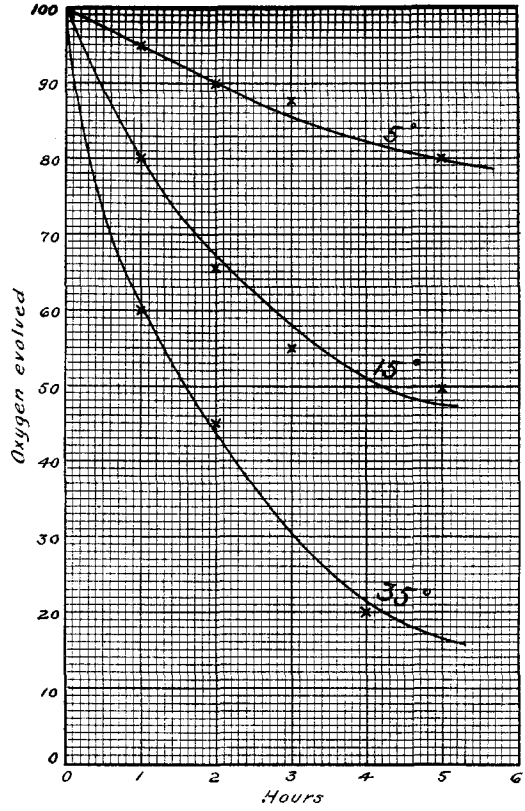
This rapid falling off in the catalytic action of cane juice on standing is probably due to some form of self-oxidation resulting from an interaction between the oxidizing and reducing enzymes.

If certain polyphenols, such as hydroquinone, or pyrogallol, are added to fresh cane juice, a rapid oxidation of these compounds is produced with an intense darkening of the juice. The

[1] Loew: "Catalase, a New Enzyme of General Occurrence," Report No. 60, U. S. Dept. Agric.

[2] Pozzi-Escot: "Reducing Enzymes," Am. Ch. J. 29, 517.

[3] Raciborski (Verslag, 1898, Proefstation voor Suikerriet in West Java, page 15) has also tested cane juice with guaiac and states that after heating above 60° C. the juice no longer gives the reaction; if, however, a little hydrogen peroxide is added the blue color disappears. This, Raciborski attributes to the presence of a substance occurring in all parts of the cane which he calls *leptomine*, and which he believes plays the part of an oxygen carrier in the respiration of all higher plants, similar to the function exercised by *haemoglobine* in the breathing of animals. We have repeated Raciborski's experiments, but have been able to confirm them only partially. Our experiments were nearly all performed upon cane which had lain several weeks in the windrow, and this may perhaps account for the differences. We found it necessary to heat the juice to from 75° to 85° C. before guaiac ceased to give the reaction. After thus destroying the *oxidase*, hydrogen peroxide was found, with some juices, to restore the color, thus indicating, perhaps, another enzyme, a *peroxidase*; with other juices no peroxidase reaction could be obtained.



latter takes on at the same time a peculiar odor, due to the formation of a quinone body, and what is more remarkable acquires a germicidal property which, in the case of the juice treated with hydroquinone, insures its preservation for weeks. Sterilized juice shows no change in color and develops no germicidal properties with any of the phenol bodies named. In connection with this oxidation of hydroquinone there is a very marked absorption of oxygen.

Two samples of cane juice (300 cc. each), one the fresh juice and the other the same juice boiled, were treated with 1 gram of hydroquinone in a closed flask. The flasks were agitated from time to time. The unboiled juice darkened rapidly, and at the end of twenty hours had turned a reddish brown; the color of the boiled juice remained unchanged. The quantity of air absorbed by each solution was determined.

	Cubic centimeters air absorbed.				
	1st day.	2d day.	3d day.	4th day.	Total.
Fresh juice	16	13	7	1	37
Boiled juice	4	5	4	3	16

The absorption in the boiled juice was probably due largely to the natural absorption of air, all dissolved gases having been previously expelled from the juice by the heating. The color of the fresh juice at the end of the experiment was almost black; the boiled juice showed no change.

The darkening of vegetable tissues on their exposure to the air, has been explained by Bertrand[1] to be due to the action of an oxidizing enzyme upon various tannin bodies, all more or less related to the polyphenols, and the query naturally arises does cane juice itself exercise any germicidal properties in connection with the natural phenomenon of darkening. The conclusion which we have reached in investigating this point is that cane juice does acquire for a time such germicidal characteristics. Counting the bacteria in the expressed juice of the cane at regular periods usually shows for several hours a uniform decrease in numbers; with juice from sterilized canes, on the other hand, the bacterial content increases from the very start.[2] The following table shows the rate of growth of bacteria in cane juices under different conditions.

TABLE I.

Experiment.	Source of juice.	Temperature.	Number of bacteria per cubic centimeter of juice. At beginning.	In 4 hours.	In 8 hours.	In 24 hours.	In 36 hours.
1	Green top	10°	2,761,920	294,336	262,080	211,800	.....
1	Green top	10°	2,681,280	241,920	231,840	187,488	.....
2	Green top	15°	106,880	96,320	98,240	.....	.....
3	Frozen cane, top	15°	260,160	274,560	320,000	960,000	.....
3	Sound cane, bottom	15°	458,880	465,600	475,840	560,000	.....
4	Steamed cane	15°	276,800	308,160	405,120	985,600	.....
4	Raw cane	15°	101,760	94,180	130,240	280,000	.....
5	Steamed cane	20°	12,480	20,160	33,600	311,040	3,840,000
5	Raw cane	20°	37,440	43,840	65,280	902,400	5,120,000

The experiments are not altogether conclusive. In Expt. 1

[1] Compt. rend. 121, 166; 122, 1132, 1215.

[2] In this connection it is interesting to recall the vast amount of work done by Hunziker (Bull. 197, Cornell Expt. Sta.) and others upon the germicidal action of fresh milk; it may be that certain enzymes, which we know to be always present in milk, exercise also here a toxic action.

the low temperature at which the juice and cultures were kept undoubtedly caused many organisms to succumb or remain dormant. Comparative experiments such as Nos. 4 and 5 are more satisfactory, and these show for several hours a marked retardation of bacterial growth in the raw juices, especially at the lower temperature. The tests for oxydase and catalase in cane juice became very feeble after ten or twelve hours and with this disappearance in enzymic power the number of bacteria begins to undergo a sudden increase. But it is more especially within the body of the cane itself that this germicidal action is most evident, and this we might expect not only from the colloidal and adherent character of the enzymes, which renders them resistant to expression, but from the facts of localization which will be discussed later.

If two stalks of sugar-cane, one raw and one sterilized, are punctured with a knife there will be observed at the end of a few days a marked difference in the character of the wounds. The surface of the wound in the raw cane will be discolored, but free from evidence of fermentation; the surface of the wound in the sterilized cane, on the other hand, will not be thus discolored, but will be badly infested with bacteria and moulds. When the sugar-cane is attacked by the borer or beetle, the pathway of the insect is much discolored, but we notice no inroad of organisms into the sound tissue. The living plant therefore does appear to protect itself against the invasion of microscopic parasites by forming toxic products. In case the sugar-cane is killed, and split open as sometimes happens during a freeze, this power of protection is lost (see Expt. 3, Table I). The formation of toxic products does not go on; hordes of bacteria invade the stalk, and finding no resistance start a fermentation which soon renders the cane worthless for milling.

The question may be asked if these toxic products are so deadly to micro-organic life, why do they not react unfavorably upon the cane itself? It is just here that the reducing or catalyzing enzymes perform their functions, for should the toxic oxidation products diffuse inward beyond the points of their formation, they are at once reduced and thus exert no action deeper than the exposed surface.

It will be seen that there is a great difference between working with enzymes before and after expression from the plant. As

Pfeffer remarks in his Plant Physiology, "The living cell must not be judged by reactions obtained with dead material or in the expressed juice." That there is a localization of the enzymes within the plant is rendered very evident if we apply the guaiac test to the cross-section of a cane stalk; the blue coloration is developed the strongest upon the peripheral parts, showing that the oxydases are, apparently, more localized in this region. Pozzi-Escot[1] has demonstrated that the same condition exists with other plants such as the potato; his experiments show, however, that the oxydases exist in the inner tissues as well, but that the greater localization of the reductases in these parts interferes with the guaiac reaction. Jacoby[2] goes even a step further and states that the localization of ferments exists not only in the tissues but within the individual cell. He sums up the situation very briefly in these words: "The work of the cell can be determined with absolute certainty only when we learn to separate in the test-tube the substances which are separated in the cell, and allow the individual ferments to do their work in proper sequence and in proper concentration." The difficulties of carrying out a reaction under these conditions seem almost insurmountable with our present methods of laboratory technique, yet it would be rash indeed to say that the execution of the above postulate is impossible.

II. THE DECOMPOSITION OF SUGAR-CANE PRODUCTS BY MICRO-ORGANISMS.

The number of micro-organisms which produce decomposition of cane products is almost unlimited and the chemical changes which develop, especially when several fermentations take place simultaneously, are necessarily very complex. In the brief space at our command we can only take up a few of the typical and more common fermentations.

The most common fermentation which the raw juice of the cane undergoes in Louisiana, is not the alcoholic, as might be supposed, but a fermentation designated variously as the viscous, mucilaginous or mannitic. This fermentation is anaerobic in character; in consequence, a most powerful reducing action takes place by virtue of which the juice is rapidly bleached. The liquid becomes thick and ropy, and if the culture be pure the juice will finally

[1] Am. Ch. J. 29, 530.

[2] Ergebnisse der Physiologie [1] 1, 213.

set to a perfectly solid jelly. Various organisms may produce this type of fermentation, but the best known member of this class of bacteria is the *Leuconostoc* or *Streptococcus mesenteroides*, the so-called "frog spawn" of the beet-sugar manufacturer. This fermentation was one of the first to attract the attention of investigators and the study of its products of decomposition constitutes an interesting chapter in the subject of biochemistry.

Vauquelin in 1822 caused four bottles of cane juice to be sent from Martinique to France. The samples arrived, however, in a very bad condition, the juice having changed to a thick mucilage. Vauquelin, therefore, busied himself with a study of the gummy matter into which the sugar had been changed, but the methods of organic analysis at that time were in their infancy and no definite knowledge of the gum seems to have been gained. Peligot, Kircher, Brüning and many others also occupied themselves with the problem, studying the gummy fermentation products of both cane and beet juices. Durin[1] regarded the constituent of this gum as cellulose and felt so certain of his ground that he took out a patent—a curiosity of its kind—for the "conversion of crystalline sugar into cellulose and for any use which such cellulose can find technically." It was Scheibler[2] who first established the real nature of the product; he proved the gum to be a body very similar to dextrin and named it dextran. Scheibler himself, however, fell into an error, for he regarded the gum not as a fermentation product, but as a substance occurring naturally in the plasma of the beet-cells.

Dextran was prepared from samples of clarified cane juice, which had undergone the viscous fermentation, by precipitating with 95 per cent. alcohol. The gum was filtered off and repeatedly purified by dissolving in dilute sodium hydroxide, filtering and precipitating with alcohol acidified with hydrochloric acid. After washing with alcohol and ether, the gum was dried first at 60° and then, after pulverizing, at 100°, and finally at 130°. The product thus obtained was perfectly white and contained 1.65 per cent. of ash, mostly sodium chloride.

The following analysis, calculated to ash-free substance, was obtained:

[1] "De la fermentation cellulosique du sucre de canne," Compt. rend. 83, 128 (1876).

[2] Z. Vereins deutschen Zucker-Industrie, 1869, p. 472; 1874, p. 309; 1875, p. 112.

Calculated for: $(C_6H_{10}O_5)_n$, H, 6.22 per cent.; C, 44.42 per cent.; $3(C_6H_{10}O_5).H_2O$, H, 6.40 per cent.; C, 42.83 per cent. Found: H, 6.54 per cent.; C, 42.50 per cent.

Other analysts have reported for dextran 41.45–43.61 per cent. carbon. The formula usually assigned to dextran is $(C_6H_{10}O_5)_n$, the same as that of cellulose. The writer, however, is inclined to the belief that dextran is a hydrated product of variable composition.

0.3848 gram of dextran was dissolved to 50 cc., a drop of ammonia being added to secure freedom from opalescence. The solution gave a polariscopic reading of +4.47° Ventzke in the 100 mm. tube, from which the specific rotation, $[\alpha]_D^{20} = \frac{0.3468 \times 4.47 \times 50}{0.3848} = +201.8$. The results recorded in the literature for the specific rotation of dextran vary from +195 to +230.

Ten grams of dextran were treated in the cold with 50 cc. of 90 per cent. sulphuric acid for twenty-four hours. The dark colored mixture was then poured into 500 cc. of water and heated on the steam-bath for five hours. After neutralizing with an excess of calcium carbonate, the solution was filtered and evaporated to a syrup, which was purified from gummy matter by shaking up with alcohol and ether. On filtering and evaporating a bright straw-colored syrup was obtained, which on several weeks' standing solidified to a thick mass of crystals. These were filtered off, and after recrystallizing from alcohol, using bone-black, a perfectly white sugar was obtained.

0.4514 gram of the sugar was dissolved to 10 cc. and polarized in a 100 mm. tube. Strong multirotation was noted.

TABLE II.

Time after solution.	Polariscope reading. °Ventzke.	Specific rotation. $[\alpha]_D^{20°}$.
5 minutes	+12.8	+98.3
5 hours	+ 6.9	+53.0

The constant rotation agrees with that of dextrose. No other sugar than this could be detected among the hydrolytic products of dextran.

The presence of dextran in sugar-cane products may introduce at times an error into the analytical work. It happens occasionally in Louisiana that the sugar-cane is damaged by a splitting freeze; on the occurrence of warm weather a fermentation sets in with the formation of considerable dextran within the cane. In

analyzing juices from such canes the inexperienced chemist is often puzzled because his juices polarize well, yet give him poor returns in the sugar-house. The following analyses of badly fermented cane juices will show the influence of dextran upon the polarization.

TABLE III.

No.	Degrees Brix.	Polarization.	Sucrose. Per cent.	Reducing sugars. Per cent.	Dextran. Per cent.	Apparent purity.
1	7.8	+18.0	0.0	0.15	5.90	232
2	4.8	+10.4	0.0	trace	3.35	216

The occurrence of dextran in cane syrups and molasses might lead the food chemist to suspect an adulteration of these products with commercial glucose, when in reality no such adulterant was present.

The viscous fermentation, as was stated, exerts a powerful reducing action upon the cane juice, and as a consequence of this reduction various deoxidation products are formed. The most common of these is mannite, which was very early recognized among the products of this fermentation and for this reason the name mannitic fermentation was sometimes applied. It was at first supposed that the mannite was the product of a special organism, but this is a mistake, for mannite may be formed in any fermentation of sugar where a reducing action takes place. The quantity of mannite in fermented juices will vary; juices which showed over 2 per cent. mannite were found on subsequent analysis to be nearly deficient in the same, owing to the fact that other fermentations had set in whereby the mannite was destroyed.

The separation of mannite from juices which have undergone the viscous fermentation is usually a simple matter. After removing the dextran by means of alcohol, the clear solution is filtered and evaporated, when the mannite will usually crystallize out in characteristic needles. When, however, there is a large residue of unfermented sugars, the crystallization of the mannite offers some difficulty. In such cases the writer has used a method proposed by Guignet.[1] The clarified juice is treated with a solution of copper sulphate containing a minute excess of ammonia. If mannite is present, a greenish white precipitate is formed. After some hours' standing, this is filtered off, washed with a little

[1] Ber. 22, 687.

cold water, and then, after solution in strong ammonia, decomposed with hydrogen sulphide. The copper sulphide is removed and the clear filtrate evaporated, when the mannite separates in the crystalline form. A preparation obtained from a fermented cane juice in the manner described gave on heating with concentrated hydrochloric acid and formaldehyde needles of mannite-triformacetal melting at 228°. After oxidizing with dilute nitric acid phenylhydrazine produced a white crystalline deposit of mannose-phenylhydrazone, m. p. 195°.

Among the products of the different anaerobic fermentations to which cane juice is subject, are various gaseous bodies. In cane juices clarified by the sulfitation process, especially such as have been afterwards treated with phosphoric acid, fermentation occasionally sets in and large quantities of hydrogen sulphide are evolved. The odor of this gas is usually very noticeable around fermenting press cakes. In other cases hydrogen is given off and serious accidents have been reported through the explosion of this gas, generated from juice or syrup that had been left standing in vacuum pans or effects. When fermentation of the juice and bagasse together takes place, as may occur in a diffusion battery, the cellulose of the cane fiber undergoes a decomposition, and the gas given off contains methane as well as hydrogen. Explosions of diffusion cells in sugar-beet factories from this cause have been reported not infrequently. The fermentation which takes place in bagasse piles is usually of the butyric order, as may be recognized by the peculiar rancid odor which is given off.

Reference was just made to Durin's patent for obtaining cellulose from sugar. Notwithstanding the fact that Scheibler proved Durin's cellulose to be an entirely different body, cellulose may be formed from sugar in large amounts by the activity of bacteria. A fermentation of this kind was reported by the writer[1] several years ago, and more recent investigations show that this fermentation is one of very general occurrence in Louisiana.

This fermentation, unlike the viscous, is aerobic. Large gelatinous lumps of leather-like toughness are formed in the juice. These lumps, which sometimes weigh several pounds, are stratified in appearance and are as a matter of fact made up

[1] The Louisiana Sugar Planter, 31, 305 (1903).

of an infinite number of closely compacted membranes. The substance of these membranes on boiling with alkali does not pass into solution as is the case with dextran, but shrivels up into a dense white body which gives all the reactions of cellulose, yielding a blue coloration with zinc chloride and iodine and being 99 per cent. soluble in cuprammonium. The percentage composition of the purified substance precipitated from cuprammonium agreed with that for cellulose.

Calculated for: $(C_6H_{10}O_5)_n$, H, 6.22 per cent.; C, 44.42 per cent. Found: H, 6.28 per cent.; C, 43.87 per cent.

The amount of dried membrane formed by this fermentation in a cane juice was about 13 per cent. and the amount of cellulose about 7 per cent. of the total sugar fermented. When examined under the microscope the membranous tissue is seen to consist of interwoven chains of streptococci embedded in the capsular matter, which constitutes the real substance of the membrane. Intermingled with these bacterial chains a great many yeast cells are usually visible, and it may be that we have here a case of symbiosis—such as occurs with the ginger-beer ferment, which was formerly employed quite extensively in the Southern States for making molasses beer. The cellulose-forming organism resembles very much in appearance old cultures of the *Bacterium xylinum*, which, according to A. J. Brown,[1] replaces its bacterium form in advanced stages with micrococci. The *Bacterium xylinum* also produces cellulose and the writer believes that the two organisms are identical.

Many bacteriologists and chemists are not disposed to recognize the formation of cellulose by bacteria. Emmerling,[2] for example, holds that what Brown regards as cellulose in the *Bacterium xylinum* is not cellulose at all, but chitine, and in proof of this claims to have obtained crystals of glucosamine hydrochloride on hydrolyzing the membranes with hydrochloric acid. The writer has been unable to confirm the work of Emmerling with the membranous substances obtained from cane juice. The dried membrane, after boiling with 10 per cent. caustic soda, contained only 0.2 per cent. of nitrogen and only 1 per cent. of residue

[1] "On an Acetic Ferment which Forms Cellulose," by A. J. Brown, J. Chem. Soc. 49, 432 (1886).

[2] Ber. 32, 541 (1899).

insoluble in cuprammonium, so that if chitine were present it occurred in but minute traces.

As constituents of the deposits and scums which always form in fermenting juices, syrups and molasses, we have a number of substances which, like dextran and cellulose, are to be regarded as of assimilative rather than of fermentative origin. Such, for example, is mannan; the mixed sediment of yeast cells, mycelia, etc., found in decomposed juices and syrups always contains this body. A collection of such deposits was filtered off and washed with successive portions of water, boiling alcohol and ether. The residue was then hydrolyzed with sulphuric acid in the manner already described for dextran. The solution, after neutralizing with calcium carbonate, filtering and evaporating, gave a dense white precipitate in the cold with phenylhydrazine. On recrystallizing from 60 per cent. alcohol a pure white hydrazone was obtained, soluble in boiling water and but slightly soluble in absolute alcohol or ether. The melting-point on slow heating was 188° and on rapid heating 195°. These properties agree with those of mannose-phenylhydrazone.

Another common ingredient of these fermentation products is the nitrogenous body chitine previously mentioned. We have found this substance to be a very important constituent of the scums[1] which form every year upon the surface of molasses left over in the hot-room. These scums, upon washing out the adherent molasses, constitute a brownish pulpy mass, a sample of which, air-dried, gave the following analysis:

	Per cent.
Moisture	10.00
Chitine	11.30
Protein	31.62
Fat	27.50
Ash	5.58
Undetermined (N. free)	14.00

After extracting the dried material with successive portions of ether, boiling sodium hydroxide, hot water and alcohol, about 15 per cent. of insoluble residue was obtained which analysis showed to be 90 per cent. chitine.

One gram of the purified scum residue was gently boiled in a

[1] These scums are produced by a fungus belonging to the genus *Citromyces*, for the identification of which the writer is indebted to Mrs. F. W. Patterson, of the Bureau of Plant Industry, U. S. Dept. of Agriculture.

small flask with 20 cc. of concentrated hydrochloric acid for one hour, a condenser being used to prevent evaporation. The dark colored solution was then filtered, clarified with bone-black, and evaporated until crystallization began. On cooling, a considerable quantity of white granular crystals were obtained, which were filtered off, washed with alcohol and ether, and then dried over sulphuric acid. 0.6 gram of product was obtained or 60 per cent. of the residue taken.

The crystals gave all the reactions of glucosamine hydrochloride, $C_6H_{11}(NH_2)O_5.HCl$. Heating with caustic soda yielded ammonia, and boiling with copper sulphate solution precipitated red cuprous oxide. 0.2092 gram of substance was dissolved to 10 cc. and polarized in the 100 mm. tube. The following series of readings were obtained:

TABLE IV.

Time after solution.	Polariscope reading. ° Ventzke.	Specific rotation. $[\alpha]_D^{20°}$.
5 minutes	+5.9	+97.80
30 minutes	+5.1	+84.54
1 hour	+4.8	+79.57
2 hours	+4.3	+71.28
3 hours	+4.25	+70.45
6 hours	+4.25	+70.45

Ledderhose[1] reported as the constant specific rotation of glucosamine hydrochloride prepared from lobster shells +69.54°–+70.61°.

The large amount of fat in the molasses scums (27.50 per cent.) is noteworthy, and what is more remarkable, the composition of this fat, as is shown from its physical and chemical constants, agrees very closely with that of butter fat.

	Fat from scums.	Butter fat.
Saponification number	223.1	228.5
Iodine absorption	28.17	33.35
Reichert-Meissl number	30.36	28.3
Melting-point	35°	33.2°
Melting-point, insoluble acids	41°	41.7°
Iodine number	30.53	29.5

This is the first instance so far as the writer can find of any other fat, either vegetable or animal, showing such a similarity, as regards the above constants, to butter fat. In certain respects, however, the fat differs from fresh butter fat.

[1] "Ueber Chitin und seine Spaltungsprodukte," Z. physiol. Chem. **2**, 213-227 (1878-1879).

	Fat from scums.	Butter fat.
Acid number	85.2	0.50
Ether number	137.9	228.0
Mean mol. wt. soluble acids	129.7	98.1
Mean mol. wt. insoluble acids	283.2	261.0

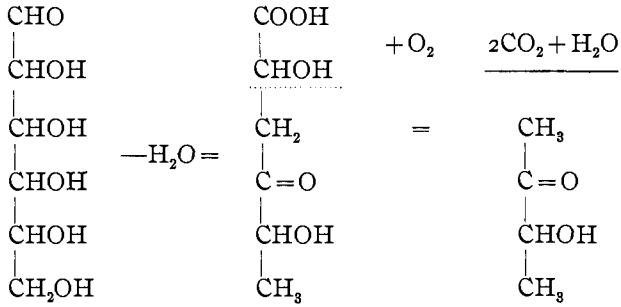
The distinguishing characteristic of the fat from the scums is the high degree of acidity and the greater preponderance of such soluble acids as caproic and caprylic. The high acid number is undoubtedly the result of hydrolysis through a lipolytic enzyme.

The physiology of fat in bacteria and other micro-organisms is a subject deserving of further investigation. The function of the fat is unquestionably that of a reserve material, and when this is utilized by the organism a series of interesting changes must result. In the hydrolysis of this fat by means of a lipase, glycerol is of course formed and this diffusing through the cell membranes enters into the fermenting liquid. Udransky[1] showed many years ago that the formation of glycerol in wines had no direct connection with the conversion of sugar into alcohol but that it was the result of metabolic activities within the yeast cell, and suggested that lecithin might be the body from which the glycerol was derived. The formation of glycerol in wines, cider, etc., by the cleavage of fatty matter within the yeast cell appears more plausible than the hypothesis of Udransky. In this cleavage some of the lower fatty acids which are liberated may unite with the alcohol forming the various fruity esters which are so marked in certain fermentations, such as that produced by *Oideum lactis*, which organism is characterized by a high content of oily matter.

It will be impossible in a paper already too long to take up other interesting fermentations of sugar-cane products, such as that produced by *Oideum lactis*, by the different varieties of *Mucor*, *Aspergillus*, and *Penicillium*, and by the various kinds of *Mycoderma*, or to discuss the character of the products formed by these various organisms. There is one fermentation product, however, which we would like to mention before concluding, as it seems to be of quite common occurrence, although not generally recognized. The presence of this compound also seems to lend support to the theory recently advanced concerning the rearrangement of the sugar molecule prior to its conversion into lactic acid or alcohol. The substance in question is dimethylketol

[1] Z. physiol. Chem. 13, 539 (1889).

or acetylmethylcarbinol, $CH_3—CO—CHOH—CH_3$. The compound was first made synthetically by von Pechmann;[1] it was discovered later by Grimbert[2] among the products produced by the fermentation of dextrose, dextrin, and mannite by *Bacillus tartricus*, and by the writer[3] among the fermentation products in cider vinegar. Pastureau[4] has also recently shown this body to be a common constituent of commercial vinegar. The writer has again found the same substance in a fermented syrup and it is his belief that this compound is always produced in small amounts whenever the alcoholic fermentation is arrested through the development of oxidizing or acid-producing bacteria. The hypothetical compound[5] into which sugar is supposed to pass before splitting up into alcohol and the oxidation of this compound to acetylmethylcarbinol can be represented as follows:



Sugar. Intermediary compound. Acetylmethylcarbinol.

It is a mistake to suppose that the fermentation of sugar-cane products is limited entirely to such dilute media as juices and syrups. Molasses is also very susceptible to fermentation, and even raw sugars during transport or in storage may undergo a gradual deterioration through the activity of yeasts and bacteria. The fermentation of such a thick menstruum as molasses, however, is confined entirely to the surface, which through the attraction of hygroscopic moisture becomes dilute enough to favor micro-organic growth. The same is true of raw sugars; the film of molasses coating the crystals undergoes a gradual fermentation

[1] Ber. 21, 2754; 22, 2214.

[2] Compt. rend. 132, 706.

[3] This Journal, 25, 31.

[4] J. pharm. chim. 21, 593 (1905).

[5] For the arguments supporting the existence of this compound see Lippmann's "Chemie der Zuckerarten," 3rd ed., p. 1890.

with the result that the underlying sucrose is slowly dissolved and inverted.

The deterioration of molasses and sugar during storage is often the cause of considerable losses in the sugar industry. A discussion of this, however, and of other economic questions, closely related to our subject, would take us far beyond the limits of our paper and must therefore be reserved for another occasion.

[CONTRIBUTIONS FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS.]

THE CHEMISTRY OF FLESH.

(FOURTH PAPER.)[1]

A STUDY OF THE PROTEIDS OF BEEF FLESH.[2]

BY P. F. TROWBRIDGE AND H. S. GRINDLEY.

Received February 17, 1906.

THE past researches upon the proteids of animal substances have been mainly devoted to the study of the proteids of blood and to the proteids of muscle freed from blood. However, the flesh of the lower animals, as it is sold for food, is a mixture of muscle and blood, each of which is a very complex substance composed of many chemical bodies in varying proportions.

Notwithstanding the large amount of valuable research which has been devoted to the chemistry of the proteids of muscle and blood, the present knowledge of these substances is very incomplete, imperfect and contradictory. This is especially true of the chemistry of muscle. The literature of this subject is at present in a state of much confusion. Hammarsten,[3] the distinguished physiological chemist, in the last edition of his text-book in considering the proteids of muscle, says: "The views of the various investigators differ so essentially and the nomenclature is so complicated that it is extremely difficult to give any correct review of the various notions. For these reasons the author is not sure whether he has understood and correctly given

[1] This Journal, 26, 1086 (1904); 27, 658 (1905); 28, 25 (1906).

[2] This research was made possible by a grant from the Elizabeth Thompson Science Fund. We wish hereby gratefully to acknowledge the aid thus received in our investigations.

[3] "Text-book of Physiological Chemistry," 4th English Ed. (1904), p. 382.