methyl-*d*-xyloside. Fifteen g. of this substance was gradually added to a boiling solution of 6 g. of anhydrous sodium acetate in about 60 cc. acetic anhydride. After boiling for a few minutes the reaction mixture was cooled, mixed with 800 cc. of cold water, and an insoluble sirupy phase separated and crystallized almost immediately. After one recrystallization of the substance from water its specific rotation was found to be $[\alpha]_D^{22} = -60.9°$, and the melting point 115° (uncorr.). These values, which remained the same after another recrystallization, agree with those previously found for β-triacetyl-methyl-*d*-xyloside that had been prepared from bromo-acetyl-xylose.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE LABORATORIES OF AGRICULTURAL CHEMISTRY OF THE UNIVERSITY OF WISCONSIN.]

BY-PRODUCTS OF THE FERMENTATION OF CABBAGE.

BY V. E. NELSON AND A. J. BECK.

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The results of this investigation are merely the outgrowth of some work which was planned in this laboratory to shed further light upon the chemical changes taking place in silage and the direct cause of these transformations.

In the fermentation of green plant tissue two dominant factors contribute to the production of the various chemical by-products, namely enzymes and bacteria. To what extent enzymes are a factor to the exclusion of bacteria is a very debatable question requiring considerable experimental investigation before a solution can be made, and one which our experiments were planned to answer. For certain specific reasons we chose the cabbage instead of the corn plant as a method of solving this problem. Before any progress could be made, it was necessary to determine the by-products resulting from the fermentation of cabbage or sauerkraut, as it is commonly called.

As far as the authors are aware no systematic quantitative study of this product has been made.

Experimental.

For the estimation of acids, alcohols and esters of fermented products, certain methods were employed which have been little utilized by biological chemists. A detailed description of the methods will not be given here as it is the intention of one of the authors to discuss these methods in a forthcoming paper on the volatile by-products of certain soft cheeses, and a determination of the volatile fatty acids, esters and alcohols resulting from the growth of certain microörganisms on synthetic media. However, a very brief summary may not be out of place. For information more in detail, it is necessary to consult Suzuki, Hastings and Hart[1] on the volatile

[1] *J. Biol. Chem.*, **7**, 431–458 (1910).

by-products of cheddar cheese, Hart and Willaman[1] of this station and Dox and Neidig[2] on the by-products of corn silage, DuClaux's[3] original article in which the principles of the method are given in detail, and the work of Richmond,[4] who verified the findings of DuClaux.

Recently certain criticisms regarding the value of this method have been published. Of particular interest, mention is made of the work of Upson, Plum and Scott[5] of Nebraska, who condemned the method because of its failure of absolutely quantitative accuracy. This paper was completed before the above work appeared, and so we will not offer any data here to refute the claims set forth by these investigators. Richmond,[6] Suzuki,[6] and Lamb[7] have verified the findings of DuClaux, and work at this station has substantiated his data. It must be borne in mind that none of these investigators hold the DuClaux method to be as accurate as a standard analytical operation, but that it does yield fairly accurate quantitative results as measured in terms of biological chemical determinations. The recent work of Gillespie and Walters[8] confirms our viewpoint and substantiates data obtained at this station.

The Determination of Acids, Alcohols and Esters.

Cans of fermented cabbage were purchased on the market, the contents removed and finely comminuted by passing the material through a meat grinder. Five hundred g. was placed in a five-liter round-bottomed long-necked flask made slightly acid to congo red with sulfuric acid, and subjected to steam distillation until 2 liters was collected. The contents of the flask was kept in a pail of boiling water during the distillation so as to prevent condensation as much as possible. The volatile acids of the distillate were then titrated with 0.1 *N* barium hydroxide using phenolphthalein as an indicator and the alcohols and esters distilled from the barium salts of the acids. The alcohols and esters were repeatedly redistilled until 50 cc. was obtained. In each case two-thirds of the original distillate was collected until the required 50 cc. resulted. This latter solution, which is called the flavor solution, was saponified with 10 cc. of 20% potassium hydroxide and the alcohols distilled off. The acids which formerly were in ester combination are now in the form of potassium salts. The alcohols were concentrated by repeated distillations to 50 cc., and oxidized on the water bath by a solution of potassium dichromate in sulfuric acid, prepared

[1] THIS JOURNAL, 36, 1619–1625 (1914).
[2] *Res. Bull.* 7, Iowa Agr. Expt. Sta.
[3] *Traite de Microbiol.*, 3, 388 (1900).
[4] *Analyst*, 33, 305–313 (1908).
[5] THIS JOURNAL, 39, 731–742 (1917).
[6] *Loc. cit.*
[7] THIS JOURNAL, 39, 746–7 (1917).
[8] *Ibid.*, 39, 2027–2055 (1917).

from 10 g. of potassium dichromate and 20 g. of strong sulfuric acid diluted with sufficient distilled carbon dioxide-free water to make 100 cc.

Finally the acids resulting from the oxidation of the alcohols were distilled from the oxidation mixture, and converted into barium salts by titrating with 0.1 N barium hydroxide. The potassium salts of the acids in ester combination were decomposed with dil. sulfuric acid, distilled and the distillate titrated with 0.1 N barium hydroxide solution. The free acids, and the acids resulting from the saponification of the esters and the oxidation of the alcohols were then subjected to the DuClaux method for analysis and estimation. Reference must be made to the original article for details concerning this process. In case the titer with 0.1 N barium hydroxide did not reach at least 5 cc., no attempt at differentiation was made.

TABLE I.—RESULTS OF ANALYSES.

Sample No.	Dry matter. %	Total acidity in cc. 0.1 N H_2SO_4 for 200 g. sample.	Total volatile acidity in 0.1 N H_2SO_4 for 200 g. sample.	Volatile acidity in cc.		
				0.1 N formic.	0.1 N acetic.	0.1 N propionic.
I.....	7.53	312.8	86.81	None	81.25	5.56
II.....	8.39	292.92	111.63	13.73	94.13	3.77
III.....	8.27	317.00	103.18	None	95.62	7.56
IV.....	8.27	317.00	109.88	None	101.94	7.94
V.....	5.05	176.32	85.95	None	51.27	34.68
VI.....	8.53	362.00	101.30	2.49	94.01	4.80
VII.....	7.47	190.00	91.19	None	83.74	7.45

Sample No.	Total volatile alcohols in cc. 0.1 N H_2SO_4.	Acids from alcohols in cc.		Acids in ester combination in cc. 0.1 N H_2SO_4.
		0.1 N acetic.	0.1 N propionic.	
I.....	86.81	81.25	5.56	1.1
II.....	Alcohols lost		..	Not determined
III.....	61.03	54.08	6.95	0.8
IV.....	61.28	56.33	4.95	0.52
V.....	105.14	95.24	9.9	Not determined
VI.....	Alcohols lost		..	1.7
VII.....	Alcohols lost		..	Lost

The results of 7 analyses are given in the table. It will be noted that the volatile acidity bears a considerable relation to the total acidity. Due to the fact that the quantitative methods for isolating lactic acid are very inadequate, it is difficult to say whether the total acidity minus the volatile acidity represents this component entirely. No succinic acid was found, and the lactic acid isolated was inactive.

Only 2 acids compose the volatile portion, namely acetic and propionic although, in 2 instances, formic acid was isolated. Acetic acid is present in the greatest quantity. No butyric or higher acids could be found. The alcohols are present in considerable amount, in fact, to the same extent as the volatile acids, and are comprised entirely of ethyl and propyl alcohols, the former being by far the most abundant.

The determination of the esters is very unsatisfactory for in the process

incident to the estimation of these substances hydrolysis destroys by far the larger proportion. For that reason they were obtained only in small amounts, and yet they contribute largely to the flavor and aroma of the original product, as is evidenced by the fact that after saponification the characteristic flavor and aroma have disappeared.

In the 5 samples analyzed for methyl alcohol, which according to Hart and Willaman[1] and Hart and Lamb[2] is a natural constituent of corn silage, none was found.

In the extraction of the material under investigation by means of boiling ethyl alcohol for the isolation of the fixed acids, it was found that on concentrating and cooling, snow white, needle-shaped crystals separated, which had a melting point corresponding to 169°. After considerable time devoted in the identification of this substance, it was found to consist of pure mannitol. A careful review of the literature developed that mannitol had been detected in fermented cabbage by Feder.[3] In the 3 samples investigated by the authors, 1.9, 2.0, and 2.5%, respectively, was found.

Our method consisted in extracting with hot alcohol, cooling and allowing the material to crystallize from 95% alcohol, which it invariably did after a time varying from a few hours to a day or more.

Thinking that the mannitol might be a natural constituent of cabbage we undertook an examination of this material. Since mannitol has been found in a number of naturally occurring food products and higher plants, including turnip, celery, onion, carrot, pineapple, June grass, etc., it was not out of the range of possibility that the mannitol had its origin in the cabbage plant. However, we found such is not the case, for no mannitol could be isolated from cabbage, and consequently, must have resulted from bacterial decomposition of carbohydrates.

Conclusions.

The volatile acidity of fermented cabbage represents a considerable proportion of the total acidity.

Since no succinic acid or acid other than lactic was isolated from the fixed portion, the fixed acidity is concluded to be this component and in an inactive form.

Acetic and propionic acids comprise the volatile portion, although in 2 cases formic was isolated. Acetic acid is present in the greatest quantity. No butyric or higher acids were present.

Alcohols are found to the same extent as volatile acids and consist entirely of ethyl and propyl alcohols. No methyl alcohol was found.

The esters though present in small amount contribute an essential part in the flavor and aroma.

[1] *Loc. cit.*

[2] THIS JOURNAL, **36**, 2114 (1914).

[3] *Z. Nahr. Genussm.*, **22**, 295–6.

Mannitol was isolated from fermented cabbage to the extent of 2.0 to 2.5%.

Since no mannitol was obtained from the natural plant, that found in the fermented product must have had its origin in the bacterial decomposition of carbohydrates.

[CONTRIBUTION FROM THE CARBOHYDRATE LABORATORY, BUREAU OF CHEMISTRY, U. S. DEPARTMENT OF AGRICULTURE.]

METHYLTETRONIC ACID AND ITS AMIDE.

BY C. S. HUDSON AND L. H. CHERNOFF.

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Methyltetronic acid has been previously prepared by Ruff and Kohn[1] who obtained it in the form of its lactone through the oxidation of methyltetrose with bromine. Methyltetrose, however, is difficult to make. We have obtained this lactone very easily by a simple method recently described by Nef, Hedenburg and Glattfeld[2] for the oxidation of arabinose and xylose. By passing a current of air through dilute solutions of these pentoses, they obtained *l*-erythronic lactone from *l*-arabinose and *d*-threonic lactone from *d*-xylose. Applying this method to rhamnose one would expect that its oxidation should yield methyltetronic lactone, and this proves to be the fact. Our yield of lactone was 9.6% of the theoretical. This is considerably more than Ruff and Kohn obtained (2%), and as the method which we have followed is much more direct and simple than theirs, it is recommended for the preparation of this lactone.

The configuration of rhamnose has been established[3] to be that of a methylpentose,

$$\begin{array}{c} \text{OH OH H H} \\ \text{CH}_3\text{.C . C . C . C . CHO} \\ \text{H H OH OH} \end{array}$$

hence the configuration of methyltetronic lactone (considered to be a γ-lactone) is $\begin{array}{l} \text{CH}_3\text{.C . C . C . CO.} \\ \text{H H OH} \end{array}$ By passing dry ammonia gas into a solution of this lactone in ether we have prepared the crystalline amide of methyltetronic acid, the structure of which must be $\begin{array}{l} \text{CH}_3\text{.C . C . C . CONH}_2\text{.} \\ \text{H H OH} \end{array}$

Since the hydroxyl group is on the right of the asymmetric α-carbon atom

[1] *Ber.*, **35**, 2365 (1902).

[2] THIS JOURNAL, **39**, 1638 (1917).

[3] Fischer and Morrell, *Ber.*, **27**, 382 (1894); Fischer and Zach, *Ibid.*, **45**, 3761 (1912); Hudson, THIS JOURNAL, **31**, 345 (1910).