PRODUCTION OF ALCOHOL AND ACETALDEHYDE BY TOMATOES[1]

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Introduction

Ever since the time of PASTEUR it has been known that under some conditions seed plants produce small quantities of alcohol. LECHARTIER and BELLAMY (**10**) were the first to show that fruits, when deprived of air, produce alcohol as well as CO_2. These workers studied a number of varieties of fruits and always found some alcohol produced. They inclosed their fruits in tight containers for several months and in many cases the fruits were considerably disintegrated although they were sterile. From his extensive studies on the maturing of fleshy fruits, GERBER (**3**) came to the conclusion that in many fruits alcohol is formed during the process of ripening, together with volatile acids and esters. MÜLLER-THURGAU and OSTERWALDER (**13**) found alcohol in pears. THOMAS (**18**) found alcohol in apples. Although GERBER studied apples he does not mention having found alcohol in these fruits. ONSLOW and BARKER (**17**) found alcohol in oranges. The concentration was particularly high when the fruits had been stored in an atmosphere high in CO_2, but even in ordinary air alcohol was present to the extent of about 0.03 to 0.10 per cent.

It is thus evident that alcohol is produced in fruits when they are surrounded by air. Under anaerobic conditions alcohol has been found in many plant structures, and reference to much literature on the subject is to be found in the monographs (**8**) on plant respiration.

It has been shown by a number of investigators that acetaldehyde is formed in plants. MAZÉ (**11**) found acetaldehyde in unripe seeds of corn and pear. This acetaldehyde he believed prevented them from germinating. In 1912 KOSTYTSCHEW (**7**) demonstrated that acetaldehyde was produced in the alcoholic fermentation of yeast. His method of demonstrating acetaldehyde consisted in adding zinc chloride to the fermenting liquid, which brought about polymerization of the acetaldehyde, preventing it from being reduced to alcohol. KOSTYTSCHEW *et al.* (**9**) found acetaldehyde in poplar blossoms. Acetaldehyde has also been found in other plants: by MÜLLER-THURGAU and OSTERWALDER (**13**) in pears and apples; by NEUBERG and REINFURTH (**14**) in alcoholic fermentation; by NEUBERG and GOTTSCHALK (**15**) in crushed peas and bananas; by THOMAS (**18**) in apples; by BODNAR and coworkers (**1**) in whole seeds of peas; by KLEIN and PIRSCHLE (**6**) in flowers, leaves, seedlings, and seeds of a number of different species of plants; by HARLEY and FISHER (**5**) in ripe pears.

[1] Paper from the Department of Botany of the University of Michigan, no. 400.

That acetaldehyde is an intermediate compound in anaerobic respiration seems to have been first shown by KOSTYTSCHEW (7), although NEUBERG is given all of the credit. He certainly demonstrated beyond a doubt that acetaldehyde is one of the intermediate products formed during alcoholic fermentation. NEUBERG and REINFURTH by their "Abfangsmethode" have been able to show that acetaldehyde is produced in large quantities during alcoholic fermentation. Their first method consisted of adding sodium bisulphite to the fermenting liquid; the bisulphite united with the acetaldehyde and thus prevented it from being reduced to alcohol. In 1920 they published a second method of binding the acetaldehyde. In this method one molecule of acetaldehyde united with two molecules of dimethylhydroresorcin (dimedon). After the fermentation had been completed the acetaldehyde was quantitatively determined.

Investigation

The work reported in this paper was undertaken as a further investigation of respiration in tomato fruits. The fruits used were picked from the vines as needed, thus insuring freshness of the material.

Both alcohol and acetaldehyde were determined in the fruits and leaves as they came from the plants and also after the fruits had been respiring anaerobically for various lengths of time. Nitrogen was used in most of the experiments to replace air, although a few experiments were conducted in which CO_2 replaced air. In four experiments the fruits were placed in a large container, which was then closed, and as respiration proceeded the oxygen was used up and replaced with CO_2.

When either CO_2 or nitrogen was used to replace the air, these gases were passed through wash bottles containing chromous chloride, to remove all oxygen. The gas was then passed through the chamber containing the fruits. In the experiments on alcohol production fruits were in a nitrogen atmosphere for 24, 48, and 72 hours. For these experiments a quantity of fruits was collected and divided into four groups, each group having fruits of the same ripeness and size. Only uninjured fruits were used. One lot of fruits was analyzed at once, and the others were placed in separate containers through which nitrogen was passed. One lot was analyzed each day. In this way it was possible to study the progressive accumulation of alcohol in similar fruits.

Several methods of determining alcohol were tried. In the beginning a method of steam distillation was used. A weighed quantity was taken and the fruits cut into small pieces, placed in a 5-liter round-bottom flask and steam-distilled for 5 to 6 hours, or until about 600 cc. of distillate had been collected. The distillate was collected in two receiver flasks, each containing 50 cc. distilled water at the beginning, and surrounded by ice. The total distillate was made up to 760 cc. each time by addition of distilled water, and 500 cc. of this were used for the alcohol determination and 260 cc. for the acetaldehyde determination.

Acetaldehyde was determined according to the method of NEUBERG and GOTTSCHALK (**16**). For the acetaldehyde determination 260 cc. of the distillate were used. To this 40 cc. of hydroxylamine sulphate (1.0 per cent.) were added and the mixture permitted to stand for an hour. The H_2SO_4 liberated was titrated with 0.1 N NaOH, using methyl orange as indicator. To insure that the same end point was always reached, two buffer flasks with pH 3.6 and 5.8 were used, each having the same amount of liquid as the titration flask. A correction was made for the neutralizing effect of distilled water. From several titrations of laboratory distilled water it was found that the addition of 100 cc. of distilled water was equivalent to 0.2 cc. 0.1 N NaOH. NEUBERG and GOTTSCHALK give the conversion factor as 1 cc. 0.1 N NaOH equivalent to 0.0044 gm. acetaldehyde.

The alcohol and acetaldehyde were oxidized to acetic acid with potassium dichromate and sulphuric acid. This was then distilled from a claison flask and the acetic acid titrated with 0.1 N NaOH, using phenolphthalein as indicator. By calculation the total alcohol in the 760 cc. of distillate was found. The acetic acid formed from the aldehyde was of course subtracted from the total in making this calculation. This method of determining alcohol gave very high values, and as other methods gave only about half as high a percentage these figures are not presented. The high concentration of alcohol is probably due to the long distillation at steam temperature. This probably distilled over some other substances, such as esters, in addition to alcohol and acetaldehyde, which later reacted with the NaOH in the titration.

If this method of the distillation vitiated the alcohol determination it did the same to the acetaldehyde analysis. The acetaldehyde analyses are not presented because of their lack of reliability. All one can say is that according to the determinations following the NEUBERG-GOTTSCHALK method, acetaldehyde was always found to be present. How much of this apparent acetaldehyde was due to acids that may have distilled over is not known. There was very little variation in the acetaldehyde noted, and there was no accumulation as the time of anaerobiosis was continued. The second method of alcohol determination did not lend itself readily to acetaldehyde analysis and it was therefore not repeated.

The alcohol was finally determined according to the method used by CANNAN and SULZER (**2**) in determining alcohol in blood. The apparatus used was modified to utilize more material than these investigators used. In these experiments a known amount (about 300 gm.) of freshly ground tomato pulp was placed in a 2-liter round-bottom flask, and between 500 and 600 gm. of anhydrous Na_2SO_4 added to prevent frothing. Distillation was carried out at a reduced pressure, which was around 6 cm. of mercury for all experiments, into two tubes 2 × 25 cm., each containing 35 cc. of concentrated H_2SO_4. At the pressure of 6 cm. mercury bubbling or boiling was produced at 40° C. In no experiment did the temperature rise as high as 45° C. After several preliminary experiments the time of distillation was placed at three hours. The H_2SO_4 from both receiving tubes was brought together and the volume made up to 100 cc. A 20-cc. portion of this H_2SO_4 was added drop by drop to a known quantity of 0.2 N $K_2Cr_2O_7$, which quantity varied with the experiment. The dichromate flask was kept cool by immersion in running water while the sulphuric acid was added. The oxidizing mixture of sulphuric acid and potassium dichromate was allowed to stand for one hour to complete the oxidation of the alcohol to acetic acid. This solution was then diluted until the sulphuric acid was 5 per cent., and an excess of 10 per cent. KI added (about 10 cc.). The iodine liberated by the excess dichromate was titrated with 0.1 N sodium thiosulphate, using starch as the indicator. In this determination 1 cc. 0.1 N $K_2Cr_2O_7$ is equivalent to 1.15 mg. of alcohol.

With the CANNAN-SULZER method much more complete and better organized experiments were conducted than with the first mentioned method. For this reason, and also because it is a more reliable method, the results obtained with it are presented in table I.

Table I shows that there is considerable alcohol in the fruits that have been in air; that this is at its maximum in the nearly ripe orange-red fruits; and that in the green fruits the amount of alcohol decreases as the fruit size is decreased, even though the small young fruits respire very much more than the larger fruits. During these investigations an experiment was conducted having as its purpose to study the relation between size and alcohol production. For this experiment orange colored tomato fruits were chosen, because among the ripening fruits they respire rapidly and for the experiment it was necessary to have fruits of the same physiological age. In one lot the fruits were 10.0 cm. in diameter and in the other lot only 3.5 cm. in diameter. Analysis was made directly after the fruits were removed from the vines. The large fruits had an alcohol content of 0.012 per cent. while in the smaller fruits only 0.0081 per cent. alcohol was found. From this experiment as well as from the experiments noted in table I it seems evident that when respiration is rapid the alcohol formation (*i.e.*, the anaerobic respiration) is greater in the large fruits than in the smaller ones.

Table I also shows that alcohol, unlike acetaldehyde, accumulates as the time of anaerobiosis lengthens; and that the alcohol increase is greatest in those fruits carrying on the most rapid respiration, namely, the small green fruits, in which the initial alcohol content was by far the smallest. There

TABLE I

PERCENTAGE OF ALCOHOL IN TOMATO FRUITS

CONDITION OF FRUITS	DIRECTLY FROM PLANT	NUMBER OF HOURS IN NITROGEN		
		24	48	72
	%	%	%	%
Red-ripe	0.0125	0.04	0.065	0.071
	0.0140	0.031	0.057	
	(1)*0.0144	(1)0.031	(1)0.054	(1)0.070
			0.054	
Orange-red	0.023	(2)0.0255	(2)0.0555	(2)0.0315
	(3)0.025	(3)0.028	(3)0.061	(3)0.046
Orange	(4)0.016	(4)0.037	(4)0.040	(4)0.062
Pink-orange	....	....	0.041	
			0.035	
Pink	0.012	(5)0.016	(5)0.034	
	(6)0.016	(6)0.017	(6)0.096†	(6)0.042
Green Large	0.016	(7)0.021	(7)0.047	(7)0.094
	0.014			
Medium	(8)0.012	(8)0.039	(8)0.077	(8)0.133
Small	(9)0.008	(9)0.066	(9)0.116	(9)0.150
2 cm. diameter	(10)0.0015	(10)0.003	(10)0.095	(10)0.185
1–2 cm. "	0.0011	(11)0.104	(11)0.135	(11)0.205

* Similar numbers in the brackets before the figures indicate that these fruits were picked at the same time and were all alike, but have been in nitrogen different lengths of time.

† Fruit very soft when taken out of nitrogen.

is one exception to the preceding statement. In the orange-red fruits there is a decrease in amount of alcohol from 48 hours to 72 hours. There is no apparent reason for this decrease.

TABLE II

PERCENTAGE OF ALCOHOL IN OTHER PLANT STRUCTURES

MATERIAL	IN AIR	IN NITROGEN 48 HOURS
Potato Old	0.0013	0.019
New	0.0012	....
Peas germinated 48 hours	0.037	0.244
Tomato leaves	0.0031	....

It should be pointed out that although table I is headed alcohol yet it actually applies to alcohol plus acetaldehyde. Both are oxidized by the sulphuric acid and the potassium dichromate. The amount of acetaldehyde, however, is a very small part of the total volatile material as given in table I.

Table II presents a miscellaneous collection and is presented only for the sake of comparison. It is usually stated in works on respiration (**8**) that potatoes do not produce any alcohol, and that germinating peas produce large quantities of it. From table II one must conclude that some alcohol is produced in potatoes although in small quantity, and that this quantity increases only very slightly when the tubers are completely deprived of oxygen for 48 hours. Peas germinating in air produce somewhat more alcohol than orange-red tomatoes, and the amount of alcohol increases very much when the peas are deprived of oxygen for a period of 48 hours. The alcohol content in tomato leaves directly from the vines is intermediate between that from tomato fruits with a diameter of 2 cm. and that from fruits with a diameter of 4 to 5 cm.

Determination of acetaldehyde depends upon titration with a base, and it is obvious that any acid which had distilled over would increase the amount of hydroxide needed to bring about neutralization and this would increase the apparent amount of acetaldehyde. Also other alcohols than ethyl alcohol would be oxidized by the H_2SO_4 and $K_2Cr_2O_7$ and the apparent amount of ethyl alcohol would thereby be increased. Therefore it was deemed appropriate to make qualitative tests for acids, other alcohols, and aldehydes to discover whether the figures just cited could be considered to represent the ethyl alcohol as stated in the tables.

Not only acids and alcohols were tested for but also other compounds with a boiling point near 100° C. and known to be associated with anaerobic respiration. These compounds were: formic and acetic acids, formaldehyde, acetaldehyde and higher aldehydes, methyl, ethyl, n. propyl, isopropyl, isoamyl and isobutyl alcohols, acetone, acrolein, and methyl glyoxal. Of these acetaldehyde, ethyl alcohol, and a trace of acids were always found. In several experiments there was a trace of methyl alcohol. In one experiment each formic and acetic acids and formaldehyde were found as a trace. The other compounds were always absent. The experimental material consisted of fresh leaves, large green fruits, also small green, large green, faintly pink or yellow, and red ripe fruits, which had been respiring anaerobically from 48 to 96 hours in nitrogen. This material includes all that was used in the quantitative analyses.

From the results of these qualitative analyses it seems fairly certain that the only aldehyde found extensively is acetaldehyde, and only ethyl alcohol is formed; and it can then be concluded that in the respiration of tomatoes (both when in air and when deprived of oxygen) acetaldehyde and alcohol are formed. Since acetaldehyde does not accumulate as the time of anaerobiosis lengthens, it is evident that it is not a final product of anaerobic respiration. On the other hand alcohol does accumulate and is therefore a final product of anaerobiosis.

The experiments bring out the fact that in the larger fruits there is a certain amount of anaerobic respiration even when the fruits are surrounded by air. In a previous paper the writer (**4**) pointed out that, from the behavior of the fruits when deprived of oxygen, it seemed very likely that there was some anaerobic respiration in tomato fruits even when in air, and these experiments prove that this assumption was correct. The experiments further show that the anaerobic respiration when the fruits are in air is much greater in large than in small fruits of the same physiological age. At least the alcoholic formation is greater in the large fruits than in the small ones, when both fruits are orange in color.

As yet no analyses have been made of the gas from the interior of tomato fruits, but the CO_2 content is probably high and the oxygen low. This is to be expected from other work, as that of MAGNESS (**12**) on apples and potatoes and some unpublished analyses of the gas from cacti by the writer. According to KOSTYTSCHEW and others, the preliminary steps in all types of respiration are the same. If that is true there is no reason why the aerobic and anaerobic respirations should not take place at the same time in a fruit, when the oxygen concentration becomes low. Some molecules of the intermediate compounds may follow the aerobic respiration path leading to the CO_2 and H_2O formation, while others may be changed to CO_2 and C_2H_5OH. The ratio between the two types of respiration would vary with the amount of oxygen present. As the oxygen became depleted the anaerobic type would increase, until finally all of the respiration was of that kind.

In his work on apples, THOMAS (**18**) distinguishes two types of respiration in which alcohol is formed. In the absence of oxygen there is little acetaldehyde formed with the alcohol. This type he calls anaerobic zymasis. The other type which he calls carbon dioxide-zymasis takes place in a high concentration of CO_2 in the presence of oxygen. Under this condition a high percentage of acetaldehyde is formed together with the alcohol. In the experiments on tomatoes there is no such distinction. THOMAS reports that in carbon dioxide-zymasis the acetaldehyde alcohol ratio was near one to two. There is no essential difference in this ratio in the absence of oxygen and in its presence, in the work of the tomato fruits. The writer's experiments on carbon dioxide-zymasis were not so extensive as those of THOMAS, but nevertheless it seems that there is a decided difference in the behavior of apples and tomatoes.

Summary

1. Acetaldehyde has been found in all tomato fruits, under all conditions of treatment. The amount of this aldehyde does not increase as the time of anaerobiosis lengthens.

2. Ethyl alcohol has also been found in all tomato fruits, under all conditions of treatment. The amount of alcohol increases with the length of anaerobiosis.

3. Fruits as taken off the vines, which had been in the greenhouse under natural conditions, contained some acetaldehyde and alcohol, although not so much as in fruits deprived of oxygen.

4. Large fruits contained more alcohol than small fruits when analyzed directly from the vines.

5. In qualitative experiments, compounds known to be associated with anaerobic respiration and having a boiling point near 100° C. were tested for. Acetaldehyde and ethyl alcohol were always found. Traces of methyl alcohol were found in several experiments, and traces of formic and acetic acids and formaldehyde were each found in only one experiment.

Acknowledgment is made of a grant from the Faculty Research Fund of the University of Michigan for most of the analytical work, which was done by Miss EDNA WARWEG and Mr. SAM GRANICK.

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LITERATURE CITED

1. BODNAR, J., SZEPESSY, CHARLOTTE, and FERENCZY, JOHANN. Die Anwendung der Neubergschen Acetaldehyd-Abfangmethode bei der alkoholischen Gärung höherer Pflanzen. Biochem. Zeitschr. **165**: 16–22. 1925.
2. CANNAN, R. K., and SULZER, R. The estimation of alcohol in blood. Heart **11**: 148–150. 1924.
3. GERBER, M. C. Recherches sur la maturation des fruits charnus. Ann. Sci. Nat. Bot. VIII. **4**: 1–279. 1896.
4. GUSTAFSON, F. G. Intramolecular respiration of tomato fruits. Amer. Jour. Bot. **17**: 1011–1027. 1930.
5. HARLEY, C. P., and FISHER, D. F. The occurrence of acetaldehyde in Bartlett pears and its relation to pear scald and breakdown. Jour. Agr. Res. **35**: 983–993. 1927.
6. KLEIN, G., and PIRSCHLE, K. Acetaldehyd als Zwischenprodukt der Pflanzenatmung. Biochem. Zeitschr. **168**: 340–360. 1926.
7. KOSTYTSCHEW, S. Über Alkoholgärung. I. Über die Bildung von

Acetaldehyd bei der alkoholischen Zuckergärung. Zeitschr. physiol. Chem. **79**: 130–145. 1912.

8. ————. Plant respiration. Transl. by C. J. LYONS. P. Blakiston's Son and Co., Philadelphia. 1927.
9. ————, HÜBBENET, E., and SCHELOUMOFF, A. Über die Bildung von Acetaldehyd bei der anaeroben Atmung der Pappelblüten. Zeitschr. physiol. Chem. **83**: 105–111. 1913.
10. LECHARTIER, G., and BELLAMY, F. De la fermentation des fruits. Compt. rend. Acad. Sci. (Paris). **69**: 466–469, 1869; **79**: 1006–1009; 1874.
11. MAZÉ, P. Maturation provoquee des graines. Action antigerminative de l'aldéhyde éthylique. Compt. Rend. Acad. Sci. (Paris). **151**: 1383–1386. 1910.
12. MAGNESS, J. R. Composition of gases in intercellular spaces of apples and potatoes. Bot. Gaz. **70**: 308–316. 1920.
13. MÜLLER-THURGAU, H., and OSTERWALDER, A. Acetaldehydbildung in Obstfrüchten. Landwirt. Jahrb. Schweiz. **29**: 397. 1915.
14. NEUBERG, C., and REINFURTH, E. Die Festlegung der Aldehydstufe bei der alkoholischen Gärung. Biochem. Zeitschr. **89**: 365–414. 1918.
15. ————, and GOTTSCHALK, A. Beobachtungen über den Verlauf der anaeroben Pflanzenatmung. Biochem. Zeitschr. **151**: 167–176. 1924.
16. ————, and ————. Quantitative Untersuchungen über die Bildung und Herkunft des Acetaldehyds im intermediären Zellstoffwechsel der Warmblüter. Biochem. Zeitschr. **146**: 164–184. 1924.
17. ONSLOW, M., and BARKER, J. The alcohol content of oranges and its diagnostic value. Report Food Investigation Board. 1927. p. 35.
18. THOMAS, M. A quantitative study of the production of ethyl alcohol and acetaldehyde by cells of the higher plants in relation to concentration of oxygen and carbon dioxide. Biochem. Jour. **19**: 927–947. 1925.