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Improved Method for Making Cider Vinegar

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SUMMARY

It has been demonstrated by the fermentation of a considerable number of samples of cider from different varieties of apples that it is possible under the conditions described to make good cider vinegar of marketable strength and uniform quality in six months or less.

The best results were secured at a temperature of 65° to 75° F. where in many cases vinegar of marketable strength was obtained in less than four months. The temperature should never be allowed to go more than a few degrees above 75° F., because of the danger of losing alcohol by evaporation.

Inoculation with pure cultures of yeast, Saccharomyces ellipsoideus, stimulated the alcoholic fermentation appreciably and decreased the chances of losing sugars through unfavorable fermentation by checking the development of the undesirable bacteria. Thus a medium was developed which was especially favorable to a good and vigorous acetic acid fermentation.

Under ordinary conditions of temperature and aeration the acetic acid fermentation did not progress to any appreciable extent until the alcoholic fermentation was practically completed. Therefore, it is better not to initiate the acetic acid fermentation by inoculation with pure cultures of vinegar bacteria, Acetobacter aceti, until active alcoholic fermentation has ceased. This is indicated by the absence of foam on the surface of the cider.

Good vinegar was obtained in one year or less when ciders were kept in an unheated cellar where the temperature of 45° to 55° F. prevailed. In this case inoculation with pure cultures of both yeast and vinegar bacteria proved very beneficial and on this basis inoculation is recommended, provided nothing but pure cultures of the proper organisms are used.

Satisfactory results were obtained by not removing the sediments after the alcoholic fermentation was completed and by allowing the fermentation to proceed undisturbed throughout the process. This suggests leaving the cider in the original containers until the process of acetic acid fermentation is complete. Then the clear vinegar must immediately be put into clean containers and sealed to exclude the air to prevent the weakening of the vinegar by destructive fermentation of the acetic acid.

Barrels and all utensils to be used for vinegar making should be thoroughly scalded with steam or boiling water and then be rinsed with plenty of clean water. The apples should be sorted, have their wormy and decayed spots removed, and be washed in clean water before pressing the cider.

*When cider vinegar is made for the purpose of selling, a permit must be obtained in compliance with the requirements of the 18th amendment. According to the Washington State law cider vinegar offered for sale must contain not less than 4 per cent of acetic acid. The apple solids contained in 100 cubic centimeters must not be less than 1.6 grams of which not more than 50 per cent are reducing sugars and not less than 0.25 grams are apple ash. The water soluble ash from 100 cubic centimeters of vinegar must contain not less than 10 milligrams of phosphoric acid (P_2O_5) and require not less than 30 cubic centimeters of decinormal acid to neutralize its alkalinity.

NOTE—Pure cultures of vinegar yeast and vinegar bacteria, together with directions for use, can be secured from the Division of Bacteriology of the Washington Experiment Station, State College, Pullman, at a cost of 50 cents for sufficient material to inoculate a 50 gallon barrel of apple cider.

IMPROVED METHOD FOR MAKING CIDER VINEGAR

by
S. C. Vandecaveye

Introduction

Many people who have cull apples are reluctant to use them for vinegar making, because of the uncertainty of success. If cider vinegar of a standard strength and superior quality could be produced on the farm with a reasonable degree of success and comparatively little labor and care, a much larger part of the cull apples now wasted by spoiling would undoubtedly be converted into vinegar. A ton of apples will yield from 100 to 150 gallons of juice suitable for vinegar, which at 20 cents per gallon is worth from $17.00 to $27.00. Good old-fashioned cider vinegar is a valuable by-product for which there is still a market in spite of the fact that thousands of gallons of vinegar are produced in a purely chemical way by converting alcohol into acetic acid. The genuine article will never be entirely replaced by the artificial product, because of the superior flavor and the many desirable ingredients other than acetic acid invariably present in good apple cider vinegar, and never to be found in synthetic vinegar. The farm production of good cider vinegar has every reason to be encouraged, as its superior flavor and quality make it more suitable for pickling and table use than the synthetic product. Moreover, it is a valuable source of revenue as a by-product from the apple orchard, and a more efficient production of home made cider vinegar from cull apples would result in a large saving for the fruit growers.

In many cases home made vinegar never reaches the legal standard strength of 4 per cent acetic acid. This is generally due to unfavorable fermentation, caused by contaminating organisms. The sugars of the apple juice are completely destroyed by these

organisms instead of being changed into acetic acid as desired. In exceptional cases the low acidity is due to a too low sugar content of the apple juice or to dilution of the cider with water. Whatever the causes of this inferior product may be, the fact remains that the strength and quality of home made cider vinegar are often so unreliable that the merchants cannot afford to handle it. Yet, when one understands the principles involved it is just as easy to make high grade cider vinegar at home as it is to make good butter or good bread.

RESULTS OF PREVIOUS INVESTIGATORS OF VINEGAR MAKING

The process of vinegar making on the farm has been studied by various investigators under a variety of conditions. In 1904 Van Slyke (6)* reported an extensive study of home made vinegar in which it took from three to six months to complete the alcoholic fermentation when the cider was kept at a temperature of 45° to 55° F. and about three months when at a temperature of 70° to 85° F. He found that the time to complete the fermentation could readily be reduced one half or more by the addition of yeast, especially when the cider was kept at the higher temperature. The same view was held by Sackett (5). Lamb and Wilson (3) followed the fermentation of cider vinegar under various conditions and observed that the alcoholic fermentation of cider kept at about 50° F. was nearly completed in one month or less and progressed more satisfactorily without the addition of yeast.

The acetic acid fermentation in Van Slyke's experiments, when the cider was kept at a temperature of 45° to 55° F., took place very slowly during the first three months after the alcohol had reached its maximum, but from that time on it progressed much more rapidly and was practically completed at the end of 24 months. At the higher temperature of 50° to 90° F. the acetic acid fermentation was somewhat more rapid, but the results were not uniform. This is in contrast to the results of Lamb and Wilson (3) who stated that at a temperature of about 50° F. it is possible to make good cider vinegar in twelve months and that in many cases the

* The numbers refer to literature cited.

vinegar reaches a marketable strength in less than six months. The effect of inoculations with vinegar or cultures of vinegar bacteria was claimed to be helpful by Van Slyke (6) and by Sackett (5), but was declared to be of little value by Lamb and Wilson (3) and also by Brierley (1).

The temperature at which the fermentation takes place is often considered an important factor in cider vinegar making. Van Slyke reported the temperature of 65° to 70° F. to be the most favorable for both the alcoholic and acetic acid fermentations. The results obtained by Lamb and Wilson (3) showed no significant difference due to temperatures. Ciders kept at 50° F. apparently fermented as satisfactorily as those kept at 75° F. They obtained the best results with clean vessels, the natural flora of the apples and undisturbed fermentation. Brierley (1) is of the opinion that the fermentation ratio is due to a certain extent to the varieties of apples from which the cider has been pressed. According to his results some varieties are inferior for vinegar purposes and temperature or inoculation with yeast or vinegar bacteria have but little effect.

Another factor which has been discussed considerably in cider vinegar production is the proper time of starting the acetic acid fermentation. Van Slyke (6) found that the acetic acid fermentation was practically inactive as long as the alcoholic fermentation was in progress. Sackett (5) Cruess (2), and LeFevre (4), stated that the alcoholic fermentation must be completed before the acetic acid fermentation is allowed to start, because the yeast fermentation is stopped by the acetic acid. LeFevre (4) found that .5% acetic acid interferes seriously with the growth of yeast and 1% is almost prohibitive. Lamb and Wilson (3) on the other hand, reported that the acetic acid fermentation begins soon after the juice is pressed from the apples and that the alcoholic and acetic acid fermentations are progressing simultaneously for a time. A significant point in their work is that they demonstrated that apples frosted or frozen on the trees yielded a cider which fermented very quickly and satisfactorily when the apples were pressed soon after freezing, without being allowed to spoil.

This brief review is sufficient to show that the results obtained by investigators in the past are not at all uniform and are some-

times contradictory. Probably the chief cause of these varied results is the difficulty of controlling the fermentation processes. Cider vinegar is a product of combined biological and chemical action, largely initiated and controlled by biological processes. In vinegar factories where large quantities of cider are handled and where the so-called "quick vinegar process", by which vinegar can be made in a few days, is used, these processes can readily be controlled. But the methods used there involve too much labor and expense to be practical on the farm. For this reason the experiments to be discussed have been confined to the old process which requires several months' time, but comparatively little labor and equipment, and is, therefore, suitable for small quantities under farm conditions.

Experiments in Fermentation Processes of Ciders

The experiments reported here were started in the fall of 1924 and were conducted for the purpose of following closely under various temperatures and treatments the fermentation processes of ciders from three of the leading varieties of Washington apples. The objects were to make an attempt to discover the reasons for the frequent failures in securing the proper vinegar fermentation under average farm conditions and to arrive, if possible, at some practical method by which a better and more uniform cider vinegar can be made on the farm. These experiments were planned in two series. One included 500 cc quantities of cider placed in bottles in triplicate and the other four gallon quantities of cider in 5 gallon kegs in duplicate. The ciders for both series were pressed from Jonathan, Wagener, and Rome Beauty apples, and from mixtures of equal parts of these three varieties. To prepare the ciders from each variety the apples were washed and sorted for rotten spots. For the mixed cider one lot was prepared from washed and sorted apples and a second lot was pressed from apples that were not washed or sorted. The total invert sugars of these various lots of fresh cider were determined gravimetrically by the official method. The total acids were determined by titration and calculated according to the official method for acetic acid in vinegar. The results of both analyses are given in Table 1 as averages of duplicate determinations.

Table 1. Total Sugars by Gravimetric Method, and Total Acidity by Titration of Fresh Ciders Pressed November 17, 1924.

Variety of Apples	Percent Total Invert Sugar Average from Duplicate Determinations	Percent Total Acidity by Titration
Jonathan	9.90	0.78
Wagener	8.83	0.62
Rome Beauty	8.64	0.44
Mixed, washed	9.60	0.62
Mixed, not washed	9.57	0.62

Fermentation of Ciders in Bottles

The 500 c.c. portions of cider placed in one-half gallon bottles and kept at a temperature of 65° to 75° F. were intended for determinations of CO_2 and total acid. They were arranged in triplicate portions, of which one was not treated, thus allowing natural fermentation to take place. A second portion was inoculated with a pure yeast culture, Saccharomyces ellipsoideus, in the proportion recommended to the vinegar makers, and a third was treated with double the recommended amount of inoculent. When active fermentation ceased, which in this case was at the end of four weeks, the second bottle of each triplicate was inoculated with a pure culture of vinegar bacteria, Acetobacter aceti, in the proportion recommended to the vinegar makers. The arrangement of the ciders was as follows:

Nos. 1, 2, 3,. Jonathans, washed and sorted for wormy and rotten spots.

Nos. 4, 5, 6. Wagener, washed and sorted for wormy and rotten spots.

Nos. 7, 8, 9. Rome Beauty, washed and sorted for wormy and rotten spots.

Nos. 10, 11, 12. Above varieties mixed, washed and sorted for wormy and rotten spots.

Nos. 13, 14, 15, 16.* Above varieties mixed, not washed and sorted for wormy and rotten spots.

* No. 16 was inoculated with an S. ellipsoideus culture three times as large as the recommended portion.

Table 2. Mgms. of CO_2 Given Off at Each Titration During Alcoholic and Acid Fermentations of Ciders Kept at 65° to 75° F.

Kind of Cider		No.	1st Week Nov. 22/24	2nd Week Nov. 29/24	3rd Week Dec. 6/24	6th Week Dec. 27/24	33rd Week July 8/25	50th Week Oct. 27/25
Jonathan	No inoculation	1	6280	10639	4832	2726	1320	822
	Inoculated	2	7150	11109	4762	1331	3850	255
	Double inoculation	3	7283	11046	5632	1503	4364	902
Wagener	No inoculation	4	6864	9452	4286	1297	5368	1249
	Inoculated	5	7223	10310	3114	1227	7809	1082
	Double inoculation	6	7211	9687	2746	1870	6160	1333
Rome Beauty	No inoculation	7	6094	10445	3976	803	6564	1438
	Inoculated	8	6742	12305	2883	924	5667	1271
	Double inoculation	9	7069	12762	2204	730	6283	1359
Mixed W*	No inoculation	10	7362	7128	5192	2979	5526	1289
	Inoculated	11	7586	7883	4770	2842	6089	1104
	Double inoculation	12	7647	7155	4766	2995	5649	902
Mixed N. W.**	No inoculation	13	5994	8274	3172	5089	4699	959
	Inoculated	14	6291	10107	2970	2152	5508	927
	Double inoculation	15	5382	10304	2367	5002	4505	1016
	Triple inoculation	16	6778	11592	2000	2988	4153	967

* W.—Washed and sorted.
** N. W.—Not washed and not sorted.

The CO_2 evolved was conducted by constant air suction into towers containing N/1 KOH, and the amounts determined at various times by double titration using Thymol blue and Brom phenol blue as indicators. The total acids were also determined by titration at definite intervals of time, using phenolphtalein as indicator and calculating the acidity according to the official method for acetic acid in vinegar. The results of the CO_2 determinations are given in Table 2. These were used as a basis for calculating the amount of alcohol formed. Such a method is probably only approximate as cider contains organic matter other than sugars from which CO_2 may be given off by bacterial action. But the total evolution of CO_2 may be considered a fair index of the rate of alcoholic fermentation and thus can be depended upon to show comparative results. The calculations of the percentage of sugars oxidized and the amounts of alcohol formed on this basis appear in Table 3. In the last column of the table the amounts of acid that should be formed theoretically are given. The acidity that was actually obtained at each titration is reported in Table 4.

The results show that the alcoholic fermentation proceeded very rapidly and had reached a well advanced stage three weeks after the cider was pressed. The fermentation was completed in six weeks, the mixed ciders progressing a little slower than the ciders from the single varieties. Theoretically speaking, there was sufficient CO^2 evolved during that short period to ferment all the sugars into alcohol . The effect of inoculation with a pure culture of yeast was to increase the alcoholic fermentation during the first two or three weeks. The results obtained from the samples receiving double the recommended portion of culture, or more than double, did not seem to favor the use of more than the standard amount of inoculent.

The acetic acid fermentation also progressed rapidly. In many cases vinegar of marketable strength was obtained in six weeks after the cider was pressed. It is interesting to note that the acid fermentation was fairly well started three weeks after pressing the cider. This is in agreement with the results of Lamb and Wilson (3) showing that it is possible for the acetic acid fermentation to begin before the alcoholic fermentation is completed and that both may proceed simultaneously for a time.

Table 3. Calculated Percentage of Sugars Oxidized With Calculations of Theoretical Equivalents of Alcohol by Weight of the Variously Treated Ciders at Each CO_2 Determination.

Kind of Ciders	No.	% Sugars Oxidized 1 week	% C_2H_5OH by weight 1 week	% Sugars Oxidized 2 weeks	% C_2H_5OH by weight 2 weeks	% Sugars Oxidized 3 weeks	% C_2H_5OH by weight 3 weeks	% Sugars Oxidized 6 weeks	% C_2H_5OH by weight 6 weeks	% Equivalent CH_3OOH
Jonathan	1	25.9	1.30	69.8	3.25	89.9	4.52	101.0	5.09	6.61
Jonathan	2	29.1	1.49	75.0	3.80	95.1	4.86	100.6	5.07	6.59
Jonathan	3	30.1	1.51	75.7	3.81	99.0	4.98	105.2	5.29	6.87
Wagener	4	31.8	1.42	75.6	3.39	95.4	4.28	101.4	4.56	5.92
Wagener	5	33.4	1.50	81.1	3.65	95.6	4.29	101.3	4.55	5.91
Wagener	6	33.4	1.50	78.3	3.51	91.0	4.09	99.7	4.47	5.81
Rome Beauty	7	28.8	1.26	78.2	3.44	97.1	4.27	100.9	4.43	5.75
Rome Beauty	8	31.9	1.40	90.1	3.96	103.8	4.52	108.2	4.76	6.18
Rome Beauty	9	33.4	1.47	93.8	4.12	104.3	4.58	107.8	4.73	6.14
Mixed, W.	10	31.3	1.53	61.7	3.01	83.8	4.09	96.5	4.71	6.12
Mixed, W.	11	31.9	1.58	62.0	3.07	83.2	4.07	95.3	4.66	6.05
Mixed, W.	12	32.1	1.59	62.8	3.08	83.3	4.07	96.0	4.69	6.09
Mixed, N. W.	13	25.8	1.24	61.4	2.97	75.0	3.62	96.7	4.68	6.08
Mixed, N. W.	14	27.0	1.31	70.4	3.41	83.3	4.02	100.4	4.48	5.82
Mixed, N. W.	15	23.1	1.12	67.4	3.26	77.6	3.76	99.1	4.79	6.22
Mixed, N. W.	16	29.1	1.41	78.9	3.81	87.6	4.24	100.4	4.86	6.31

Table 4. Observed Acidity at Various Times During the Process of Acetic Acid Fermentation and After the Completion of the Acetic Fermentation of Ciders kept at 65° to 75° F.*

Kind of Apples	No.	% CH_3CooH Fresh 11/17/24	% CH_3CooH After 3 Weeks	% CH_3CooH After 6 Weeks	% CH_3CooH After 9 Weeks	% CH_3CooH After 12 Weeks	% CH_3CooH After 18 Weeks	% CH_3CooH After 30 Weeks	% CH_3CooH After 50 Weeks
Jonathan	1	0.78	1.78	4.68	6.15	6.50	6.47	6.22	6.22
Jonathan	2	0.78	1.85	5.08	6.85	6.49	6.81	6.58	6.18
Jonathan	3	0.78	1.61	4.26	5.49	6.62	6.75	6.67	6.01
Wagener	4	0.62	1.91	4.03	5.32	6.84	6.80	6.53	6.53
Wagener	5	0.62	2.40	5.38	6.54	5.80	4.88	2.16	0.23
Wagener	6	0.62	2.47	4.86	6.50	6.46	5.85	4.88	3.72
Rome Beauty	7	0.44	1.86	4.51	5.98	5.98	5.26	4.23	3.30
Rome Beauty	8	0.44	1.79	4.57	6.22	5.86	4.59	1.67	0.10
Rome Beauty	9	0.44	1.54	4.63	5.94	5.36	4.71	4.26	2.89
Mixed, W.	10	0.62	1.78	5.26	6.35	6.40	6.30	5.82	5.32
Mixed, W.	11	0.62	1.72	4.95	5.91	5.50	5.19	2.91	0.65
Mixed, W.	12	0.62	2.05	5.77	6.78	6.73	6.69	6.60	6.49
Mixed, N. W.	13	0.62	2.18	4.66	6.56	7.26	7.21	7.10	6.87
Mixed, N. W.	14	0.62	1.01	2.50	4.13	7.01	6.43	5.13	2.39
Mixed, N. W.	15	0.62	0.92	2.01	3.60	6.41	6.72	7.69	6.27
Mixed, N. W.	16	0.62	1.81	3.40	4.99	7.18	7.42	7.33	7.08

* Samples were analyzed at intervals of three weeks, but the data included in this table are sufficient to show the general progress of the results.

In the majority of cases, the inoculation of bottles 2, 5, 8, 11, and 14 with pure cultures of Ace. aceti caused a slight increase in acetic acid fermentation during the first six weeks after inoculation. However, all the bottles, with the exception of Nos. 9 and 11, attained the theoretical maximum of acetic acid irrespective of inoculation. This is indicated in Table 4 by the actual results of titration. The data also show that the actual acidity obtained corresponds closely with the theoretical, the latter being taken on a basis of 100 parts of alcohol being equal to 130 parts of acetic acid. In some instances the theoretical amounts were slightly exceeded by the actual amounts. This is partly due to the limitations of the methods used and also to the possibility of starch hydrolysis by which additional acetic acid may be formed.

It frequently occurs that vinegar decreases in strength by standing or that a weak vinegar results following an apparently successful alcoholic fermentation. An experiment was carried on to determine the cause of this state of affairs. Ten weeks after the pressing of the apple juice, when most of the alcohol was fermented into acetic acid, 200 c.c. samples of the clear vinegar were taken from bottles Nos. 2, 5, 8, 11, and 14. These samples were placed in bottles of exactly 200 c.c. capacity, corked, and sealed with paraffin to exclude the air. The bottles were kept at the same temperature as those of the original experiment and left there for 40 weeks. At the end of this period the acidity was determined in the usual manner and compared with that in the original bottles. The results which are given in Table 5 show that some of the original samples decreased in strength and that this decrease must be due to aerobic organisms, since it could readily be checked by excluding the air from the vinegar. An attempt was made at isolating the bacteria responsible for this destructive action. A number of organisms were isolated from the various bottles affected. Several gram positive and gram negative forms were found. Each one was planted in sucrose and in lactose broth, but none produced gas from these sugars. They were then planted in sterile vinegar of various strengths. All of the gram positive organisms failed to grow in vinegar of 6% acetic acid. Some of the gram negative organisms developed and used up the acid. None of these formed a pellicle or film, but in morphology they resembled some of the Ace. aceti group. It is

possible that they belong to that group, but that they are the kind that continue the oxidation of acetic acid to CO^2 and water when there is no longer any alcohol to be oxidized. It was hoped that this reaction would be indicated by the amounts of CO_2 produced in the original bottles, after the alcoholic fermentation was completed. The data in Table 2 do not show any marked increase in CO_2 production as a result of large decreases in acetic acid. All the samples produced small amounts of CO_2 after completion of the alcoholic fermentation. This resulted very likely from the decomposition of the organic matter precipitated from the cider. Further work is in progress on this project.

Table 5. The Effect of Anaerobic Conditions on the Acid Contents of Vinegar Samples Kept at 65 to 75° F.

No. of Samples	% CH_3CooH After 10 Weeks	% CH_3CooH in Airtight Bottles After 50 Weeks	% CH_3CooH in Original Open Bottles After 50 Weeks
2	6.85	6.48	6.18
5	6.54	6.85	0.23
8	6.22	6.25	0.10
11	5.91	5.97	0.65
14	4.13	4.23	2.38

Fermentation of Ciders in Kegs

The four gallon portions of cider in the five gallon kegs were prepared in duplicates and kept at a temperature of 65° to 75° F. One of each duplicate portions received no inoculation and the other was inoculated with a standard quantity of yeast, S. ellipsoideus, at the beginning and with a standard amount of vinegar culture, Ace. aceti, six weeks later when active alcoholic fermentation had ceased. The two mixed ciders were prepared in quadruplicate so that two kegs of each—one inoculated and the other not inoculated—could be kept in a cellar at a temperature of 45° to 55° F. The purpose of these experiments was to follow closely the processes of fermentation of these ciders at two different temperatures, one approximating the optimum for both the yeast and the acetic acid

Table 6. Per Cent of Alcohol by Volume and Total Invert Sugars at Different Times of Sampling of Various Ciders Kept at 65° to 75° F.

Kinds of Ciders	No.	% C_2H_5OH After 3 Weeks	% C_2H_5OH After 6 Weeks	% C_2H_5OH After 9 Weeks	% C_2H_5OH After 12 Weeks	% C_2H_5OH After 15 Weeks	% Total Invert Sugars After 6 Weeks
Jonathan	17	4.7	4.2	2.6	0.6	0.0	0.08
Jonathan, inoculated	18	4.8	6.1	5.3	1.7	0.0	0.08
Wagener	19	3.4	5.6	3.4	0.6	0.0	0.09
Wagener, inoculated	20	4.5	5.1	3.7	0.6	0.0	0.06
Rome Beauty	21	4.8	5.8	5.0	3.5	0.5	0.07
Rome Beauty, inoculated	22	5.0	5.8	4.7	2.3	0.0	0.08
Mixed, W.	23	4.9	5.9	3.8	0.6	0.0	0.08
Mixed, W., inoculated	24	5.6	6.0	5.2	0.7	0.0	0.08
Mixed, N. W.	25	4.4	5.9	5.4	2.6	0.7	0.06
Mixed, N. W., inoculated	26	5.1	6.0	5.3	3.3	0.6	0.09

bacteria and the other being that which prevails in unheated farm cellars. The kegs were placed on their sides and were not filled to capacity, so that plenty of air was admitted through the bunghole which was simply covered with cheese cloth to keep out dust and insects. The arrangement was such that samples could be taken with the least amount of disturbance to the fermenting material and the surface films. In this series the alcohol was determined by distillation and the acetic acid in the same way as in the first series.

The Behavior of Ciders at High Temperatures

This experiment consisted of ciders of the single varieties and mixed varieties of apples. The ciders were kept at a temperature of 65° to 75° F. Alcohol determinations were made every three weeks until the fermentations were completed and acetic acid determinations were made at regular intervals until the end of the experiment which lasted 47 weeks. Six weeks after the cider was pressed duplicate samples taken from all the kegs were analyzed for sugars. The results of the analyses together with the results of the alcohol determinations are given in table 6. Due to the possibility of the interference of the volatile reducing substance, acetyl methyl carbinol, which may be formed after several weeks of fermentation, some of the later data may not be absolutely accurate, but since they are to serve only for comparative purposes they are considered satisfactory.

The data in this experiment show that more than 75% of the sugars were fermented in three weeks and that the alcoholic fermentation was entirely completed in five to six weeks after the cider was pressed. This was shown by the small traces of sugar which were secured in the analysis at that time. The effect of inoculation with yeast was once more to speed up the alcoholic fermentation during the first three weeks. However, all the samples, irrespective of inoculation, produced approximately the theoretical maximum of alcohol. The data also show that the alcohol was practically all converted into acetic acid at the end of 15 weeks at which time vinegar of marketable strength was obtained in every sample. The progress of the acetic acid fermentation is recorded in Table 7. It is shown that in this experiment practically no acid was formed

Table 7. Observed Per Cent of Acetic Acid at Different Times of Sampling of Various Ciders Kept at Temperatures of 65° to 75° F.*

Kind of Ciders	No.	% CH_3CooH Fresh 11/17/2	% CH_3CooH After 3 Weeks	% CH_3CooH After 6 Weeks	% CH_3CooH After 9 Weeks	% CH_3CooH After 12 Weeks	% CH_3CooH After 15 Weeks	% CH_3CooH After 18 Weeks	% CH_3CooH After 30 Weeks	% CH_3CooH After 47 Weeks
Jonathan,	17	0.78	0.81	2.60	4.63	6.54	7.80	7.81	8.18	8.54
Jonathan, inoculated	18	0.78	0.40	0.85	1.06	3.97	6.29	6.75	6.76	7.03
Wagener	19	0.62	0.61	0.45	1.67	4.89	5.11	4.84	4.28	4.17
Wagener, inoculated	20	0.62	0.46	0.38	1.08	3.96	5.87	5.41	4.77	4.65
Rome Beauty	21	0.44	0.32	0.27	0.30	0.93	4.40	4.79	2.88	1.86
Rome Beauty, inoculated	22	0.44	0.61	0.79	1.06	3.24	6.00	6.28	6.19	6.55
Mixed, W.	23	0.62	0.69	1.06	0.99	4.95	6.85	6.89	6.72	6.99
Mixed, W., inoculated	24	0.62	0.66	0.86	0.96	2.54	6.23	6.95	6.46	6.26
Mixed, N. W.	25	0.62	0.42	0.71	2.38	2.46	4.74	6.55	6.12	6.27
Mixed, N. W., inoculated	26	0.62	0.67	0.90	1.18	3.18	5.28	6.30	6.44	6.72

* Samples were analyzed at intervals of three weeks, but the data included in this table are sufficient to show the general progress of the results.

until the alcoholic fermentation was completed. This is contrary to what was observed in the the first series and the possible reason for this contrast is given in the discussion of the results. Inoculation with pure cultures of Ace. aceti did not have any appreciable effect on the acetic acid fermentation. The theoretical maximum amounts of acetic acid were secured in all cases except Nos. 19 and 21. These samples together with No. 20 were subject to a gradual decrease of acetic acid due to destructive fermentation. The same thing was observed in some of the samples in the first series. The majority of the samples so affected in both series were ciders from Wagener and Rome Beauty apples. Thus, these results support the contention of Brierley (1) that certain varieties of apples, although they contain sufficient sugar, are inferior for vinegar making.

Some of the samples apparently yielded more than the theoretical equivalent of acetic acid and increased in acidity as time progressed. This was probably due to evaporation and to some extent to starch hydrolysis from which additional acid was formed.

The Behavior of Ciders at Low Temperatures

Only the mixed ciders were used for this experiment and the temperature at which they were kept was 45° to 55° F. During the hottest part of the summer the temperature raised to 60° F. for a short time, but the prevailing temperature throughout the time of the experiment was below 50° F. The methods and procedure were similar to those used in the preceding experiment. One of each of the two fresh ciders was inoculated with a pure culture of yeast, and nine weeks later when active alcoholic fermentation ceased, with a pure culture of vinegar bacteria, Ace. aceti. Alcohol and acetic acid determinations were made at definite intervals, taking special precautions at sampling to leave the ciders as much as possible undisturbed. The results of the alcoholic determinations are reported in Table 8 and those of the acetic acid determinations in Table 9.

The data in Table 8 show that the alcoholic fermentation was progressing rapidly and was practically completed in nine weeks at this low temperature. This does not agree with the results of Van Slyke (6) who found at this temperature a uniform progression of alcoholic fermentation during several months. Although the

Table 8. Observed Percent of Alcohol at Different Times of Sampling of Mixed Ciders Kept at a Temperature of 45° to 55° F.

Kind of Cider	No.	% C_2H_5OH After 6 Weeks	% C_2H_5OH After 9 Weeks	% C_2H_5OH After 12 Weeks	% C_2H_5OH After 15 Weeks	% C_2H_5OH After 19 Weeks	% C_2H_5OH After 23 Weeks	% C_2H_5OH After 27 Weeks	% C_2H_5OH After 47 Weeks
Mixed, W.	27	4.7	6.2	6.2	5.7	5.4	4.8	4.2	2.9
Mixed, W., inoculated	28	5.1	6.1	5.7	5.4	4.7	3.8	2.8	0.0
Mixed, N. W.	29	3.8	6.1	6.2	5.7	5.7	5.8	5.1	0.0
Mixed, N. W., inoculated	30	6.0	6.1	6.2	5.7	5.1	4.5	4.1	0.0

Table 9. Observed Percent of Acetic Acid at Different Times of Sampling of Mixed Ciders Kept at a Temperature of 45° to 55° F.*

Kind of Cider	No.	% CH_3CooH Fresh 11/17/24	% CH_3CooH After 6 Weeks	% CH_3CooH After 12 Weeks	% CH_3CooH After 18 Weeks	% CH_3CooH After 27 Weeks	% CH_3CooH After 36 Weeks	% CH_3CooH After 48 Weeks	% CH_3CooH After 69 Weeks
Mixed, W.	27	0.62	0.53	0.53	0.58	0.88	1.06	2.95	4.61
Mixed, W., inoculated	28	0.62	0.48	0.48	0.73	1.88	4.81	6.76	6.43
Mixed, N. W.	29	0.62	0.54	0.54	0.52	0.84	3.92	6.00	4.88
Mixed, N. W., inoculated	30	0.62	0.42	0.42	0.54	1.32	3.98	6.68	6.32

* Samples were analyzed at intervals of three weeks, but the data included in this table are sufficient to show the general progress of the results.

fermentation progressed normally in this experiment, and was practically completed in all samples in nine weeks, it was stimulated considerably during the first six weeks in the samples that were inoculated with the pure culture of S. ellipsoideus. The acetic acid fermentation, on the other hand, was extremely slow during the first four months. The data in Table 9 show that at the end of 19 weeks the acid content in three of the four samples was lower than in the fresh cider. Yet the alcohol was gradually decreasing during that same period, for in some of the samples more than 1% of the alcohol had disappeared. This shows that probably some destructive fermentation was going on and that the acid was used up faster than it was produced. After the fourth month, however, the reverse action occurred and more acid was produced than was destroyed. From that time on the acetic acid fermentation progressed more rapidly. One reason for this is that vinegar bacteria work better in an acid medium and another reason may be that the temperature in the cellar was gradually increasing at that time.

It is significant to note that of the three ciders which completed the acetic acid fermentation in less than one year two received inoculation with pure cultures of yeast and vinegar bacteria. The acid fermentation in the fourth which was uninoculated was still in progress after sixteen months, and the other uninoculated sample gradually decreased in acetic acid content soon after the acid fermentation was completed. The results of this experiment show that the inoculation with pure cultures of yeast and subsequently with pure cultures of vinegar bacteria caused the fermentations to progress more rapidly and apparently checked the development of the bacteria responsible for the destruction of acetic acid.

DISCUSSION

The fermentation of a considerable number of samples of cider from different varieties of apples, prepared under clean and unclean conditions and fermented at different temperatures demonstrated that inoculation with pure cultures of yeast, S. ellipsoideus, appreciably stimulated the alcoholic fermentation. This is significant because by stimulating the alcoholic fermentation the chances for loss of sugar by unfavorable fermentation are decreased; the development

of the undesirable bacteria which destroy the sugars is more or less inhibited; and favorable conditions for a good and vigorous acetic acid fermentation are developed. The results of the experiments show further that under ordinary temperatures and aeration the acetic acid fermentation does not begin until the alcoholic fermentation is practically completed. This is supported by the results of Van Slyke (6) and by the contentions of Sackett (5), Cruess (2), and LeFevre (4). The fact that in the first series the acetic acid fermentation started before the alcoholic fermentation was completed, was probably due to the unusual air supply which those samples received. The exposed surface of these ciders was exceptionally large in proportion to the volume and in addition a current of fresh air was constantly drawn over the surface. When the total air supply is that which is supplied through the bunghole of a barrel, as is ordinarily the case, the acetic acid bacteria develop very slowly while the alcoholic fermentation is active, and consequently it takes some time before any appreciable amount of acid is formed.

Washing and sorting the apples had very little effect on the fermentations. This is taken to indicate that not all the detrimental organisms are eliminated by washing and sorting, but that probably about equal proportions of desirable and undesirable organisms are removed. However, this should by no means be taken as an encouragement for using unclean fruit. Too much emphasis cannot be placed on cleanliness of all utensils and raw materials. Barrels that are to be used for either cider or vinegar should first be thoroughly scalded with steam or boiling water and then rinsed with an abundance of clean water. The cider press should also be well cleaned before and after each pressing. The cull apples should be sorted, have their wormy and decayed spots removed, and be washed in clean water before pressing the cider. This is necessary because vinegar which is the direct product of this fruit is a food article for human consumption.

It has been shown in these experiments that it is possible to make good vinegar in four months and that some varieties of apples are better than others for this purpose, although all may contain the required amounts of sugar. Ciders from Jonathan apples proved

to be superior to those of the Wagener and Rome Beauty varieties, in that under no circumstances were they subject to undesirable fermentation. Mixed ciders from these three varieties of apples also proved satisfactory. The best results were obtained at a temperature of 65° to 75° F. and fairly good results were secured at a temperature of 45° to 55° F. The inoculation with pure cultures of vinegar bacteria, Ace. aceti, did not prove very effective at the higher temperature, but was quite helpful at the lower temperature. The point that is especially significant is that the time for making vinegar of marketable strength can be reduced from a year or more to four months by holding the temperature of the ciders around 70° to 75° F.

The removal of the sediment of the ciders after the alcoholic fermentation is completed is often advocated, but was not found necessary here. Time and effort can be saved by allowing both fermentations to progress undisturbed. However, the completion of the acetic acid fermentation should be watched carefully. When the alcohol is all converted into acetic acid, the clear vinegar should immediately be siphoned into clean containers which must be filled to capacity and stoppered air tight to prevent destructive fermentation. It should also be remembered that fresh vinegar has a sharp acid taste, but it will become mild and acquire desirable flavors when allowed to age from some months to a year or more. The strength of vinegar cannot be judged very readily. It must be ascertained by analysis. For this, special equipment and a certain degree of laboratory skill are required. The simplest way to handle it is to fill a small clean bottle with a sample of vinegar from each barrel in which the fermentaton seems to be well advanced, and send these samples to the nearest chemical laboratory; or better yet, make use of the services of the State College by sending them to the Bacteriology Division of the Experiment Station where they will be analyzed free of cost.

Literature Cited

1. Brierley, W. G. "Cider and Vinegar Making Qualities of Minnesota Apples," Bul. 185, Minn. Agr. Exp. Sta., Aug. 1919.

2. Cruess, W. V. "Vinegar From Waste Fruits," Bul. 287, Calif. Agr. Exp. Sta., Oct. 1921.

3. Lamb, A. R., and Wilson, Edith. "Vinegar Fermentation and Home Production of Cider Vinegar." Bul. 218, Iowa Agr. Exp. Sta., Aug. 1923.

4. Le Fevre, E. "Making Vinegar in the Home and On the Farm"—Farmer's Bul. No. 1424, U. S. D. A., June, 1924.

5. Sackett, W. G. "Home Made Cider Vinegar"—Bul. 192, Colorado Agr. Exp. Sta., Nov., 1913.

6. Van Slyke, L. L. "A Study of the Chemistry of Home Made Cider Vinegar," Bul. 258, New York Agr. Exp. Sta., Dec., 1904.