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EFFECT OF ETHYLENE ON CERTAIN CHEMICAL CHANGES ASSOCIATED WITH THE RIPENING OF PEARS¹

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(WITH TWO FIGURES)

Introduction

Recent discoveries (7, 8, 10) have shown that ethylene occurs in small amounts as a natural metabolic product of certain fruit and other plant tissues. The fact that a gas of this nature is produced by plants is of great interest, especially in view of the fact that ethylene has been used extensively in the past as an artificial treatment to hasten ripening in various fruits. The results obtained from use of this treatment, however, have not always been uniform, and for this reason there apparently has developed a diversity of opinions as to the exact relation, if any, of ethylene to the ripening of fruit. In some cases respiration (6), starch hydrolysis (14), and other chemical changes are reported to have been affected. In other cases (5, 16, 26), the results obtained have indicated that there is no apparent benefit from the use of ethylene for increasing the rate of ripening in various fruits.

As an explanation for these differences, HARVEY (15) has suggested that the concentration of gas used in some experiments might have been too low. In other cases (1, 27) maturity of the fruit has evidently been a factor. It is apparent, however, that in many of these previous experiments the fact has been overlooked that during maturation and after storage, fruits naturally undergo progressive physiological changes which may greatly modify the response shown to ethylene treatment. Thus, in a former experiment with pears (12) it was found that the respiration of the fruit showed a definite, well-defined trend during maturation and after storage, and the response obtained with ethylene varied greatly according to the respiratory activity of the fruit at time of treatment.

In the present investigation it was considered desirable to study the effects of ethylene on certain chemical changes occurring in pears during the ripening process. By observing the comparative responses of a single variety treated at different stages of maturity and after various periods in cold storage, it was thought that any chemical changes which were definitely affected by this gas during any specific stage in the life of the fruit would become apparent. Information of this nature would be of value in determining what the effects of a natural accumulation of ethylene in fruit tissues would have upon initiation of the ripening process.

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Materials and general methods

COLLECTION AND HANDLING OF SAMPLES

The fruit used in these experiments were Bartlett and Anjou pears, obtained from the Hood River and Medford (Oregon) districts. Fruit intended for treatment immediately after picking was gathered at approximately two-week intervals, beginning 5 weeks after blooming and extended several weeks beyond the regular commercial harvest season. Fruit intended for storage studies was picked on one date when the proper picking stage for the variety had been reached.

As soon as the fruit had been brought from the field or removed from storage, two uniform lots were sorted out and placed in 5-gallon glass jars. To one set of jars was added ethylene to make a concentration of 1:1000 by volume; the second set of jars was kept under constant ventilation with fresh air to prevent any accumulation of gases affecting ripening. Details of the method used to maintain comparable temperatures, humidities, and carbon dioxide and oxygen tensions in the treated and untreated jars are described in a previous publication (12). To compare the trend of chemical changes occurring after picking in the ethylene-treated and untreated lots, a sample of 15 pears was withdrawn for analysis at definite intervals during the course of ripening. With immature fruit, a sample was taken every 4 days during a period of 12 days. With mature and storage fruit, which ripened more rapidly, only two analyses were made: the first immediately after picking or removal from storage, and the second after a period of 8 days.

Methods of analysis

SAMPLING.—Samples for chemical analysis were prepared by cutting longitudinal sectors of 15 whole pears, grinding them through a food chopper, and finally weighing out duplicate 50-gm. samples of the finely ground tissue.

EXTRACTION.—Sugars were extracted with 95 per cent. alcohol in a Soxhlet extractor for 24 to 30 hours. The alcohol was then distilled off under a 26 to 28 inch vacuum at 40° C., the water extract cleared with neutral lead acetate, de-leaded with potassium oxalate, and made to volume.

SUGAR DETERMINATIONS.—Reducing sugars before and after inversion were determined by the method of LANE and EYNON (19). Inversion was carried out by the method described by MARTIN (21) and sucrose was calculated by the usual method.

ALCOHOL INSOLUBLE RESIDUE.—The alcohol insoluble residue was determined as the dry weight of the material remaining in the thimbles after extraction.

STARCH.—This was determined on 0.5-gm. samples of the alcohol insoluble residue, finely ground with quartz sand, and digested with fresh saliva

according to the method described by LOOMIS and SHULL (20). The reducing values of the solutions after hydrolysis were determined by the iodometric method of SHAFFER and HARTMANN (24); and converted to starch by the factor 0.90.

PECTIC SUBSTANCES.—Soluble pectin and protopectin were determined by the method of CARRÉ and HAYNES (4) on 50-gm. samples of the fresh tissue, and are expressed as impure calcium pectate.

ACID.—Acid was determined on 50-ml. aliquots of the water solution of the alcohol extract used for sugar determinations and calculated as citric. Because of the difficulty in determining the end point with phenolphthalein indicator, the solutions were titrated electrometrically to pH 7.2 with 0.01N sodium hydroxide on a YODEN quinhydrone electrode.

Results

EXPERIMENTS WITH BARTLETT PEARS RIPENED BEFORE STORAGE

In order to have fruit representative of different stages of maturity, approximately 200 pears were picked at intervals throughout the later part of the growing season. The dates on which the samples were collected and the average weights of 25 fruits at time of picking are shown in table I.

TABLE I
AVERAGE WEIGHT OF FRUIT COLLECTED AT DIFFERENT PERIODS

DATE PICKED	AVERAGE WEIGHT PER FRUIT
July 14	<i>gm.</i> 43.1
July 29	99.3
August 16	138.6
August 25*	150.5
September 14	160.5

* Commercial picking date.

The first picking, made on July 14, was extremely immature. The amount of ethylene produced at this stage of development was barely detectable by epinasty of potato leaves, and the tissues of the untreated fruit probably contained only traces of this gas naturally produced. Fruit picked later showed increasing evidence of ethylene production, and the tissues of the untreated fruit were probably not free from this gas, even though constant aeration was provided during the entire ripening period.

The changes in total and reducing sugars, sucrose, starch, pectic substances, alcohol insoluble residue, and acid are shown in table II, and are represented graphically, with the omission of acid and alcohol-insoluble residue, in figure 1. The percentages of increase or decrease in each material

TABLE II
EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF BARLETT PEARS PICKED AT DIFFERENT STAGES OF MATURITY

No. DAYS RIPENED	TOTAL SUGARS		REDUCING SUGARS		SUCROSE		SOLUBLE PECTIN		INSOLUBLE PROTOPECTIN		STARCH		ALCOHOL- INSOLUBLE RESIDUE		ACID AS CITRIC	
	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED
1st picking—July 14																
0	4.20	4.20	4.06	4.06	0.13	0.13	0.10	0.10	0.85	0.85	1.24	1.24	6.65	6.65	0.23	0.23
4	4.86	5.85	4.62	5.32	0.23	0.50	0.10	0.31	0.84	0.36	1.29	0.76	6.32	5.41	0.17	0.17
8	5.29	6.39	5.18	5.80	0.11	0.56	0.12	0.65	0.80	0.25	1.14	0.21	5.98	5.91	0.28	0.27
12	5.39	5.72	5.18	5.26	0.20	0.44	0.09	0.21	0.85	0.18	1.01	0.14	6.13	5.42	0.19	0.17
2nd picking—July 29																
0	6.28	6.28	5.70	5.70	0.55	0.55	0.09	0.09	0.86	0.86	1.09	1.09	4.95	4.95	0.15	0.15
4	6.54	6.82	6.08	6.43	0.44	0.38	0.08	0.57	0.90	0.42	0.85	0.60	4.70	4.45	0.18	0.19
8	6.82	7.31	6.30	6.60	0.50	0.67	0.32	0.71	0.46	0.20	0.66	0.45	4.38	3.98	0.20	0.19
12	7.72	7.60	7.15	7.00	0.55	0.57	0.51	0.38	0.13	0.10	0.39	0.28	3.54	3.59	0.23	0.23
3rd picking—August 16																
0	6.68	6.68	6.04	6.04	0.61	0.61	0.21	0.21	0.64	0.64	0.71	0.71	4.35	4.35	0.20	0.20
4	7.29	7.59	6.54	6.75	0.71	0.80	0.26	0.39	0.68	0.52	0.85	0.68	4.05	3.76	0.20	0.21
8	7.72	7.58	6.99	6.52	0.70	1.00	0.42	0.64	0.26	0.10	0.60	0.34	3.63	3.39	0.22	0.19
4th picking—August 25																
0	7.21	7.21	6.24	6.24	0.93	0.93	0.19	0.19	0.61	0.61	0.64	0.64	3.22	3.22	0.17	0.17
8	8.42	8.44	6.67	6.90	1.66	1.46	0.51	0.59	0.23	0.19	0.37	0.10	3.22	3.38	0.22	0.21
5th picking—September 14																
0	8.49	8.49	6.45	6.45	1.94	1.94	0.19	0.19	0.63	0.63	0.34	0.34	2.90	2.90	0.26	0.26
8	8.87	8.97	6.49	6.56	2.26	2.29	0.65	0.68	0.19	0.15	0.21	0.19	2.42	2.41	0.15	0.21

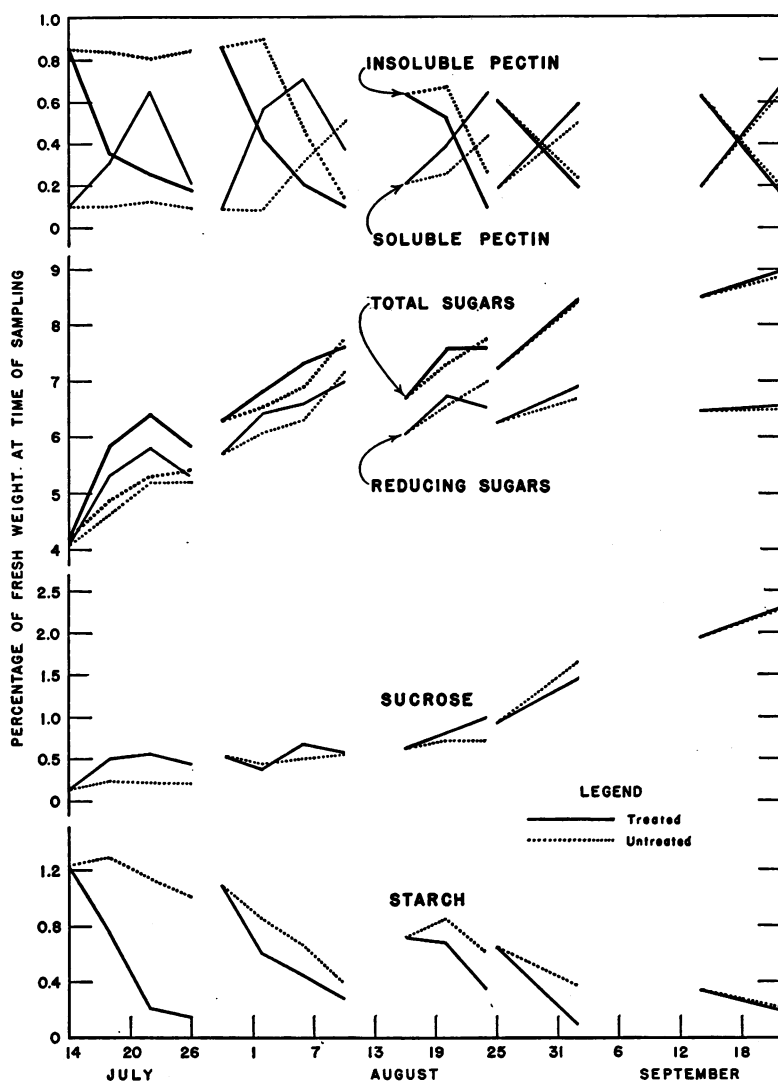


FIG. 1. Effect of ethylene on chemical changes of Bartlett pears picked at different stages of maturity.

during ripening of treated and untreated lots are shown in table III. These values were calculated on the basis of the percentage increase or decrease, at the end of 8 days, over or under the original amount of the material present at time of picking.

CHANGES IN SUGARS.—Total sugars in the green fruit increased throughout the growing season. During ripening, definitely greater increases were

TABLE III

COMPARATIVE CHANGES IN THE CONSTITUENTS OF ETHYLENE-TREATED AND UNTREATED BARLETT PEARS, EXPRESSED AS THE PERCENTAGE INCREASE OR DECREASE DURING EIGHT DAYS' RIPENING, OF THE AMOUNT ORIGINALLY PRESENT

DATE OF PICKING	INCREASE IN TOTAL SUGARS			INCREASE IN REDUCING SUGARS			INCREASE OR DECREASE IN SUCROSE			INCREASE IN SOLUBLE PECTIN			DECREASE IN INSOLUBLE PROTOPECTIN			DECREASE IN STARCH		
	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE
7/14	52.15	25.95	26.20	42.86	27.59	15.27	331.00	-15.39	346.4	550.0	20.0	530.0	70.60	5.88	64.72	83.06	8.07	74.99
7/29	16.40	8.60	7.80	15.79	10.53	5.26	21.82	-9.09	30.91	689.0	255.6	433.4	76.74	46.51	30.23	58.73	39.46	19.27
8/16	13.47	15.57	-2.10	7.95	15.73	-7.78	63.95	14.75	49.20	204.4	100.0	104.4	84.56	59.37	25.19	52.11	15.49	36.62
8/25	17.07	16.78	0.29	10.58	6.89	3.69	56.99	78.49	-21.50	210.6	168.4	42.2	68.85	62.30	6.55	84.39	42.19	42.20
9/14	5.65	4.47	1.18	1.71	0.62	1.09	18.04	16.49	1.55	237.9	247.4	10.5	76.19	69.84	6.35	44.12	38.28	5.89

observed in the ethylene-treated than in the untreated lots in the first and second pickings; but in more mature fruit these differences were much less pronounced. As can be noted in table III, the increase in total sugars during ripening was 26 per cent. greater in the treated fruit of the first picking but only 1.18 per cent. greater in the last picking.

Reducing sugars showed changes similar to those found for total sugars. In the fruit of the first picking the increase in the treated sample was 15.27 per cent. over the untreated. In the final picking, however, an increase of only 1.09 per cent. was found in favor of ethylene treatment.

Very little sucrose occurs in immature green fruit but this sugar builds up constantly during maturation. During ripening more sucrose developed in the treated than in the untreated pears in all but one of the samples collected. In the first picking this increase amounted to over 300 per cent. in favor of ethylene treatment. Much smaller differences were found in fruit picked and ripened later in the season.

STARCH.—The maximum concentration of starch was found in the first sample collected, and the amount contained in the tissues at time of picking decreased thereafter. After ripening of the earliest collected samples, it became evident that ethylene treatment was very effective in increasing the rate of starch hydrolysis. In the treated samples of the first picking practically all of the starch had disappeared after a period of 8 days, while the untreated lot still retained all but a small fraction of the amount originally present. When the last fruit was collected late in the season, there was little starch remaining, and during ripening this disappeared almost as rapidly in the untreated as in the treated fruit.

ALCOHOL INSOLUBLE RESIDUE.—The amount of this material decreased throughout the growing season, and fruit treated early in the season contained less after ripening than did untreated fruit held for a similar period of time. These differences were not noticeable after ripening of more mature fruit picked at later dates.

PECTIC CHANGES.—The amount of soluble pectin in the green fruit at the time of picking was very small, never exceeding 0.2 per cent., even in pears collected two weeks later than the period when commercially mature. After picking and during ripening, the increase in soluble pectin closely parallels the increase in softening of the fruit and is, therefore, a very good index of progress in ripening.

That ethylene has a very pronounced effect on pectic changes is evident from the results obtained. Untreated fruit of the first picking was held for 12 days without any increase in soluble pectin. In the ethylene treated lot, however, there was a very rapid increase which at the end of 8 days amounted to more than 500 per cent. over the amount originally present.

In the untreated sample of the second picking, soluble pectin showed no increase during a 4-day period, but increased rapidly thereafter. In all fruit

collected at later dates, soluble pectin began to increase immediately after picking in the untreated lots, but the rate of this increase was always less rapid than that observed in the treated fruit. Even in the post-mature pears, the amount of soluble pectin developed was higher in the treated fruit, but the difference was much less than that found in earlier picked samples.

The amount of insoluble protopectin in the green fruit decreased slightly throughout the season. During ripening there was a further decrease which occurred more rapidly in the treated fruit. Judging from the data obtained, it is assumed that this decrease in insoluble protopectin is related to the increase in soluble pectin since the percentage decrease in one can approximately be accounted for on analysis as the percentage increase in the other.

The relation of protopectin to pectin has been investigated extensively in apples by CARRÉ (3). APPLEMAN and CONRAD (2) have reported that in peaches the rate of softening parallels the transformation of protopectin into pectin. Similar observations have been made on pears by EMMETT (9).

That ethylene increases the rate of softening in fruit is a common observation. As far as the writer is aware, however, specific data to show the effect of ethylene in increasing the rate of transformation of protopectin to pectin have not been reported previously.

ACID.—The amount of acid found in the Bartlett pears used in these experiments was small, never exceeding 0.3 per cent. During the course of ripening, the acid content showed considerable variation; and no well-defined trends in either treated or untreated lots were apparent. In some lots of fruit titratable acidity appeared to increase during ripening, but in most cases the changes observed were very small.

EXPERIMENTS WITH BARTLETT PEARS RIPENED AFTER STORAGE

The pears used for this series were held at a temperature of 31° F. Samples for analysis were withdrawn after periods of 10, 20, 30, 70, and 120 days, and then ripened at 65° F., as in all previous experiments. The chemical changes occurring in treated and untreated lots during ripening are shown in table IV.

CHANGES IN SUGARS.—Total and reducing sugars increased throughout the storage period. During ripening they showed a tendency to increase, but with the probable exception of the earliest withdrawn samples there were no significant differences observed between treated and untreated lots.

Sucrose increased during the early storage period but declined slightly thereafter. Ethylene treatment did not appear to have any significant effect in increasing or decreasing the concentration over that found in untreated lots after ripening.

STARCH.—Starch was hydrolyzed rapidly during storage. Although microchemical tests showed this material evenly distributed throughout the

TABLE IV
EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF BARLETT PEARS RIPENED AFTER STORAGE AT 31° F.

No. days of storage	No. days ripened	Total sugars		Reducing sugars		Sucrose		Soluble pectin		Insoluble protopectin		Starch		Alcohol-soluble residue		Acid	
		Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	0	7.65	7.65	6.79	6.79	0.82	0.82	0.10	0.10	0.78	0.78	0.99	0.99	4.33	4.33	0.19	0.19
	8	9.10	9.20	7.54	7.81	1.48	1.32	0.39	0.57	0.48	0.21	0.30	0.21	3.00	3.06	0.23	0.24
10	0	8.22	8.22	6.72	6.72	1.43	1.43	0.10	0.10	0.61	0.61	0.68	0.68	3.58	3.58	0.19	0.19
	8	9.15	9.15	7.52	7.46	1.54	1.61	0.53	0.59	0.24	0.17	0.27	0.13	3.23	3.15	0.19	0.19
20	0	8.73	8.73	7.56	7.56	1.11	1.11	0.18	0.18	0.73	0.73	0.41	0.41	3.45	3.45	0.22	0.22
	8	8.74	8.96	7.20	7.43	1.46	1.46	0.61	0.69	0.18	0.16	0.23	0.17	3.49	3.53	0.21	0.20
30	0	8.33	8.33	7.34	7.34	0.94	0.94	0.09	0.09	0.87	0.87	0.19	0.19	3.64	3.64	0.18	0.18
	8	9.07	9.11	7.60	7.62	1.40	1.42	0.71	0.79	0.45	0.41	0.16	0.19	3.48	3.57	0.21	0.22
70	0	8.87	8.87	7.92	7.92	0.90	0.90	0.11	0.11	0.75	0.75	0.15	0.15	3.71	3.71	0.20	0.20
	8	9.07	9.03	7.41	7.53	1.57	1.43	0.65	0.64	0.14	0.12	0.15	0.14	3.42	3.42	0.18	0.20
120	0	8.97	8.97	7.92	7.92	0.99	0.99	0.36	0.36	0.37	0.37	0.08	0.08	3.37	3.37	0.21	0.21
	8	9.00	9.03	7.41	7.53	1.51	1.43	0.36	0.37	0.34	0.32	0.07	0.09	2.78	3.00	0.19	0.20

tissues of the fruit at the time of harvest, only traces could be detected after 30 days of cold storage. The hydrolysis of starch, therefore, does not enter into the ripening changes of pears withdrawn after delayed periods of storage. This fact has been observed by MARTIN (22).

During ripening the rate of hydrolysis tended to be more rapid in the treated lots, but starch also disappeared rapidly in the untreated fruit, and the differences observed, therefore, were not as large as those found in newly-picked fruit.

ALCOHOL-INSOLUBLE RESIDUE.—The alcohol-insoluble residue decreased throughout the storage period, and no significant differences were found in the amount of this material remaining in treated and untreated lots after ripening. This would be expected since starch was hydrolyzed almost as rapidly in the untreated as in the treated lots. Other undetermined hydrolyzable materials, such as hemicellulose, would probably respond in a similar manner.

ACID.—The changes in acid during ripening of the storage fruit were very small, and no significant differences were observed between treated and untreated samples.

CHANGES IN PECTIC SUBSTANCES.—Ethylene continued to affect pectic changes for a longer period of time than starch hydrolysis or any other chemical changes observed. In the initial storage sample, protopectin disappeared twice as fast in the treated fruit, and the increase in soluble pectin was almost twice as great. In fruit which had been held for various periods in storage, the rate of protopectin transformation in untreated fruit was more rapid than that observed in pears ripened before storage, and this rate could be increased but little by ethylene treatment. After 30 days of storage, however, slightly more soluble pectin was found in the treated fruit when ripened.

The decrease in effect of ethylene treatment in relation to the increase in maturity of fruit has been observed by ALLEN (1). He found that this gas was more effective in influencing sugar, acid, and starch changes in Gravenstein apples collected early in the season than in those picked at later dates. With pears he also found that the rate of softening and ripening was affected mostly in fruit treated prior to storage. Chemical analysis made after the fruit had been held in storage for 10 and 15 weeks showed no differences in acid or in total and reducing sugars between treated and untreated lots. The suggestion was made, however, that in view of the results on softening and color, it would appear that greater differences in chemical composition might have been found had samples been analyzed a short time after harvesting. The results obtained in the present experiments show clearly that this assumption was correct.

EXPERIMENTS WITH ANJOU PEARS RIPENED AFTER STORAGE

Some studies of the Anjou pear were included because this variety keeps longer and, as shown formerly (12), responds to ethylene treatment for a longer period of time after storage than the Bartlett pear. The fruit for this series was held at 31° F., and samples were withdrawn for analysis after 20, 60, and 120 days. In addition to chemical analysis of treated and untreated lots, respiration determinations were made during ripening, using the method of HARDING and MANEY (13). Pressure tests before and after ripen-

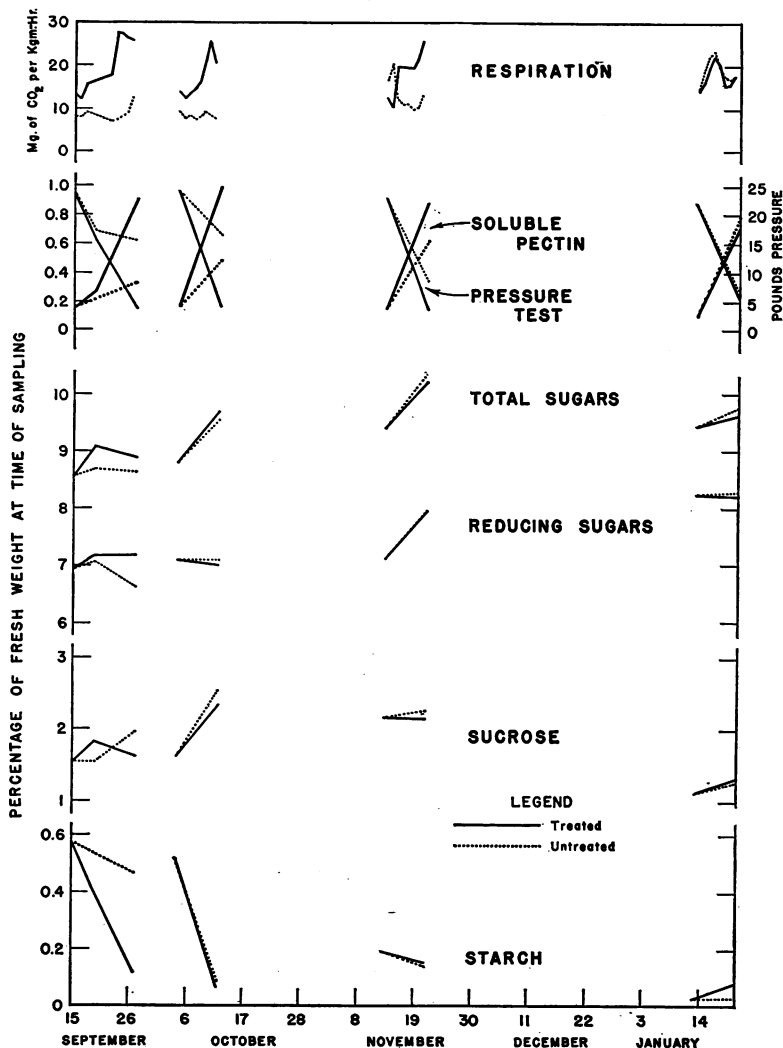


FIG. 2. Effect of ethylene on chemical changes of Anjou pears ripened before and after storage at 31° F.

ing also were made, using the Oregon pressure tester (23). The data presented in table V show the comparative chemical composition and the pressure tests of the ethylene-treated and untreated lots before and after ripening. Comparative changes in sugar, starch, pectin, pressure test, and respiration are represented graphically in figure 2.

The results obtained with the Anjou are similar to those found for the Bartlett, and therefore, will not be discussed in detail.

CHEMICAL CHANGES.—The greatest effects from ethylene were obtained early in the storage period. Total and reducing sugars increased more rapidly in the treated lot ripened immediately after harvest; but fruit ripened after being held for various periods at 31° F. showed no differences which could not be attributed to experimental error. In one or two instances sucrose was found to be slightly lower in the treated lots but in most cases the differences were not significant. Since the hydrolysis of sucrose does not occur in the early stages of ripening, as shown by MARTIN (22), it is possible that had the ripening process been observed over a longer period, ethylene might have shown more effect on sucrose hydrolysis. This appears likely, since HIBBARD (16) has reported that he found less sucrose in several kinds of fruits as a result of ethylene treatment.

Starch disappeared more rapidly in the treated fruit ripened at time of storage, but ethylene had little effect upon increasing the rate of its hydrolysis after the pears had been held at a low temperature for even short periods of time.

In the Anjou pear, as in the Bartlett, the pectic changes were influenced by ethylene treatment for a longer period of time than starch hydrolysis or any other chemical change observed. As can be noted in table V, starch hydrolysis was not affected by ethylene even after only 20 days' storage, although there was still considerable of this material in the fruit tissues at this time. Decidedly higher amounts of soluble pectin, however, developed in the treated fruit ripened at this period, and also in the treated lot ripened 40 days later. The rate of pectic changes, though, was eventually unaffected by ethylene, as shown by similar amounts of soluble pectin found in the untreated and treated fruit ripened after 120 days' storage.

PRESSURE TEST.—The pressure test decreased only slightly during 120 days of storage, indicating that very little softening of the tissues had occurred while the fruit was held at 31° F. Pressure tests of the ethylene-treated lots were much lower than those of the untreated fruit ripened before and after 20 and 60 days of storage, indicating a much more rapid softening of the tissues as a result of treatment. Treated and untreated fruit which was ripened after 120 days' storage showed very little difference in pressure test.

It is apparent from these results that softening of the tissues and the development of soluble pectin are closely correlated. This is indicated by

TABLE V
EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF ANJOU PEARS RIPENED BEFORE AND AFTER STORAGE AT 31° F.

NO. DAYS RIPENED	TOTAL SUGARS		REDUCING SUGARS		SUCROSE		SOLUBLE PECTIN		PRESSURE TEST IN POUNDS		STARCH		ALCOHOL IN- SOLUBLE RESIDUE	
	UN- TREATED	TREATED	UN- TREATED	TREATED	UN- TREATED	TREATED	UN- TREATED	TREATED	UN- TREATED	TREATED	UN- TREATED	TREATED	UN- TREATED	TREATED
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	lb.	lb.	gm.	gm.	gm.	gm.
	When stored													
0	8.57	8.57	6.95	6.95	1.54	1.54	0.14	0.14	23.9	23.9	0.58	0.58	4.51	4.51
4	8.69	9.09	7.07	7.17	1.54	1.82	0.21	0.27	17.0	15.6	0.54	0.41	4.19	4.25
12	8.64	8.90	6.63	7.19	1.96	1.62	0.33	0.92	15.6	3.7	0.47	0.12	4.46	3.97
	After 20 days' storage													
0	8.80	8.80	7.08	7.08	1.63	1.63	0.15	0.15	24.0	24.0	0.52	0.52	4.09	4.09
8	9.55	9.70	7.08	7.00	2.34	2.56	0.50	0.99	16.6	4.0	0.07	0.09	3.92	3.90
	After 60 days' storage													
0	9.40	9.40	7.11	7.11	2.17	2.17	0.15	0.15	23.0	23.0	0.20	0.20	3.89	3.89
8	10.36	10.23	7.97	7.96	2.27	2.16	0.62	0.89	8.4	3.7	0.16	0.15	4.05	3.89
	After 120 days' storage													
0	9.44	9.44	8.25	8.25	1.13	1.13	0.11	0.11	22.2	22.2	0.03	0.03	4.05	4.05
8	9.75	9.65	8.30	8.23	1.28	1.34	0.80	0.73	6.5	5.5	0.03	0.08	3.54	3.79

the fact that higher pectin content was always associated with lower pressure tests in the ethylene-treated fruit as compared to lower pectin content and higher pressure tests in the untreated fruit. This correlation is also indicated by the fact that fruit ripened under treatment after 120 days of storage showed no increase in soluble pectin over that found in untreated fruit; neither did this fruit show any significant decrease in pressure test as a result of similar treatment.

RESPIRATION.—Increases in respiration were obtained long after starch and sugar changes ceased to be affected by ethylene. As can be observed in figure 2, rate of respiration was consistently higher in the ethylene-treated lots ripened before and after 20 and 60 days' storage; but no significant differences were observed between treated and untreated fruit ripened after being held at 31° F. for 120 days. It will be recalled that the development of soluble pectin was also no longer affected by ethylene after a similar storage period, and it is therefore apparent that respiration and pectic changes cease to respond to ethylene treatment at approximately the same time.

Considerable importance has been attributed by KIDD and WEST (18) to the increase in respiration occurring prior to ripening in apples. They have applied the term "climacteric" to this respiratory increase, since they consider that it marks a transition to senescence in the life of the fruit. Ripening of the fruit, *i.e.*, softening of the tissue, the development of flavor and aroma, are considered senescent phenomena; since they were observed not to occur in apples until the climacteric had passed.

Various theories have been advanced to account for this increase in respiration observed to take place at a definite period in the life of the fruit. KIDD and WEST (17) suggested it was associated with a decrease in acidity; WARDLAW and LEONARD (25) to an increase in the internal concentration of oxygen; GUSTAFSON (11) to a decrease in hydrogen-ion concentration. The results of the present experiments indicate that the increase in respiration in pears is not associated with a particular concentration of any specific chemical substance, especially sugars. Neither does it appear to be associated with any change in titratable acidity. The fact that this increase in respiratory activity can be initiated so readily with ethylene would indicate that the basic changes brought about by this gas are undoubtedly causal factors of this phenomenon. In addition, the fact that ethylene is so effective in initiating ripening changes other than the climacteric indicate that possibly the basic changes occurring in all cases may be similar in nature. For this reason, further experiments with ethylene, especially in relation to enzyme reactions, appear justified.

Discussion

It is assumed from the results obtained in these investigations with pears that ethylene treatment applied at certain periods in the life of the fruit

definitely affects the principal reactions associated with the ripening processes. This is shown by the increased rate of starch hydrolysis, the higher sugar content, the more rapid transformation of protopectin to pectin and by the increase in respiratory activity in the fruit to which ethylene has been applied. The initiation of these chemical ripening processes could be brought about by ethylene treatment of pears picked at a very immature stage, long before the fruit had naturally developed to the period when ripening would normally occur. In addition, the reactions occurring in the presence of ethylene were identical to the changes that were observed to take place in mature fruit ripened naturally under normal conditions. In the light of these facts, the question is raised as to what would be the effects of a natural accumulation of ethylene in the tissues of the fruit.

The magnitude of the chemical changes affected by ethylene during ripening is determined by the maturity of the fruit and by the length of time it is held in storage prior to treatment. As previously pointed out, pears picked while still in an early stage of development ripened readily when subjected to ethylene, while similar fruit, not so treated, failed to ripen altogether or was markedly delayed in ripening beyond the period required for treated fruit. In pears collected at more mature stages, the ripening changes naturally progressed at a more rapid rate, and the effect observed from ethylene, therefore, was less pronounced. After the pears had been held in storage for comparatively short periods of time, the chemical changes resulting in ripening occurred very rapidly when the fruit was removed to a higher temperature, and little if any benefit could be observed from the use of ethylene.

The length of time during which the individual chemical changes associated with ripening are influenced by ethylene varies greatly, being shortest with sugar and starch changes, and longest with pectic transformations and respiration. Starch disappears rapidly after picking or storage, and the hydrolysis of this material does not enter into the ripening process of pears withdrawn after long periods of storage. Pectic changes and respiratory activity cease to respond to ethylene at approximately the same period.

Summary

1. Ethylene treatment, when applied at certain periods, was found to increase the rate of starch digestion, the concentration of total and reducing sugars, and the transformation of protopectin to pectin in Bartlett and Anjou pears. No changes in titratable acidity were found.
2. The increase in rate of softening observed in pears treated with ethylene is definitely correlated with an increase in rate of pectic changes.
3. The magnitude of the response obtained with ethylene is determined by the maturity of the fruit and by the length of time held in storage prior to treatment.

4. The length of time during which the individual chemical ripening changes are influenced by ethylene varies greatly, being shortest with sugar and starch changes, and longest with pectic reactions and respiration.

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