

VOLATILE PRODUCTS OF APPLES

II. PRODUCTION OF VOLATILE ESTERS BY GRANNY SMITH APPLES

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[Manuscript received May 10, 1951]

Summary

Granny Smith apples were stored at 0°C., and samples were removed at intervals for determination of volatile ester production at 20°C.

In early removals ester production at 20°C. increased to a maximum and then decreased. In later removals the increase was much less and finally became negligible. Ester production at 0°C. appeared to increase steadily.

Early picking reduced ester production and a higher rate of air flow increased it. Reducing the oxygen concentration to 6 per cent. first increased and then decreased ester production in comparison with air.

The metabolic significance of the results is discussed.

I. INTRODUCTION

Evidence has been presented that superficial scald, a functional disorder of cold-stored apples, may be caused by the volatile substances produced by the fruit itself. In view of the possible importance of esters in relation to scald, as indicated by the experiments of Brooks, Cooley, and Fisher (1919), it was decided to measure the production of volatile esters. The ester fraction has not previously been determined separately, although other workers have determined the production of "odorous" volatiles after absorption in concentrated sulphuric acid. The absorbed volatiles were determined by wet oxidation with chromic acid and measurement of the carbon dioxide produced (Potter and Griffiths 1947; Griffiths and Potter 1949). This method, however, is purely empirical.

The identification of the acids and alcohols, free and combined as esters, given off by Granny Smith apples has been described in an earlier paper (Thompson 1951).

The measurements of volatile ester production have all been made with Granny Smith apples. The first measurements were made in 1949 on a series of samples removed to 20°C. after various periods at 0°C. Definite trends in ester production were observed, and further measurements were made in 1950, when the effect of reducing the oxygen concentration was investigated. Some determinations of volatile acids and alcohols in the fruit were also made in 1950.

II. EXPERIMENTAL TECHNIQUE

(a) *Collection of Esters*

Air, purified by combustion of organic matter and absorption of carbon dioxide, was passed over 5-10 kg. of Granny Smith apples held at 20°C.

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either in a series of four desiccators or in a galvanized iron drum. The rate of flow varied from 10 to 40 l./hr. in different experiments. The volatile substances were absorbed by passing the air first through a U-tube cooled in an ice-calcium chloride mixture and then through two spiral absorbers, each containing 30 ml. of ether and cooled in a solid carbon dioxide-alcohol mixture. After 6-24 hr. the absorbers were disconnected, emptied into a separating funnel and washed out with ether. The aqueous layer was then separated off and extracted with ether. The combined ether extracts were dried over calcium chloride and made up to volume (usually 100 ml.) in a volumetric flask.

Initially the contents of the second absorber were made up and analysed separately. It was found that, with a flow rate of 10 l./hr., an average of 95 per cent. of the esters was collected in the U-tube and first spiral absorber. Although little ester was collected in the U-tube, some water condensed which would otherwise have blocked the spiral absorber.

(b) Determination of Esters

The apple esters were determined colorimetrically in 5 ml. of the ether solution by the procedure described previously (Thompson 1950). This method involves conversion of the esters to hydroxamic acids, which are determined as the coloured ferric hydroxamate complexes.

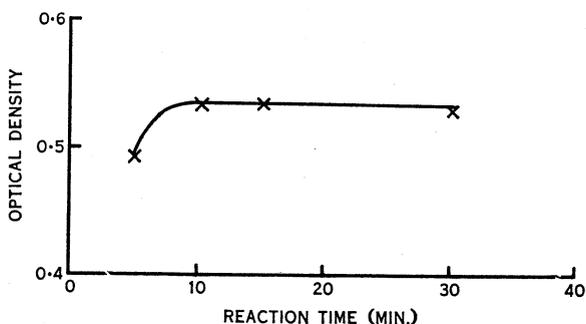


Fig. 1.—Formation of hydroxamic acids from apple esters.

The method is applicable to the mixed apple esters, since tests have shown that the reaction with hydroxylamine is complete in 15 min. (Fig. 1) and Beer's Law is obeyed in the same way as for pure esters. Other tests have shown that the procedure of drying with calcium chloride, together with other manipulations, does not result in a loss of more than 3 per cent. Acetaldehyde does not interfere if its concentration in the ether solution does not exceed 0.1M.

Since *n*-butyrate appeared to predominate in the apple esters during 1949 (Thompson 1951), the apple ester concentration was calculated by assuming a ratio of 2.12×10^{-3} M per unit optical density, as previously found for ethyl butyrate (Thompson 1950). This ratio does not differ appreciably from that for acetate or propionate.

(c) Determination of Volatile Acids and Alcohols in Fruit

Cut sections of whole apples (approx. 2 kg.) were steam-distilled for 3 hr. through a double surface condenser into a receiver cooled in ice. The receiver was connected to a second trap containing water cooled in ice. The distillate was mixed with the water from the trap and divided into two equal portions.

Total volatile acids (free and esterified) were determined in one portion. Sodium hydroxide was added to a concentration of approximately 0.1N, and the solution was allowed to stand overnight. The solution was then acidified with sulphuric acid and re-distilled. After concentrating to approximately 100 ml., a further 100 ml. of water was added and distillation continued to the same final volume. Nitrogen was bubbled through the distillate (to remove carbon dioxide) and the acid determined by titration with 0.1N sodium hydroxide, using a mixture of neutral red and methylene blue as indicator.

Total volatile alcohols were determined by the method of Fidler (1934). The second portion of the first distillate was oxidized with chromic acid and re-distilled. The figure obtained by titration of the final distillate included acid originally present as well as that produced by oxidation of alcohol. By subtracting the figure for volatile acids, the figure for volatile alcohols was obtained.

(d) Respiration and Composition of Internal Atmosphere

The respiration (CO_2 production) of approximately 5 kg. lots of apples was determined by the Pettenkoffer method. The concentration of oxygen and carbon dioxide in the internal atmosphere of individual apples was determined by the method of Trout *et al.* (1942).

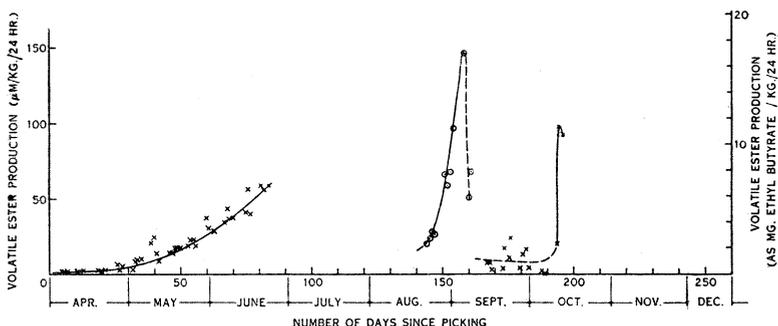


Fig. 2.—Production of volatile esters by Granny Smith apples at 20°C. after removal from storage at 0°C. (picked March 30, 1949).

III. VOLATILE ESTER PRODUCTION IN AIR

Two pickings of Granny Smith apples were made at Orange, N.S.W., on March 30 and April 12, 1949. The first picking was made a few days before commercial picking and the second was commercially mature. Apples of both

pickings were stored at 0°C. and samples were removed at intervals for determination of volatile ester production at 20°C. Apples of the first picking were removed after 0, 20, and 23 weeks and apples of the second picking after 1, 6, 11, 16, 22, 26, and 31 weeks. Measurements were continued for as long as the fruit was sound and free from rots. The values of volatile ester production for the first picking are given in Figure 2 and for the second picking in Figure 3. In these experiments measurements were made with 10 kg. of fruit and a rate of air flow of 10 l./hr. There was frequently considerable fluctuation in individual readings, but curves of best fit were drawn.

Volatile ester production shows a similar trend in both pickings, although the data for the second picking are more complete. The ester production of the first picking was generally lower than that of the second picking. In the first removal the ester production increased steadily from a very low value over a period of about 10 weeks. In subsequent removals an initial delay was followed by a rapid rise to a maximum and a fairly rapid fall. In later removals the rise became much less and finally negligible.

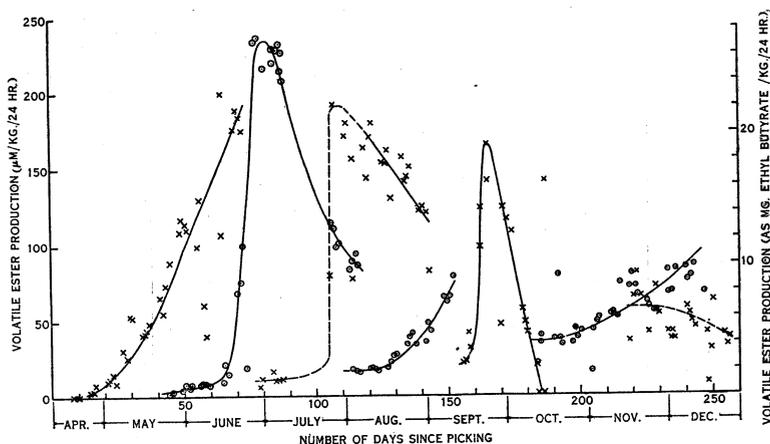


Fig. 3.—Production of volatile esters by Granny Smith apples at 20°C. after removal from storage at 0°C. (picked April 12, 1949).

Determination of carbon dioxide in the air leaving the desiccators gave a concentration of about 2 per cent. It has been shown by Potter and Griffiths (1947) and Griffiths and Potter (1949) that accumulation of carbon dioxide or organic volatiles can reduce the volatile ester production. For comparison with the sample of the second picking removed after 26 weeks, an additional sample was removed after 27 weeks. The ester productions were measured with rates of air flow of 10 and 40 l./hr., and the results are given in Figure 4. Increasing the rate of flow to 40 l./hr. approximately doubled the ester production over a period of 7 weeks. Subsequently the production at the higher rate of air flow appeared to decrease earlier.

The effect of accumulated carbon dioxide or organic volatiles associated with the lower rate of air flow must be considered as a possible factor in interpreting the results of Figures 2 and 3. However, as the higher rate of air flow increased the volatile ester production in approximately constant ratio over several weeks, it is probable that the general form of the curves has not been significantly affected.

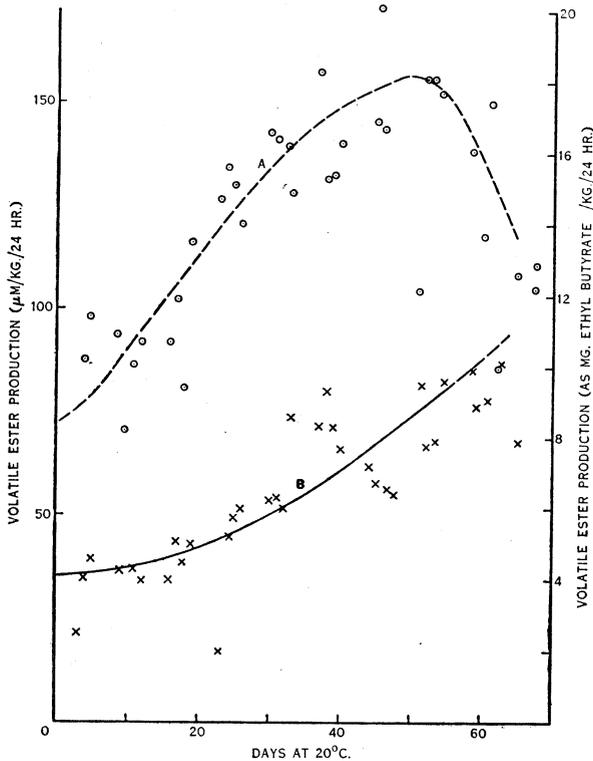


Fig. 4.—Effect of flow rate on production of volatile esters by apples.

A, flow rate 40 l./hr. Apples stored 27 weeks at 0°C.
B, flow rate 10 l./hr. Apples stored 26 weeks at 0°C.

Measurements of respiration were made on apples of the second picking removed to 20°C. after 6, 11, 16, and 22 weeks at 0°C. The results are given in Figure 5. The curves of respiration and ester production followed quite different trends. Respiration fell rapidly at first and then more slowly. In the first three curves the end of the rapid fall in respiration coincided approximately with the beginning of the rapid rise in ester production.

The measurements of volatile ester production were all made at 20°C., as the production at 0°C. is very low. However, the trend of ester production at 0°C. can be determined by assuming a constant temperature coefficient, i.e. a constant ratio between initial ester production at 20°C. and the corresponding

production at 0°C. The values of initial ester production at 20°C. in the second picking are plotted in Figure 6. The curve obtained shows a steady rise, which suggests a continuous increase in ester production at 0°C.

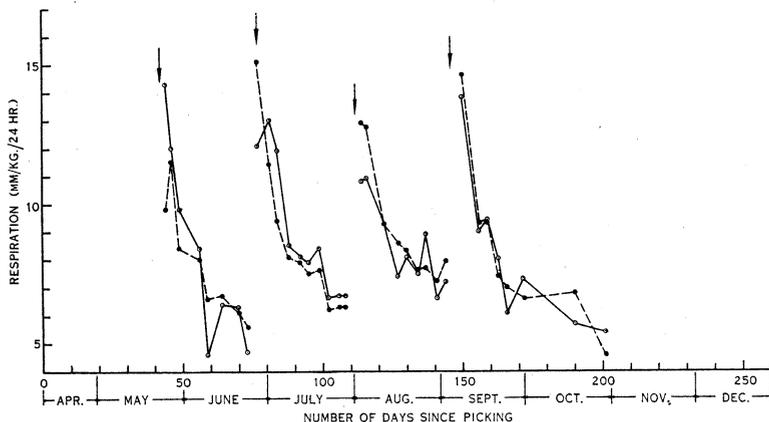


Fig. 5.—Respiration of Granny Smith apples at 20°C. after removal from storage at 0°C. (picked April 12, 1949). Arrows indicate the day on which the apples were removed from 0°C. to 20°C. Curves show duplicate estimations.

The changes in volatile ester production are difficult to interpret without further data. In view of the possibility of a relation between ester production and internal oxygen concentration, which has been shown to decrease with length of storage (Hackney 1943), it was decided to lower the concentration of oxygen in the external atmosphere. The concentration of alcohol, which may be a factor in ester production, usually increases if the concentration of oxygen is reduced sufficiently.

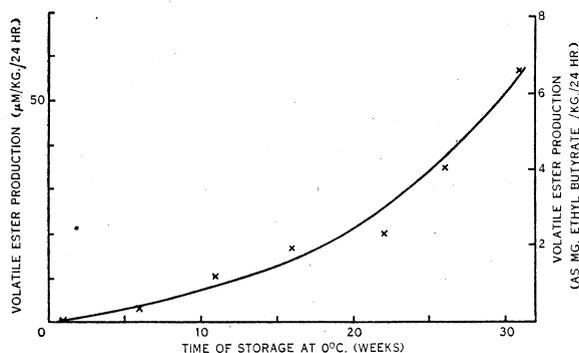


Fig. 6.—Production of volatile esters immediately after removal from storage at 0°C.

IV. EFFECT OF REDUCED OXYGEN CONCENTRATION

Granny Smith apples were picked at Orange on April 19, 1950, and stored at 0°C. After 3 and 8 weeks samples were removed to 20°C. for measurement.



ments of volatile ester production. One 5 kg. sample of each removal was held in air and the other in an atmosphere containing 6 per cent. oxygen and 94 per cent. nitrogen. The rate of gas flow was 20 l./hr. The results are given in Figure 7.

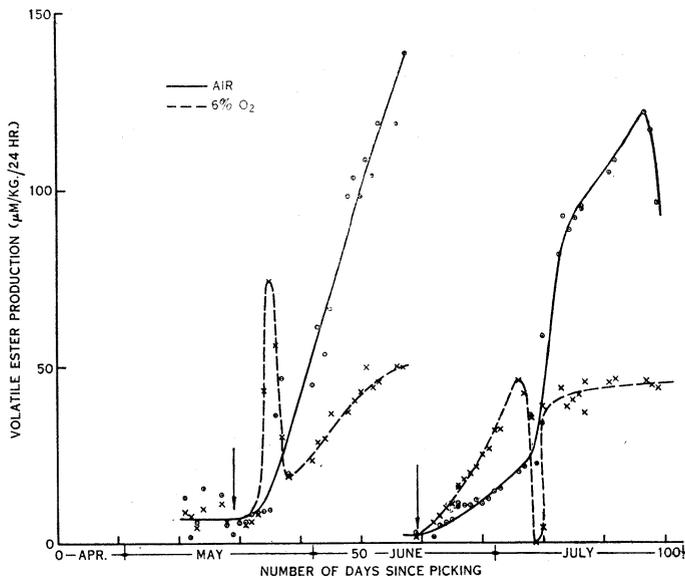


Fig. 7.—Production of volatile esters in air and 6 per cent. oxygen at 20°C. after removal from 0°C. (picked April 19, 1950). Arrows indicate the change from air to 6 per cent. oxygen.

The curves of ester production in air were similar to those obtained in 1949. After an initial delay, ester production increased rapidly. The measurements on the second removal were continued long enough to reach the maximum. Ester production in 6 per cent. oxygen increased rapidly at first to a maximum well above the production in air at that time. It then fell to a minimum and finally increased again. In the final stage the rate of ester production in 6 per cent. oxygen was well below the rate in air.

Measurements of respiration and the composition of the internal atmosphere were made on corresponding samples and the results are given in Figure 8. Reducing the external oxygen reduced the internal oxygen to a similar concentration but had little effect on the internal carbon dioxide or respiration. The respiration tended to fall but not nearly as rapidly as in the previous year.

V. VOLATILE ACIDS AND ALCOHOLS

On completing the measurements of volatile ester production in air and 6 per cent. oxygen, the samples were used for determination of volatile acids and alcohols in the whole fruit. In addition, data were obtained on samples which

had been stored at 0°C. for 10 weeks, and these were regarded as representing the approximate level of acids and alcohols before removal to 20°C. The results are given in Table 1.

TABLE 1
VOLATILE ACIDS AND ALCOHOLS IN GRANNY SMITH APPLES

Days at 20°C.	Acid (mE/kg.)		Alcohol (mE/kg.)	
	Air	6% O ₂	Air	6% O ₂
0	0.33	—	0.20	—
38 (1st removal)	0.72	0.20	1.59	0.64
44 (2nd removal)	0.11	0.13	2.22	0.52

The most important result is that the alcohol content of the fruit increased at 20°C. as well as the ester production. At the end of each experiment the alcohol content in 6 per cent. oxygen was below that in air and corresponded to a lower rate of ester production. This result will be discussed more fully in the next section.

Volatile acid content showed some increase in the first removal in air, but not in the other samples.

VI. DISCUSSION

The drift in volatile ester production in air can be briefly summarized as follows. At 0°C. ester production increased steadily for several months. In samples removed to 20°C. ester production rose to a maximum and then fell. In later removals the rise became less and finally negligible. The increase in ester production appeared to be associated with a greater production and accumulation of alcohols by the fruit.

Production of volatile alcohols can probably be regarded as due to comparatively slow side reactions of the normal aerobic respiration. One would expect the rates of such reactions to depend on the concentration of reduced diphosphopyridine nucleotide (DPNH₂) and other reducing agents. Pyruvic acid, an important intermediate of respiration, can be converted to ethanol by decarboxylation to acetaldehyde and subsequent reduction. The reduction of acetaldehyde is effected by DPNH₂, which results from the reduction of DPN by respiratory substrates such as 3-phosphoglyceraldehyde. One can postulate the formation of *n*-propanol by reduction of pyruvic acid and the formation of longer-chain alcohols by linkage of shorter fragments. Primary alcohols up to caproic have been identified as products of apples (Thompson 1951; Walls 1942; White 1950).

Fidler (1933) has shown a continuous increase in the alcohol content of apples during storage, and attributes it to a gradual replacement of normal oxidative respiration by "zymasis" or fermentation. This and other changes associated with ripening and subsequent deterioration are known collectively as senescence. In oxidative respiration DPNH₂ is oxidized through a series of linked enzyme systems, the last of which uses atmospheric oxygen. A decline

in activity of these oxidative systems (due to decreasing efficiency or lowering of internal oxygen concentration after prolonged storage in air) would increase the concentration of DPNH₂ and lead to increased alcohol production. This is the most probable explanation for the increase of alcohol content in air.

The changes in ester production obtained in 1949 (Fig. 3) can now be considered further. The initial increases in ester production could be due to an increasing concentration of alcohol, but the subsequent fall and the lack of increase after prolonged storage must have another explanation. The available evidence indicates a continuous rise in alcohol content during storage, hence the fall in ester production could be due to a decline in volatile acid production or subsequent esterification.

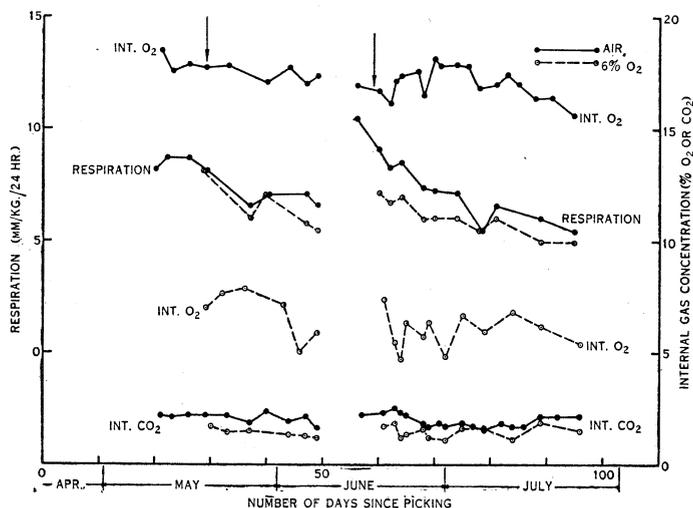


Fig. 8.—Respiration and internal gas concentration in air and 6 per cent. oxygen at 20°C. after removal from 0°C. (picked April 19, 1950). Arrows indicate the change from air to 6 per cent. oxygen.

The effect of reducing the oxygen concentration from 21 to 6 per cent. (Fig. 7) is difficult to interpret. The ester production in 6 per cent. oxygen can be considered in three stages: rise to maximum (1), fall to minimum (2), and subsequent rise (3). Reducing the oxygen might be expected to reduce oxidative metabolism and increase the production of alcohol and esters. This appeared to be the first result of the low oxygen (stage 1), but subsequently an adjustment (stage 2) occurred to nullify this effect.

In stage 3, alcohol content and ester production in 6 per cent. oxygen were less than in air. Ester production increased in 6 per cent. oxygen but much less rapidly than in air. The effect of reduced oxygen at this stage is possibly not a direct effect on oxidative metabolism but a more general effect in delaying senescence. Senescence involves, among other changes, a gradual decline in the efficiency of oxidative systems and an increase of alcohol production.

Reduction of oxygen concentration to 6 per cent. could arrest this decline, at least in the early stages of senescence. However, after prolonged storage the oxidative systems may be unable to function adequately in 6 per cent. oxygen.

As previously mentioned, the ester production of the first picking in 1949 was generally lower than that of the second picking. As earlier pickings are more liable to superficial scald, this result does not support the theory that volatile esters are directly concerned in this disorder. This theory is now somewhat doubtful, as experiments with synthetic esters in this laboratory have failed to provide evidence in its support.

VII. ACKNOWLEDGMENTS

The authors wish to thank Dr. R. N. Robertson for many helpful discussions and suggestions; Mr. T. J. Riley for determinations of respiration and composition of internal atmospheres; and Miss H. Smith and Mr. B. Kennett for technical assistance.

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