CCXX. STUDIES IN ZYMASIS¹.

IV. THE ACCUMULATION OF ZYMASIC PRODUCTS IN APPLES DURING SENESCENCE.

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(Received July 26th, 1933.)

ZYMASIS may be measured either in terms of the CO₂ which escapes or in terms of the products which accumulate². In the work described in the present and in the next two papers we have usually employed the "alcohol number" to evaluate zymasis. This number denotes the sum of the concentrations (in g. per unit mass fresh weight of tissue) of ethyl alcohol and of acetaldehyde found in the fruit by analysis at any time.

The apples used throughout this work were stored for us in air for two seasons (1929-30 and 1930-31) at the Low Temperature Research Station, Cambridge, and for one season (1931-32) at the Ditton Laboratory, East Malling, whence they were sent to Newcastle by post³.

In the present paper we report the changes we have found in the alcohol number by analysing apples (*a*) immediately after their arrival from low temperature stores at different times during the storage season (see Table I and Fig. 1), and (*b*) after subsequent exposure to air under the experimental conditions employed in the work described in the next two papers⁴ (see Table II and Fig. 2).

This is the first time that data concerning zymasis in air by apples have been collected throughout a storage season.

It had earlier been reported that whereas zymasic products do not accumulate in Newton Wonder apples in early storage [Thomas, 1925], zymasis occurs in several varieties of apples and pears during the oxidase browning that follows injury or physiological disease [Thomas, 1929]. So far as we know, however, no data have been published for older apples before browning begins, although for Bartlett pears it is known that "ripening" is accompanied by a type of zymasis during which acetaldehyde accumulates in relatively high concentrations [Harley and Fisher, 1927].

¹ The following three papers constitute Parts I, II and III of "Studies in Zymasis": Thomas, *Biochem. J.* 1925, **19**, 927; *Ann. Appl. Biol.* 1929, **16**, 444; 1931, **18**, 60.

² By zymasis we mean the process of zymase cleavage of carbohydrates that leads to the production and accumulation of ethyl alcohol and often of acetaldehyde (but in relatively smaller amounts) in plant tissues and the simultaneous production and escape of carbon dioxide.

³ We are indebted to Dr Kidd of Cambridge, and Dr West of East Malling, for arranging that these apples should be stored for us in rooms kept at a constant temperature at these Stations.

⁴ The measurements of the alcohol number which are given in Section III constitute the control figures to the experiments carried out during the storage seasons 1929-30, 1930-31, 1931-32 on the production of ethyl alcohol and acetaldehyde by apples in nitrogen, and in nitrogen-oxygen mixtures. The results of these experiments form the subject matter of papers V and VI of this series.

I. MATERIAL.

Newton Wonder and Bramley's Seedling apples picked from trees in Mr Caleb Lee's orchard at Swanley, Kent, have been used in the major experiments which are described in this and in the two following papers. The apples were stored for us in air at 1° in the season 1929-30, and at 4° in the seasons 1930-31 and 1931-32. Samples have been sent to us by post for these researches¹.

II. METHODS.

(i) *The estimation of the alcohol number.*

The method of estimation of the alcohol number will be reported in a later paper in this series. It is essentially that used by Thomas [1925], but modified so that ethyl alcohol and acetaldehyde may be estimated in the same steam distillate.

In the present paper we generally express our results in terms of the "percentage alcohol number." This means the sum in g. of the masses of ethyl alcohol and of the ethyl alcohol equivalent of acetaldehyde found in 100 g. fresh weight of fruit at the time of the analysis. The data in Table III, show that in these experiments the amount of acetaldehyde which accumulated was usually negligible when compared with that of ethyl alcohol. Hence, in the present work, the alcohol number is virtually a measure of the ethyl alcohol content of the fruit².

(ii) *Experimental procedure.*

When apples were received from cool storage, a sample was immediately analysed to determine the percentage alcohol number, *i.e.* the amount of zymasic products which had accumulated in 100 g. fresh weight of the fruit during storage at 1° or 3-4°, *plus* a day in the post. This number will be termed the percentage alcohol number of the "storage controls."

The remaining apples were then used to determine the percentage alcohol numbers of what we shall term in this present paper and in Part VI of this series the "conditions controls." To obtain this number, the apples, before they were analysed, were exposed for a period to the conditions stated below.

The sample of fruit was weighed and placed under a glass hemisphere with a wide equatorial flange, which was then fixed to a ground glass plate by means of paraffin wax. Through the pole of the container passed inlet and outlet tubes for the air stream. The fruit chamber was secured to an iron frame and immersed in a water-bath, the temperature of which was kept within 0.2° of 23° by means of a thermostat. CO₂-free air was drawn through the chamber at the rate of 2 litres per hour for a period of 120 to 140 hours. The issuing gas was bubbled through 50 cc. of concentrated sulphuric acid in a wash-bottle to trap any transpired alcohol. At the end of the period of exposure to the higher temperature, the fruit was re-weighed and analysed. The acid from the wash-bottle was added to aqueous potassium dichromate, and when oxidation was complete the mixture was distilled. The amount of alcohol transpired was calculated from the acetic acid value of this distillate. In Table II allowances have been made for this transpired alcohol.

¹ We are deeply indebted to Dr West for arranging to secure these varieties from a single orchard, and for sending samples from the air stores at the times we wanted them for our experiments.

² This is not always the case. Thus, for example, after CO₂-zymasis [Thomas, 1925], the ratio ethyl alcohol/acetaldehyde is sometimes less than 2/1. The alcohol number would then be over 50 % greater than the ethyl alcohol content of the fruit.

III. RESULTS.

The results for the storage controls are given in Table I and Fig. 1. Table II and Fig. 2 deal similarly with the conditions controls. In Table III are given the amounts of acetaldehyde and ethyl alcohol found in apples at different times during the season 1931-32. The figures in this table have been extracted from the figures for the percentage alcohol number given in Tables I and II.

Table I (see Fig. 1). *Percentage concentration of products of zymasis in air-stored apples (storage controls).*

Variety of fruit	Date	Days in store	Percentage alcohol number	Remarks (state of fruit, etc.)
Newton Wonder:				
Season 1929-30	21. iv. 30	195	0.008	Sound
	16. v. 30	225		
	5. vi. 30	250	0.030	Superficial scald
	12. vi. 30	255	0.025	"
	19. vi. 30	265	0.030	"
	25. vi. 30	270	0.025	"
Season 1930-31	17. vii. 30	From orchard	0.004	Very young. Green. Av. wt. 12 g.
	25. ix. 30	—	0.003	Young. Green. Av. wt. 120 g.
	20. x. 30	27	0.005	Green-yellow. Sound
	9. i. 31	105	0.010	Yellow-green. Wilted
	15. i. 31	112	0.011	"
	23. i. 31	120	0.020	"
Season 1931-32	1. vii. 31	From orchard	0.004	Very young. Green. Av. wt. 8.5 g.
	18. vii. 31	"	0.004	" 20.0 g.
	30. viii. 31	"	0.004	" 70.0 g.
	28. x. 31	28	0.005	Hard. Green
	9. xii. 31	70	0.003	Green to yellow-green
	6. i. 32	97	0.007	Yellow. Internal breakdown
	27. i. 32	119	0.019	Green-yellow. First sign of wrinkling
	25. ii. 32	147	0.018	Yellow. Wilted
Bramley's Seedling:				
Season 1930-31	13. xi. 30	50	0.015	Hard. Green
	20. xi. 30	55	0.010	"
	7. i. 31	105	0.015	Green
	21. i. 31	120	0.030	Green. Wilted. Keeping badly, soft spots on fruit
	28. i. 31	127	0.033	Green-yellow. Slightly wilted
	6. ii. 31	135	0.025	"
	11. ii. 31	142	0.030	Green-yellow. Badly wilted
	4. iii. 31	165	0.050	Badly wilted and scalded
Season 1931-32	1. vii. 31	From orchard	0.003	Small. Green. Av. wt. 23 g.
	18. vii. 31	"	0.004	" 40 g.
	30. viii. 31	"	0.004	Young. Green. Av. wt. 125 g.
	10. xi. 31	40	0.018	Green
	6. i. 32	98	0.014	"
	16. iii. 32	170	0.025	Green-yellow
	1. iv. 32	185	0.045	Green-yellow. First sign of wilting

IV. CONCLUSIONS.

(i) *The increase in ageing apples of the alcohol number of storage controls.* The data reported in Table I, and charted in Fig. 1 confirm the earlier observations of Thomas [1925] that the concentration of zymasic products in Newton Wonder apples early in the storage period does not rise above 0.005 % in cool storage at 1-4°. The alcohol numbers for the storage controls of Bramley's Seedling apples during this same period were usually higher than those for

Table II (see Fig. 2). *Increase in the percentage alcohol number in apples in air at 23° in the respiration chamber (conditions controls).*

No. of exp. 1	State of fruit 2		Date exp. begins 3	Duration of exp. (hrs.) 4	No. of apples in sample 5	Wt. of sample		Percentage alcohol number after 100 hrs.' exposure to air at 23° following low temperature storage (allowing for transpired alcohol) 8	Increase in percentage alcohol number during 100 hrs.' exposure to air at 23° 9
						Initial g. 6	Final g. 7		
Newton Wonder.									
Season 1930-31:									
7	Wilted. scald	Superficial	11. vi. 30	114½	1	—	72.0	0.150	0.120
Season 1930-31:									
20	Green. Hard. Young		1. x. 30	142	1	—	100.5	0.017	0.014
A	Yellow-green, wilted		9. i. 31	117½	1	—	133.5	0.025	0.015
B	Yellow, wilted		16. i. 31	121½	1	—	97.5	0.150	0.140
Season 1931-32:									
76	Green. Hard. Very young		8. vii. 31	116½	12	67.0	60.0	0.024	0.02
C			17. vii. 31	113	6	134.5	132.5	0.019	0.014
84		"	26. viii. 31	122	3	212.0	201.0	0.019	0.015
98	Green		28. x. 31	140	2	321.0	312.5	0.011	0.007
112	Green to yellow-green		10. xii. 31	141½	2	279.0	270.0	0.013	0.010
128	Green-yellow; first sign of wrinkling		27. i. 32	140½	1	97.5	97.0	0.034	0.015
H			10. ii. 32	119½	1	—	—	0.077	0.060
133		"	24. ii. 32	138	1	87.0	83.5	0.040	0.022
Bramley's Seedling.									
Season 1930-31:									
D	Green. Hard		20. xi. 30	118½	1	141.0	138.0	0.022	0.013
E	Yellow-green, slightly wilted		23. i. 31	117	1	—	125.5	0.090	0.060
46		"	29. i. 31	116	1	—	170.0	0.093	0.060
47		"	29. i. 31	116	1	—	130.0	0.100	0.060
48	Green-yellow		30. i. 31	116½	1	—	177.0	0.076	0.043
49	Yellow-green		30. i. 31	116½	1	—	160.0	0.073	0.037
55	Yellow. Badly scalded		5. iii. 31	116	1	—	147.0	0.200	0.150
56		"	6. iii. 31	118	1	—	233.0	0.220	0.170
Season 1931-32:									
75	Green. Hard. Very young		8. vii. 31	116½	8	172.0	161.5	0.010	0.007
F			17. vii. 31	113	5	192.5	189.5	0.010	0.007
88		"	2. ix. 31	140½	2	218.5	218.0	0.016	0.012
105	Green		11. xi. 31	115½	1	207.5	202.0	0.070	0.052
120		"	6. i. 32	138½	1	158.0	157.5	0.074	0.060
137	Green-yellow		16. iii. 32	140	1	167.5	164.0	0.110	0.090
141	Green-yellow; first sign of wilting		31. iii. 32	116½	1	127.5	126.0	0.100	0.050

Up to May 1931, the weight used in arriving at the percentage concentration of products of zymesis was the final weight of the apple. After this it was realised that the use of the final weight was open to objection, and since then the initial weight has been used. The loss of weight is never more than 6 % in a sound apple and is usually of the order of 1 to 3 % and, as all the figures in any series are calculated to the same basis, the error is not serious.

The original experimental numbers are retained for the convenience of such readers as may have access to Fidler's Ph.D. thesis [1932].

The percentage alcohol numbers given in column 9 represent the difference between column 8 and the corresponding percentage alcohol numbers in Table I. They constitute the conditions control values which were used as a basis for measuring zymesis owing to oxygen shortage in the work which is described later [Thomas and Fidler, 1933, Section II].

Newton Wonder apples, but did not significantly exceed 0.015 %. We infer that the rate of production of ethyl alcohol in storage controls during the earlier phase of storage is very slow. If zymesis and cell oxidations are linked processes, this slow rate may be due to the vigour with which some precursor of ethyl alcohol is oxidised during this phase.

Table III. *Production of acetaldehyde and ethyl alcohol in air by Newton Wonder and Bramley's Seedling apples during 1931-32.*

Time of analysis	Variety of apple	Number of exp.	Date	g. per 100 g. apple	
				Acet-aldehyde	Ethyl alcohol
When received from store	Newton	—	1. vii. 31	0.0005	0.003
	Wonder	—	18. vii. 31	0.0006	0.003
		—	30. viii. 31	0.0005	0.004
		—	28. x. 31	0.0003	0.004
		—	9. xii. 31	0.0004	0.003
		—	6. i. 32	0.0005	0.007
		—	27. i. 32	0.0008	0.018
		—	25. ii. 32	0.0028	0.015
	Bramley's	—	1. vii. 31	0.0002	0.003
	Seedling	—	18. vii. 31	0.0005	0.003
		—	30. viii. 31	0.0010	0.003
		—	10. xi. 31	0.0009	0.017
		—	6. i. 32	Nil	0.014
		—	16. iii. 32	0.0019	0.024
		—	1. iv. 32	0.0026	0.042
Following 100 hours' exposure to air at 23°	Newton	76	8. vii. 31	0.0018	0.022
	Wonder	84	26. viii. 31	Nil	0.019
		98	28. x. 31	0.0007	0.011
		112	10. xii. 31	0.0006	0.013
		128	27. i. 32	0.0036	0.030
	H		10. ii. 32	0.0032	0.074
	Bramley's	75	8. vii. 31	0.0005	0.009
	Seedling	88	2. ix. 31	0.0006	0.015
		105	11. xi. 31	0.0024	0.067
		120	6. i. 32	0.0033	0.071
		137	16. iii. 32	0.0043	0.108
		141	31. iii. 32	0.0047	0.090

The change in colour of an apple to a yellow-green, and, at a later date, the incidence of severe wilting and superficial scald, which occurs when the apple is full yellow, are the only external indications of the physiological state of the fruit. In wilted apples the skin can readily be pushed into ridges and later becomes noticeably wrinkled. Superficial scald means that there are dry brown patches just under the skin: this condition may be succeeded by deep scald, in which the whole of the skin is involved, and the browning extends deeply into the flesh of the fruit.

It will be seen from Fig. 1 that as Newton Wonder and Bramley's Seedling apples change in colour from green to green-yellow, the alcohol numbers of the apples rise, and as the apples become more yellow and wilted, the graph of the alcohol number takes on a steep upward path¹. The data of Table III indicate that this rise in the alcohol number is compounded of a rise in both the ethyl alcohol and acetaldehyde contents of the apples. The highest recorded values for the percentage alcohol numbers in apples which show no browning, from an air store, are 0.03 in Newton Wonder apples in June 1930, and 0.05 in Bramley's Seedling apples in March 1931.

¹ A few experiments permit us to compare the Cleopatra apple, a variety shipped from overseas, with these two varieties of home-grown apples. Thus whereas the percentage alcohol number of green-yellow Cleopatras was less than 0.007, this number had increased to 0.023 by the time this variety had turned yellow. We note that the alcohol number of apples showing no browning from ships' holds which have been well ventilated may indicate the occurrence of recent zymasis. But there is no evidence yet of the alcohol number in apples from well ventilated air stores reaching the magnitudes found in the flesh tissue after faulty ventilation [Thomas, 1931].

It appears, therefore, that the rate of zymasis after a slow initial period of varying extent becomes increasingly accelerated as the apples age further. Possibly there is always a critical phase in the storage life of the fruit when certain changes become autocatalytic. Thus in a rough grouping of Newton Wonder apples we might place in one class those apples in which the percentage alcohol number is not greater than 0.005, and in the other those apples in which the alcohol number is steadily increasing beyond this figure. Similarly, for Bramley's Seedling apples, the line of demarcation may be placed at 0.015. In our view apples in the second class (*i.e.* those which have reached the critical phase) cannot be regarded as physiologically healthy, for, in our experience, when zymasis becomes relatively rapid the browning of the flesh tissue is not far distant.

Fig. 1. Increase in percentage alcohol number in apples during storage.

Fig. 2. Percentage alcohol number in apples after 100 hours' exposure to air at 23°, following (a) removal from the tree during the growing period, or (b) low temperature storage (1930-31)¹.

(ii) *The increase in ageing apples of the alcohol number of conditions controls.* The data in column 9 in Table II and the differences in the heights above the x-axis of comparable points in Figs. 1 and 2, show that the effect of placing

¹ We have never observed the alcohol number of an apple to rise until some months after it is removed from the tree. Accordingly, part of the graph for Bramley's Seedling apples during 1931-32 has been drawn with a broken line at a level which we consider approximately to represent the true state of affairs.

Newton Wonder and Bramley's Seedling apples¹ in a closed space in moving air at 23° at any time in the storage season is to increase the alcohol number². Thus, for example, except in young Bramley's Seedling apples, the alcohol number for apples after 5 days' exposure to air, under experimental conditions at 23°, is higher than in apples received from a 4° store 1 to 2 months later. The cause of this may only be suggested. The two factors that, either together or singly, may influence zymasis are humidity and temperature. Of the effect of humidity as a single factor, nothing is known. It may well be that the question is related to temperature alone. Although the temperature of the experiment is 20° above that of the store, yet it is only 8° above the average temperature of the laboratory, so that the oxygen content of the intercellular spaces of the fruit should still be adequate for aerobic respiration.

It is well known that increase in temperature accelerates the rate of senescence in apples. Possibly one of the features of senescence is the progressive retardation of oxidative processes, and this may be the factor which leads to the observed increase in the alcohol number.

Changes in the alcohol number of the conditions controls at different periods in the storage season point to the same conclusions that we reached from the data given in the last sub-section. Thus the form of the graphs for the conditions controls is similar to that of the graphs in Fig. 1 for the storage controls, the rise in the alcohol number occurring at the same state of the fruit³. Once more the results (see Table II) may be divided into two groups. Before a certain critical phase the alcohol number of a conditions control, although higher than that of a storage control, remains relatively low. Later, when senescent characters are seen the disturbance in metabolism leading to zymasis becomes increasingly marked, as is evidenced by the rapid increase in the alcohol number of the conditions controls.

SUMMARY.

1. Little zymasis occurs in Newton Wonder and Bramley's Seedling apples at the beginning of the storage season and up to the beginning of the change from green to yellow-green, when these varieties are stored in air at 1-4°.

2. Senescence is accompanied by a change in the respiratory metabolism of the cells of the fruit, leading to the accumulation in an apple stored in air of considerable amounts of ethyl alcohol and smaller amounts of acetaldehyde, *i.e.* the alcohol number of the apple rises.

3. Increase of temperature, which is known to accelerate the rate of senescence in an apple, leads to an increase in the alcohol number of the apple.

The work described in this, and in the following paper, was carried out in the Botany Department, Armstrong College, under the supervision of

¹ The Cleopatra apple responded similarly to this transfer to experimental conditions, thus in green to green-yellow apples the percentage alcohol number increased by 0.005 during 100 hours' exposure to air under experimental conditions at 23°.

² Hence, to eliminate the effect of experimental conditions as such, conditions controls and not storage controls should always be used in quantitative researches in which zymasis is induced by shortage of oxygen or by the presence of foreign substances [see *e.g.* Thomas and Fidler, 1933].

³ It is not claimed that zymasis in air at 23° may be calculated from Fig. 2. Regarding the increased zymasis in air, there are two alternatives: (*a*) that the increased rate may be maintained over long periods, and (*b*) that the increase may take place only at the beginning of the exposure to the new experimental conditions. The present research is not concerned with the elucidation of these questions.

Mr Meirion Thomas, to whom I am indebted for continued advice and helpful criticism. My thanks are due to Armstrong College for a College Research Studentship during 1929–30, and to the Department of Scientific and Industrial Research for maintenance grants during 1929–30, and 1930–31, and for payment as a Research Assistant during 1931–32.

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