100. THE FERMENTATION PROCESS IN TEA MANUFACTURE

7. THE INFLUENCE OF EXTERNAL FACTORS ON FERMENTATION RATE

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DURING the course of investigations on the mechanism of tea fermentation carried out in this laboratory in the past four years many incidental observations have been made on the effect of various factors on the rate and extent of fermentation. In this communication are summarized such of these observations as appear to have some general scientific appeal.

Factors affecting fermentation may conveniently be divided into two classes, those modifying the nature of the leaf plucked for tea manufacture, and those in which variations in the course of manufacture affect the nature of the finished product. These two classes will be considered separately.

Factors affecting the nature of the green leaf

A full study of such factors would involve variations due to season and climate, to forms of manuring, to the presence or absence of shade, and to different techniques of plucking and pruning the bushes, to mention only some of the possibilities. No one of these factors has been studied exhaustively but it is possible to state that variations in oxidase activity over the whole season show small but erratic differences, and that the effect of manuring, if it exists, is but a small one. Two other factors, coarseness of plucking and variations associated with individual bushes, have received some attention.

Coarseness of plucking. Although shoots plucked for manufacture generally approximate to 'two leaves and a bud', coarser forms of plucking are sometimes found. In the coarse forms of plucking there may be shoots consisting of three or four leaves and a bud. A shoot consisting of four leaves and a bud is illustrated in Fig. 1.

Fig. 1: Tea shoot, four leaves and a bud.

One of the most convenient methods of studying the effect of coarseness of plucking on fermentation is to determine the gradient of tannin and oxidase, that is to say, the distribution of these substances in the component parts of the shoot. A large number of shoots are divided into bud, 1st leaf, 2nd leaf etc., and

stalk. Oxidase activities of such samples are determined manometrically and expressed as initial rates of uptake per hr. for 1 mg. dry weight (Q_{O_2}), and tannin contents are estimated by the Lowenthal method.

Table 1

	Tannin % dry weight	Q_{O_2}	Moisture
Bud	26.5	20.1	75.5
1st leaf	25.9	17.5	73.0
2nd leaf	21.9	17.7	73.8
3rd leaf	18.6	17.5	73.8
Stalk	14.4	31.2	85.1

Table 1 records typical results found for these gradients. The coarser the plucking the greater is the percentage of 3rd (and 4th) leaf and stalk in the shoots and this results in a net reduction in the percentage of tannin in the shoots but the oxidase activity remains relatively unaffected. One would expect therefore that with increasing coarseness of plucking the rate of fermentation would be unchanged, but that with less tannin to be oxidized, the process would require less time.

Confirmatory experiments on shoots showed that the tannin content of the whole shoot decreased appreciably with increasing coarseness of plucking whereas oxidase activity increased only very slightly, although significantly.

The extent of tannin oxidation at a given stage in fermentation may be determined indirectly. The partly fermented tissue is ground to a paste, suspended in water, and the uptake of O_2 determined manometrically (residual uptake). If the amount of O_2 consumed in the fermentation of the fresh or withered leaf has been previously determined, subtraction of the residual uptake from the figure for fresh leaf gives the O_2 consumption, and hence proportionately the extent of tannin oxidation, in the partly fermented leaf. In this way it was shown that the percentage oxidations in two samples of leaf, differing only in their coarseness of plucking, were almost identical (64%) after 90 min. fermentation. At no time have significant differences been observed between the times required for fermentation of finely and coarsely plucked leaf.

This is contrary to expectation and it is evident that some other factor is involved in addition to enzymic activity and tannin content. It is suggested that a permeability factor is concerned, and that on account of lower permeability in the coarser tissue, fermentation is slowed down. The possibility that permeability factors play some part in determining the rate of tea fermentation is also indicated by the variation of this rate with different degrees of withering. This will be discussed in more detail in a later section of this paper.

Varietal effects. In a previous communication [Roberts, 1939, 2] the variation of fermentation rate with jat, or commercial brand, was discussed. Significant differences between some jats were found, and this work has since been abundantly confirmed. The work of the Botanical Department at Tocklai, however, has led to the conclusion that the species, *Camellia Thea* Link, is to be considered as a linear series of forms between two extreme types, with possibilities of hybrid formation between the extremes. As one jat may contain several of such forms it is clear that the jat is not a satisfactory means of classification for scientific purposes.

It is as yet too early to state whether taxonomic intervals in such a series have any effect on oxidase activity in the tea leaf but experiments will shortly be started to investigate this possibility. Meanwhile it has been established that oxidase activity within a jat varies much more than between two jats.

Thus three jats were compared over a whole season and the average Q_{O_2} figures obtained were as follows:

Betjan	China	Kharikatia
20.8 ± 0.5	20.8 ± 0.6	17.6 ± 0.6

The Kharikatia has a significantly lower oxidase content than the other jats.

Results for leaf from single bushes within the Betjan jat show that the Q_{O_2} may vary from 18.6 to 31.4, a much greater range than the difference between fast- and slow-fermenting jats. With such heterogeneity of material within a single jat it is clear that the establishment of differences, such as are found between Kharikatia and Betjan for example, is dependent upon accurate sampling. While it is considered that sufficient attention was paid to sampling in the experiments in which fermentation rates of different jats were compared, it is now emphasized that these differences represent a comparison of two heterogeneous populations, and have no varietal significance.

Relation between Q_{O_2} of fresh leaf and its rate of fermentation. So far in these studies it has been more or less assumed that the Q_{O_2} of fresh leaf as measured manometrically gives a reasonably accurate estimation of its oxidase activity. In a series of empirical experiments, designed to measure the effects of variations of time and temperature of fermentation on the quality of different commercial varieties of tea, measurements were incidentally made of the Q_{O_2} of the fresh

Fig. 2.

leaf and of the extent of oxidation after $2\frac{1}{2}$ hr. fermentation at 80° F. A very highly significant correlation ($r=0.8663$, $P<0.1\%$) was obtained between these two variables. It is evident that despite errors introduced by the impossibility of obtaining the same degree of wither in each experiment the Q_{O_2} value largely determined the rate of fermentation of the leaf under factory conditions (Fig. 2).

Variations in the course of manufacture

Withering. In the manufacture of tea in N.E. India the leaf is generally subjected to a preliminary withering process before mechanical rolling. The leaf is spread thinly, about 1 lb. per square yard, on racks and allowed to wither for about 18 hr. This process is generally uncontrolled and the extent of loss of moisture is determined by the temperature and humidity of the atmosphere and the exposure of the leaf to the wind. A normal good wither results in the reduction of the moisture content of the leaf from about 77 to 65–70 %. The leaf has then lost its turgidity and can be twisted without smashing the tissue. In addition to this physical preparation of the leaf for rolling, chemical changes occur and there are also modifications in the activities of both hydrolytic and oxidizing enzymes [Kursanov, 1935].

When oxidase activity is measured by the manometric method it is observed that withering is always associated with a loss in oxidase activity, and this loss increases progressively with the degree of wither. In one experiment the wither was controlled by varying the thickness of spread of the leaf, and in this way three different withers were obtained. Table 2 shows the variation in Q_{O_2} as a result of the different withers.

Table 2

Sample	Moisture content	Q_{O_2}
Fresh	79.10	18.4
Withered	65.35	16.6
Withered	61.50	14.2
Withered	55.60	13.4

Each determination of Q_{O_2} is a mean of triplicates in good agreement.

In all, 16 experiments were carried out in which the oxidase content of fresh leaf was compared with that of withered leaf and in each case there was a significant decrease in Q_{O_2} as a result of withering. The extent of this decrease is irregular and is probably determined to some extent by the nature of the leaf and the rate of moisture loss.

As a result of the correlation found between Q_{O_2} values for fresh leaf, and the rate of fermentation under factory conditions, withered leaf would be expected to ferment more slowly than unwithered leaf. This is not realized except in the cases where the leaf is definitely over-withered (i.e. to 50 % moisture).

Previous experiments [Roberts, 1939, 1] had shown that withering has no detectable effect on the rate of oxidation of tannins, but these results must be qualified as a result of the development of the above method for estimating the extent of tannin oxidation in partly fermented leaf by measurement of residual O_2 uptake, which gives much more accurate results than the Lowenthal titrimetric method. It now appears that withering at first slightly increases the rate of fermentation but that as the degree of wither becomes higher the rate of fermentation again falls, and that finally with very high withers (i.e. to 50 % moisture or less) the rate is very appreciably diminished.

Two series of experiments have formed the basis of these conclusions.

In the first of these a normal wither, i.e. to about 70 % moisture is compared with a very decided under-wither. The latter was realized by thick spreading of the leaf, 3 lb. to the square yard, during the whole process. Table 3 gives the residual uptakes recorded, after 2 hr. fermentation, for the two types of wither.

Table 3

Date	$\mu\text{l. O}_2$ per mg. dry weight of tissue	
	Normal wither	Under-wither
14. vi. 39	4.00	4.95
	4.19	4.99
	3.82	4.81
	4.46	5.08
4. vii. 39	2.74	3.50
	3.08	3.61
	3.88	4.28
	3.05	4.06
21. vii. 39	3.72	3.84
	3.33	3.94
	3.64	3.89
	Mean 3.63	4.27

Sig. diff. ($P=0.1\%$) 0.33

Subjecting these figures to an analysis of variance it may be shown that the residual uptake of the under-withered leaf is significantly greater than that of the normally withered leaf, on the 0.1 % level of probability. The higher residual uptake of the under-withered leaf implies that more fermentation has taken place in the normally withered leaf. The total uptake for withered leaf after fine mincing averages about 12.0 $\mu\text{l. O}_2$ per mg. dry wt. so that the uptakes at the time of sampling were 8.37 and 7.73 $\mu\text{l. O}_2$ respectively. The rate of fermentation was therefore about 7–8 % faster in the higher-withered samples. A significant difference of this order would not easily be found by the Lowenthal method with this number of repeats.

The second experiment was not primarily designed to show the relation between withering and rate of fermentation. However, in a series of manufacturing experiments carried out during 1940, data were incidentally accumulated on the extent of oxidation after 2 hr. fermentation, and the degree of wither, this latter being measured as the weight of the withered leaf as a percentage of the fresh. The results are recorded in Fig. 3 which shows a significant correlation between these two variables ($r=0.7885$, $P=2\%$). Although this correlation rests on eight observations only, it has frequently been observed during the recent manufacturing season that with over-withered leaf, i.e. leaf in which the moisture has fallen to 55 % moisture or less, the extent of fermentation in a given time is distinctly less than obtained after a normal wither. In all cases where the wither was over-high the colour of the aqueous extract of the fermented leaf was much paler than normally observed for the same time and temperature of fermentation. This indicates a lower extent of fermentation.

These experiments show therefore that the effect of withering on fermentation is not a simple one. It is suggested that as oxidase activity decreases progressively

Fig. 3.

with increasing degree of wither some permeability factor may be involved. In the early stages of withering increased permeability may reverse the effect due to decreased oxidase activity but as oxidase inactivation increases such factors can no longer compensate for this and rate of fermentation commences to fall.

I am intentionally vague about the nature of this permeability factor, but experiments indicate that it may be associated with an increase in the solubility in water of the oxidase and hence with its diffusibility within the cell.

Although the oxidase activity of tea leaf is largely associated with insoluble matter the juice expressed from green leaf contains quite a considerable amount of the oxidase. As no high-speed centrifuge is available in this laboratory it is impossible to state whether the oxidase in the juice is in true solution or in a high state of dispersion. In the following discussion therefore water-soluble oxidase will include any of the enzyme too highly dispersed to be separated out at 2000–3000 r.p.m. in the centrifuge.

During withering there is a considerable loss of moisture so that there is a concentration of solutes in the juice expressed from the leaf. The juice from withered leaf would therefore be expected to have a higher oxidase activity per ml. than juice from fresh green leaf. Measurements of the rate of O_2 uptake by juice show that the increase is distinctly greater than can be accounted for by concentration of solutes only. Results of two such experiments are quoted in Table 4.

Table 4

Leaf	Solid matter mg. per ml. juice	Initial rate of O_2 uptake μ l. per hr.	
		Per mg. dry weight	Per ml. juice
Exp. 1. Fresh	88	2.5	220
Withered	164	5.3	868
Exp. 2. Fresh	71	8.8	624
Withered	141	14.8	2088

In Exp. 1 uptakes are measured for 3 ml. juice without dilution. In Exp. 2 0.5 ml. juice, diluted to 3 ml. with water, is measured into each Warburg vessel.

The faster rates of uptake in Exp. 2 are due to the concentration effect referred to in a previous paper in this series [Roberts, 1940, 2]. As the enzyme concentration in the juice from withered leaf is greater than that from fresh leaf the recorded activities in the former case are probably too low, but even without making corrections for this effect it is clear from the above figures that the water-soluble oxidase has increased considerably as a result of withering, and is at least double that to be expected if concentration of the juice by evaporation were the only effect of withering. Where the concentration of the juice, in mg. solid matter per ml., has been doubled by withering, the increase in the enzymic activity is a fourfold one. It is evident that the withering process is associated with increased dispersion of the oxidase.

Confirmation of this is found in the recently published work of Lamb & Sreerangachar [1940] who also observe an increase in the water-solubility of the tea oxidase from Ceylon leaf as a result of withering.

It is possible that the increased solubility of the enzyme, by favouring its greater dispersion, results in a slightly faster rate of tannin oxidation in the fermenting tea leaf despite a net reduction in enzymic activity. Whether this is the true explanation of the 'permeability' effect remains uncertain but until more definite information is available it will serve as a hypothesis to account for the observed facts.

Lately, in attempting to separate chloroplast material from green tea leaf by the method of Neish [1939], it has been noticed that the fractions obtained have a very considerable oxidase activity. Unfortunately these fractions were not of purely chloroplastic origin but it is tempting to conclude that oxidase activity within the tea leaf may be associated with chloroplastic matter. Now it has been shown by Bokuchava [1935] that there is a marked fall in the chlorophyll content of the tea leaf as a result of withering. There is thus some evidence suggesting that the net fall in oxidase activity as a result of withering, and possibly the increased dispersion of the enzyme, are associated with degradation of the chloroplasts.

Temperature. The rate of tannin oxidation in suspensions of finely ground tea leaf tissue was found by Harrison & Roberts [1939] to be increased by only some 30 % when the temperature was raised from 25° to 40°. The probable effect of temperature differences on the degree of condensation of the tannins in fermented tea was also mentioned.

During the manufacturing seasons of 1939 and 1940 these effects have received considerable attention and the original statements can now be somewhat amplified. Discussion of the effect of temperature difference on condensation of tannins will be reserved for a communication now in course of preparation.

It must be emphasized that the results of manometric experiments on the variability of fermentation rate with temperature do not necessarily apply to factory conditions, as substrate concentrations and degree of condensation of tannins are both affected by the suspension of the tissue in excess of water. Failing direct methods the residual uptake method has been employed to study the effect of temperature on fermentation. Measurements of the extent of fermentation are made after a period of 2 hr. The employment of a difference method, such as the determination of the residual uptake, affects the accuracy of these measurements and there are further obstacles to the accurate determination of the extent of oxidation in the absence of strict control of temperatures in the earlier stages of manufacture.

In the miniature factory at Tocklai the withered leaf is mechanically rolled for 30 min. and further bruising is then caused by passing through a McKercher C.T.C. machine. The leaf is then bulked and sampled before transferring to air-conditioned cabinets for the fermentation at controlled temperatures. Reference to Tables 5 and 6 will show that after a fermentation time of 2-2½ hr., which includes the preliminary rolling and subsequent treatment, considerably more than half the total oxidation has taken place. This period of fermentation is overlong to compare rates with accuracy, but shorter periods cannot be used owing to the absence of proper control over the temperature for the 1st hr.

As yet we have found no rapid method of bringing about the necessary damage to the shoots. A coarse mince is not completely effective as a large proportion of the leaf escapes damage as is shown by the persistence of anaerobic fermentation [cf. Deb & Roberts, 1940]. As a means of estimating the variability of enzymic activity at different temperatures the author therefore makes no claim of high precision for this method. However for practical purposes in the factory it does measure the effect of different temperatures of fermentation with some accuracy, and as such is of considerable value.

The results obtained by this method will now be discussed and it will be seen that, despite its imperfections, conclusions of some importance can be drawn.

In the 1939 experiment 16 comparisons were made between the extent of oxidation after 2½ hr. fermentation at 80° and 90° F. respectively. These results are recorded in Table 5.

Table 5

$\mu\text{l. O}_2$ per mg. dry weight.				
Total uptake of fresh leaf	Residual uptakes after 2½ hr. fermentation		Uptakes up to the time of sampling	
	80° F.	90° F.	80° F.	90° F.
11-64	2.24	1.98	9.40	9.66
11-30	1.93	1.70	9.37	9.60
11-92	1.59	1.22	10.33	10.70
12-67	2.75	1.40	9.92	11.27
11-09	2.00	1.86	9.09	9.23
12-55	2.69	2.58	9.86	9.97
11-81	2.76	2.62	9.05	9.19
12-59	3.27	2.94	9.32	9.65
11-58	1.52	1.81	10.06	9.77
12-23	2.54	2.48	9.69	9.75
11-60	3.55	3.05	8.05	8.55
11-75	3.95	3.76	7.80	7.99
11-95	1.89	1.93	10.06	10.02
12-09	3.03	3.34	9.06	8.75
12-82	3.48	3.17	9.34	9.65
12-72	3.70	3.78	9.02	8.94
Mean 9.34			9.54	

The increase in oxidation at the higher temperature of fermentation just falls short of significance. The value for 't' is 2.115 whereas 't' should equal 2.131 for significance on the 5% level of probability.

In the 1940 experiments two sets of comparisons were made at 60° F. compared with 80° F., and 70° F. with 90° F. Each treatment was replicated three times on three different occasions. It was impossible to compare the four temperatures of fermentation on the same day so that each pair of comparisons must be considered separately. Table 6 records the uptakes, obtained by difference as in Table 5, after 2 hr. fermentation at each temperature.

Table 6

$\mu\text{l. O}_2$ per mg. dry weight.					
Date	60° F.	80° F.	Date	70° F.	90° F.
20. viii. 40	6.70	8.04	13. viii. 40	8.30	8.77
	7.35	8.20		8.01	8.89
	7.81	8.10		8.01	9.01
10. ix. 40	5.80	7.53	3. ix. 40	7.59	8.82
	7.08	7.58		8.14	8.32
	5.62	7.75		7.98	8.28
24. ix. 40	6.70	9.06	17. ix. 40	6.68	6.88
	7.46	9.51		6.93	7.38
	7.08	9.47		6.68	7.51
Mean 6.84		8.36	7.59		8.21
Sig. diff. $P=5\%$ 0.45			0.25		

The conclusions we can draw from these results are as follows:

- (1) There is a marked increase in the rate of fermentation when the temperature is increased from 60° to 80° F.
- (2) The acceleration in the rate for the 20° F. rise between 70° and 90° F., although significant, is distinctly less than between 60° and 80° F.
- (3) An increase in fermentation rate above 80° F. is small and also just short of statistical significance.

The data are insufficient to construct an enzymic activity-temperature curve but it is clear that the maximum temperature for the enzyme system is not far removed from 85° F. This low maximum temperature accounts for the abnormally small increase in enzymic activity between 25° and 40° previously established for suspensions of ground tea leaf tissue.

It is rather unusual to find so low a maximum temperature as this for an enzyme. Experiments were therefore undertaken to determine the extent of enzymic inactivation during the course of fermentation.

The method employed for estimating oxidase activity of partly fermented leaf consists of adding 3 ml. of a green leaf infusion (50 g./500 ml.) to 150 mg. of the finely ground fermented leaf and recording O₂ uptakes over a period of 30 min. As unoxidized substrate is present in excess the uptake should be proportional to oxidase activity.

Results for three different times and two temperatures of fermentation are given in Table 7. All results are means of triplicates in good agreement.

Table 7

Uptakes, μ l. O ₂ /30 min. per mg. dry weight.			
hr. ...	2	4½	5½
70° F.	9.5	8.9	8.9
90° F.	8.4	6.6	5.1

Very little enzymic inactivation is apparent at 70° F., the figures being very little lower than those recorded for withered unfermented leaf. At 90° F., however, enzymic inactivation is quite marked and after 5½ hr., about half the original activity only is retained. It is suggested that the higher condensation products of oxidized tea tannin are responsible for the inactivation of the enzymes. At 70° F. even after 5½ hr. fermentation the degree of condensation is not high and the oxidase remains, comparatively speaking, uninhibited.

Although marked inactivation of oxidase occurs at 90° F. this is not sufficient after 2 hr. fermentation to account for the relatively low temperature coefficient between 80° and 90° F. It therefore appears that the oxidase of tea leaf has in fact an abnormally low maximum temperature, and that inactivation of the enzyme by the products of its activity is only of secondary importance.

The maximum temperature of the tea oxidase has also been studied by Lamb & Sreerangachar [1940], and it was shown that the water-insoluble form of the oxidase had a maximum temperature of 27° in excellent agreement with my figure for the whole fermentation process. The conditions under which the Ceylon experiments were carried out were rather different from those in my experiments, for the rate of tannin oxidation was determined in relatively dilute solution and the leaf was obtained from bushes living under much more temperate conditions. Although it is not certain that these differences would materially affect the maximum temperature of an enzyme it is possible that the agreement found may later prove to be less complete.

Fermentation in O₂. Results have already been published [Roberts, 1940, 1] which show that tea fermentation as measured manometrically is accelerated in an atmosphere of O₂. Factory experiments confirm this acceleration. If mechanically rolled leaf is placed in a drum through which O₂ is passing, oxidation of the tannins is brought to the same stage in 25 min. as in a period of 2 hr. or more in a normal atmosphere (Table 8).

The extents of oxidation in the two processes are comparable, amounting to 77 and 75 % completion in the control and oxy-fermentation processes respectively.

Table 8. *Residual O₂ uptakes of normally fermented teas and teas fermented for 25 min. in O₂*

$\mu\text{l. O}_2$ per mg. dry weight.		
Date	Control	Fermented in O ₂
24. v. 40	3.60	4.40
28. v. 40	3.10	3.00
29. v. 40	2.53	3.23
19. vi. 40	2.51	2.23
23. vii. 40	2.51	3.03
24. vii. 40	2.48	2.48
15. xi. 40	2.73	2.97
15. xi. 40	2.07	2.17
Mean	2.69	2.94
Uptakes during fermentation		9.31
		9.06

Bacterial contamination. The bacterial flora normally found associated with the tea leaf have no significant effect on the rate or extent of fermentation although Benton [1938] has recorded the adverse effect on quality resulting from heavy contamination and has observed a sharp drop in bacterial numbers on crushing the leaf. This the author attributes to the bactericidal effects of *o*-quinones formed in the course of fermentation. The concentration of *o*-quinone, as a short-lived intermediate product, will be proportional to its rate of production and hence to the rate of O₂ uptake. In agreement with this it is found by Benton that bacterial numbers tend to rise again in the closing stages of fermentation, i.e. when *o*-quinone concentration is again low.

If the bacterial population is brought to levels considerably greater than would normally be encountered in the course of manufacture, the rate of tannin oxidation is accelerated, as is indicated by a significant decrease in the residual O₂ uptake of partly fermented samples. In one experiment the residual O₂ uptake of the sample with the high bacterial population was $2.0 \pm 0.23 \mu\text{l.}$ per mg. dry wt. after 2 hr. fermentation, whereas the residual O₂ uptake of the control sample was $3.0 \pm 0.17 \mu\text{l.}$ This difference indicates that oxidation of tannins in the artificially infected sample is distinctly more rapid. The direct manometric method shows no effect of bacteria on fermentation rate. It is thought that the effect may be due to local development of NH₃ leading to autoxidation of the tannins. If the tissue is suspended in excess water such effects cannot be realized as any NH₃ formed would be immediately diluted and rendered non-effective.

DISCUSSION AND SUMMARY

A consideration of all the above results leads to the conclusion that the oxidase activity, as determined by the direct manometric method, does not always completely determine the rate of tea fermentation. Other factors may also play some part and the only accurate method of measuring fermentation rates is that referred to in this communication as the residual uptake method. The reliability of this method is shown by the fact that when the later stages of fermentation are carried out in the Warburg apparatus the rate and extent of oxidation is the same no matter how the initial stages were carried out. Thus if factory-treated leaf is fermented until half of the tannins are oxidized, and then ground to a paste and suspended in water in Warburg vessels, the O₂ uptakes then recorded are precisely the same as those found for the latter half of the fermentation when the process is carried out entirely in the Warburg apparatus.

The residual uptake method enables us to determine how far the oxidase content controls the rate of fermentation.

The high correlation ($r=0.8663$) found between the Q_{O_2} values for fresh green leaf and the rate of fermentation of this leaf under factory conditions shows that it is the oxidase activity which largely determines the rate of fermentation. In these experiments the wither was a variable and uncontrolled factor, but in no case was the leaf withered to less than 65 % moisture so that the effect due to withering was a comparatively small one. The differences in fermentation rates between different jats (Betjan and Kharikatia) so frequently referred to in this series is another case where the oxidase activity is the most important factor controlling the process. The increase in rate of fermentation in O_2 is also approximately proportional to the increase of Q_{O_2} of the fermenting leaf as determined manometrically.

These however are the only cases where a direct measurement of Q_{O_2} affords an accurate estimate of the speed of the fermentation process under factory conditions.

In the other cases investigated, other factors in addition to oxidase activity are affected and rate of fermentation is no longer so closely correlated with the Q_{O_2} of the leaf, as determined manometrically. Permeability factors may vary as in the case of shoots varying in their coarseness of plucking. A loss in total oxidase activity during withering may be partly offset by a greater ease of diffusion of the enzymes within the tissue. Where rates of fermentation vary with temperature entirely different results may be obtained by the direct manometric and the residual uptake methods. At 36° little oxidase activity is lost by the time tannin oxidation is complete when the fermentation is carried out in Warburg vessels containing 200 mg. of tissue suspended in 3 ml. water. On the other hand at 32° (90° F.) the results show that roughly half of the oxidase is destroyed when the fermentation takes place under factory conditions. It appears that, as a result of the dilution with water, the tannins are less readily condensed in the former case and hence enzymic inactivation is less marked. The increase in oxidase activity with temperature is therefore likely to be less marked under factory conditions than would be expected from the results of manometric experiments.

A third way in which fermentation rates may be affected without apparently affecting the Q_{O_2} values is shown in the experiment with bacteria. The significant increase in the rate of tannin oxidation that occurs after a heavy inoculation with bacteria is not observed when the tissue is suspended in water.

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