TEA FERMENTATION—PART IV.

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THEORIES PUT FORWARD TO EXPLAIN THE FERMENTATION OF TEA.

It was pointed out in the first of these articles that fermenting tea requires oxygen for the process, and that the utilisation of oxygen was accompanied by certain changes in the leaf constituents, especially in the tannin fraction of the leaf. A good deal of time has been given to the study of the mechanism of this action, but it must be acknowledged at the start that work on this subject has not yet clarified the position to any great extent. In previous articles an account has been given of the investigations dealing with the effect of different conditions of temperature, humidity, etc., on the final product without probing very closely into the process step by step. This empirical method of investigating tea manufacture, is of great service, but its limitations prevent the elucidation of the problem as a whole, and a complete control of the process of manufacture cannot be obtained until a good deal more is known of the chemistry of the leaf and of the mechanism which is responsible for the rapid oxidation, and the development of those characteristic properties of black tea.

Certain hypotheses have been put forward to explain how the reactions taking place during tea manufacture occur, and experiments have been carried out to test these hypotheses but, although the results of these experiments were not very conclusive, and the authors generally guarded in stating their conclusions, I find that in many quarters one or other of these unproved hypotheses is accepted as definitely proved, whereas the real position is very indefinite, and nothing has been proved one way or the other. An attempt will therefore be made to give a short account of some of the experiments carried out, and to demonstrate the real contribution which such work has made towards a solution of the problems concerned. In the meantime, progress will be on surer lines if the question of the mechanism of tea fermentation is approached with an open mind, and adhesion to hidebound conceptions given up in favour of strict observation and careful experiment.

The first speculation concerning the process of tea fermentation contended that it was a putrefactive change, but it was shown by the experiments of van Romburgh, Lohmann and Bamber that oxygen is necessary for oxidation, since rolled leaf could be kept in an atmosphere free of oxygen without undergoing change. Tea fermentation is thus an oxidation of the leaf material and it is possible that this oxidation is set in action as soon as the leaf sap is exposed to the oxygen of the air, *i.e.*, all that is necessary for tea fermentation is to expose the oxidisable material to the air. However, it was shown that temperature was a controlling factor in the process, fermentation being very slow below 40°F, and that heating the leaf to 212°F. rendered the leaf incapable of fermenting on being crushed. This property of the leaf is utilised on those estates where green tea is made, for when the fresh leaf is steamed at 212°F. it will not lose its green colour during the subsequent manufacturing processes. Again the production of colour in fermenting tea is usually accompanied by a development of aroma, but it was shown that although colour production does occur above 113°F, no aroma is developed at such temperatures. The original idea that tea fermentation was a process of putrefaction was thus given up for that of rapid oxidation, and the results of observation of the effect of temperature on the reaction led to the idea that the oxidation was brought about by some agent or agents which are inhibited or destroyed by unfavourable temperature conditions.

Colour production in other plants had been shown to be due to the presence of small quantities of some accelerating principles called catalysts and it was suggested that similar substances were present in the tea leaf. Attempts were made to isolate such bodies from the fresh leaf, and these attempts were successful. This isolation of organic compounds possessing catalytic activity led to the adoption of the idea that tea fermentation is due to the presence of these catalysts, and it is generally referred to as the enzyme action in tea fermentation.

On the other hand it was known that other fermenting processes were due to the action of yeasts, and that other complicated changes were carried out by means of bacteria and fungi. Further, it was known that plant products such as the grape were associated with their yeasts on the plant, so that when the skin of the grape is punctured, the yeasts come in contact with the juice. This mixing of the two results in a fermenation which is generally accompanied by the development of flavouring and aromatic substances. It is, therefore, permissible to suggest that the same thing occurs when the tea leaf is crushed in the rollers, provided it can be shown that micro-organisms do exist in the fresh leaf, and that their presence or absence can affect the process of fermentation. It was soon shown that micro-organisms were always associated with the tea leaf, but the investigators have not been so successful in their study of the action of these micro-organisms on the fermenting leaf. The presence of yeasts, fungi and bacteria on the fresh tea leaf forms the basis of the argument that tea fermentation is due to the activity of micro-organisms.

It will thus be seen that there are grounds for maintaining either the enzyme or micro-organic theory of tea fermentation, and in the present article a short account of the investigations carried out to test the enzyme theory is given. An account of the investigations concerning the micro-organic theory will be given in the concluding article of the series.

FERMENT OR ENZYME THEORY OF TEA FERMENTATION.

(a) *Enzymes and their general properties.*—According to Waldschmidt Leitz (*Enzyme Actions and Properties* 1928), "Enzymes may be described as definite material catalysers, formed indeed by living cells, but independent of the latter in their operation." The term catalyser requires further explanation to make the above clear, and this is best done by giving an example of what a catalyst does.

Cane sugar is a compound made up of two other simpler sugars, but under certain conditions it will split into the two simpler sugars which compose it. The rate at which it will break down into the simpler sugars is very low in neutral aqueous solutions, but it becomes very rapid if a little mineral acid is added. The acid can be recovered at the end of the reaction unchanged in any way and does not appear to have taken any part in the reaction. Since the same change was going on very slowly in a neutral solution the addition of acid has not started the reaction, but its presence has acted as an accelerator. There are many substances, which possess the property of accelerating chemical reactions without apparently undergoing any change themselves during the process, and the discovery of such substances has been of great value for many industrial processes. This acceleration of any chemical change by means of a foreign substance, which does not start the reaction and does not itself take part in the reaction is termed catalysis, and the substance responsible for the action is called the catalyst.

It was well known that living organisms are capable of carrying out their reactions with ease at comparatively low temperatures, and that some of these reactions could not be reproduced in the laboratory at all, or at least without the application of very high temperatures. It was thus possible that the ease with which living organisms produced these changes was due to the utilisation of catalysts by them.

Plants and animals do actually contain a large number of organic complexes which can act as catalysts, and many of these have been isolated and studied. The plant or animal catalysts are termed enzymes, and they differ from the inorganic catalysts like the mineral acid referred to previously in being sensitive to heat and light. They are all destroyed at 212°F., while many cannot stand a temperature of 140°F., and exposure to strong sunlight in the presence of oxygen often destroys them.

It is not known how these enzymes are formed in the plant, but it was soon discovered that any particular plant generally contains several types of enzymes capable of accelerating different reactions, and among these have been found several groups which are capable of accelerating oxidations. These classes of enzymes are collectively termed oxidases. Their action is often accompanied by colour changes, such as the browning of the cut surface of certain types of apples when exposed to the air, and of the expressed sap of species of *Rhus*. These saps rapidly turn brown and then black on exposure to the air owing to the presence of an oxidising enzyme laccase, and the lacquer industry of China depends on this oxidase activity.

The changes produced during the manufacture of black tea, such as the rapid browning of the leaf when it is crushed, appears to bear some similarity to these colour changes produced in other plant saps, and which were known to be due to oxidase activity. Again the way in which the fermentation of tea responded to temperature conditions was a further reason for suspecting that the action concerned was of enzymic origin, and attempts were made to isolate oxidising enzymes from the tea leaf.

(b) *Early work on the enzymes of tea.*—Bamber (*The Chemistry and Agriculture of Tea* 1893) reports having isolated small quantities of oxidising enzymes from tea and he and Wright give a fuller account of their work in the *Proceedings of the Planters' Association* 1902. According to these two authors the enzyme or enzymes exist in all types of tea, there being no difference in the amount of enzyme found in low-country tea as compared with high grown tea, or in leaf a few weeks from pruning as compared with leaf two years from pruning. Climatic conditions of growth and time of day when the leaf was plucked had no effect on the amount of enzyme determined in the leaf. Aso (*Bull. Coll. Agric., Tokyo Univ.* IV. 1901.) was also able to isolate a substance from tea leaf which gave the usual oxidase reactions, and which was able to produce a brownish colouration in an alcohol extract of fresh tea leaf. However, although these investigators were able to isolate a substance having the properties of an oxidase, they were not able to report that addition of their enzyme produced any difference on a tea fermentation, and their conclusion that tea fermentation is the result of oxidase activity depends on the fact that they found oxidising enzyme in the leaf. This may be true, but until it has been shown that addition of the enzyme to fermenting tea can produce a difference in the rate of the reaction or the degree of fermentation produced it must be accepted as an unproved hypothesis.

Mann. (*The Ferment of the Tea Leaf and its relation to Quality. Bull. Ind. Tea Assoc.* 1901. *et. seq.*) describes his experiments on the enzymes of the tea leaf. The isolated enzyme had the properties of an oxidase, and it is stated that its addition to fermenting tea leaf produced a normal but quicker browning of the leaf than in its absence. The same change was produced if it was added to tea juice instead of tea leaf, and the enzyme preparation was also able to oxidise other easily oxidised compounds with great rapidity. Mann concluded that the enzymes separated by him from fresh tea leaf did play a part in tea fermentation. He found that a temperature of 160-165°F. maintained for 3 minutes had no appreciable effect on the activity of the enzyme, but that it was destroyed if it was subjected to temperatures above 180°F. for the same period. The enzyme was very active at 130°F., but feeble at 143°F., and because of this Mann points out that it is necessary to fire tea above 150°F. in order to destroy the enzyme and thus check the fermentation. The enzyme, which acted best in a slightly acid medium was destroyed by strong acid or alkali, and could be divided into two components. One of these components gave an oxidase reaction with his guiacum reagent immediately, and was termed the free enzyme. The other component did not give the oxidase reaction until it was mixed with hydrogen peroxide. The oxidase reaction of the enzymes in the presence of hydrogen peroxide was therefore greater than in its absence and this was designated the activity of the total enzyme.

According to Mann the amount of enzyme in the different parts of the flush varied, the greatest concentration being found in the tip and stalk, but this decreased in successive leaves below the tip. On this finding Mann claims that apparently the best tea is produced from those parts where the enzyme is present in greatest amount. If a high concentration of enzyme should give a better tea than a low concentration, then the stalks should give the best tea of all, but

Mann explains away this discrepancy by showing that the relative amount of acidity, tannin and phosphoric acid is so low in the stalks as to account for the poorer quality tea made from them. This argument, however, can be put in another way, with equal justification if not more, *i.e.*, the best tea is made from those parts which contain the greatest quantity of acid, tannin and phosphoric acid. Whether the concentration of enzyme present has anything to do with it or not is not indicated by the figures given.

He states that on examining teas from various gardens it was found that there was a close relationship between the amount of oxidase present and the flavour of the tea, provided other things were equal. No figures are given to show that the other things were equal since the chemical composition of the various teas is not given, and the difference in quality may have been due to a difference in the composition of the tea. Mann himself (*The Tea Soils of Assam and Tea Manuring* 1910) says that a large amount of phosphoric acid must be present in the soil for the production of good quality tea, and that phosphoric acid is present in greater quantities in good than in poorer quality leaf. At the same time it is clear from Mann's data that a high enzyme content is not always associated with a high phosphate content since a high enzyme content is associated with a low phosphate concentration in the stalk. The few results given are not enough to draw any conclusions relative to this point, but in justice to Mann it must be said that he was aware of the fallacy in the argument, and ends his paper by saying that it is very doubtful if it would ever be possible to improve the flavour of tea by the addition of prepared enzyme, since the action of the enzyme itself depended on the presence of the material to be changed, and because the nature of the substances responsible for flavour were not known.

In his second paper Mann describes the effect of withering on the amount of enzyme to be determined in the leaf. It appeared from the results of previous experiments as if the quantity of enzyme increased as a result of withering, and on carrying out further experiments the results obtained supported this contention. Thus in one experiment carried out at a temperature between 76°F. and 86°F. he obtained the following results:—

Relative amount of Enzyme present in withering leaf. (Mann).

Time leaf was withered	Active Enzyme.	Total Enzyme.
1 hour	1·00	1·00
5 hours	1·14	0·96
18½ "	1·71	1·77
23½ "	1·29	1·38

The leaf used for the experiment was deemed to be properly withered at about 18-hours when the enzyme seemed to be present in maximum amount, and the conclusion arrived at that a proper wither is obtained when the correct physical wither corresponds with the maximum development of enzyme in the leaf. The author gives no indication of his criterion of a proper wither as judged by empirical methods, but if the 18-hour wither he obtained was the 70 per cent. usually favoured in India, then his leaf was very different from the 55 per cent. withered leaf found to give excellent results in Ceylon.

In other experiments Mann investigated the effect of period of wither, light conditions during growth and withering, and the time of plucking on the concentration of the enzyme. His conclusions were:—

(a) During withering the amount of oxidising enzyme or ferment increases up to a certain point corresponding under normal withering conditions with the point at which the leaf is ready for rolling.

(b) The two processes of loss of moisture and production of ferment by no means necessarily take the same length of time, and the leaf may be withered (in very dry weather) long before it is chemically ready to roll, and in very wet weather may be ready chemically to roll long before it is withered.

(c) At the temperature studied (76°F. to 86°F) the normal time which the leaf requires in order to be chemically ready for rolling is 18 to 20 hours with normally withered leaf, and several hours longer (it may be up to 25 hours) with leaf prevented from withering by a very wet atmosphere.

(d) Leaf withered in the light contained a little more enzyme than leaf withered in the dark.

(e) Leaf plucked in the morning contained more enzyme than leaf plucked in the evening.

Although Mann's conclusions regarding the best period of withering may agree with practical experience (it is doubted whether this is really true) the data given are not sufficient or accurate enough to substantiate his conclusions. It is not quite clear whether the figures for the total enzymes bear any relation to those for the active enzymes or not, and nothing is said about the variation found with different samples of leaf. The data for active enzyme and total enzyme vary so irregularly and the actual differences found are so small that it is doubtful whether his results are anything more than chance variations. It would appear as if the sampling error was very great from the figures given for the two samples of leaf plucked in the early morning, where one contained 3·37 and the other 1·29 of enzyme, and yet the differences found in the withering experiments were very much less than this. It is impossible to place any reliance on the data obtained by means of methods which are liable to such errors, and much as we would like to accept Mann's conclusions, it is to be regretted that they are not justified by the data he reports.

In his third pamphlet (1904), Mann reports the results of his practical experiments to test the above conclusions. There are three sets of experiments recorded, one of these appeared to support his hypothesis, one was not very satisfactory, and the other proved nothing at all. In spite of this the author claims proof of his theory concerning the time necessary for withering. When his method of experimentation is considered it will be seen that the results he obtained could be easily ascribed to other factors than time. Thus one set of experiments was carried out with four maunds of leaf in the following way:—

No. 1. Ordinary wither with leaf brought in at noon, spread on chungs, rolled at 6 a.m., leaf withered to 64 per cent. in 18 hours.

No. 2. Leaf spread *4 inches deep*, left for 18 hours and then spread in a "*high temperature house*" and quickly withered in 4 hours. This leaf was withered to 70 per cent. in 22 hours.

No. 3. Leaf spread in the same way as No. 2, but withered slowly from 6 a.m. onwards. This leaf was withered to 70 per cent. in 26 hours.

The results of tasting were as follows: "The ordinary wither was slightly, though very slightly, preferable in flavour and liquor. No. 2 (22 hours) gave slightly thicker and fuller liquors than No. 3, and the out-turn of leaf was slightly brighter. The difference, however, between No. 1 and No. 3 was very slight in any point." In each case they were described as giving, "a full brisk pungent liquor, good coppery infusions." Yet No. 3 was only withered to 70 per cent, while No. 1 was withered to 64 per cent, and No. 3 had taken *8 hours longer to wither than No. 1*. The slight difference found, if any, may have been due to the thicker spread, or the lighter wither, and these disturbing variations in the conduct of the test make it impossible to draw any conclusions as to the effect of time on the condition of the withered leaf.

The results obtained from No. 2 cannot possibly be used to draw any conclusions as to the effect of time of withering on the quality of the made tea, because the deciding factor in this experiment was evidently temperature and not time. Having retarded the wither for 18 hours by resorting to thick spreading, the leaf was placed in a high temperature house and quickly withered in 4 hours. The actual temperature in the house is not recorded, but the mere fact that the leaf was subjected to a higher temperature than the others during the process of withering is sufficient to rule this experiment out of court right away as a test of the effect of time on withering.

In another set of experiments the leaf was withered to 67·4 per cent in 5 hours at a temperature of 100°F. It is no wonder that the resulting tea gave "a thin, dull liquor, almost sour," but it cannot be said that this test contributes much to the problem of the proper time required for withering, because the temperature is the controlling factor. The obvious conclusion from this experiment is that when high temperatures are used to wither the leaf in a very short time, then loss of quality will occur to a marked extent, but it does not show what would happen to quality if the leaf was withered to this extent in the same period as a result of the use of drier air at a low temperature. If the above conclusion is true it is useless to try and wither large quantities of leaf by means of a withering machine in a short time, because such machines depend on a high temperature to produce their withering effect.

It is a pity that Mann does not report any experiments with leaf withered during a period ranging between 5 and 18 hours, or 26 and 48 hours. It is possible that the longer period would not have been practicable under the conditions he had to contend with, but this period is of great interest to Ceylon planters many of whom prefer to prolong their wither as long as 48 hours rather than resort to any hot air. The results obtained with these long withers have proved excellent in many cases, but this is not universally true, and it cannot be generalised that a long wither is better than a shorter wither, because excellent teas have also been produced with leaf withered in a short period. These experiments of Mann, however, do not supply any evidence in support of his contention that the enzyme increased during withering and that the proper period of withering should coincide with the maximum production of enzyme.

(c) *Recent work on the Enzymes of Tea.*—In spite of the failure of Mann to prove his theory of enzyme action as a cause of tea fermentation, the idea still persists that the oxidising enzymes found in the tea leaf do play an important rôle in tea fermentation. Bernard and Welter (*Annales du Jardin Botanique de Buitenzorg* 2e Serie. X. 1911) in Java reinvestigated the methods of isolating these enzymes with a view to obtaining a purer product than had been isolated so far for studying their properties, but this work is too academic in character to be fully dealt with in the present article.

On repeating Mann's method of preparation they found that all the tannin could not be removed by the use of hide powder, and that the guiacum reaction used by Mann to estimate his enzyme concentration could be produced by other substances than the enzyme. Thus scratches on the reagent glass, the presence of sand, pumice stone or any fine powders produced the same effect on the guiacum reagent as the enzyme did, and the method becomes very difficult to apply in a quantitative way. The conclusions they arrived at as a result of their very extensive investigation were:—

(1) No practical or theoretical conclusions of importance can be drawn from the investigation.

(2) Peroxidase (Mann's combined enzyme) can be found in every part of the plant, but oxidase in the true sense of the term is not found.

(3) Nothing could be said concerning the action of the enzyme on flavour or aroma.

Deuss (*Chemisch. Weekblad* XX. 19. 1923.) gives a short synopsis of the investigations into the part played in tea fermentation by oxidising enzymes, and gives the facts which had been demonstrated by the experiments without further comment or discussion, as he considers that would have been premature considering the incomplete knowledge of these enzymes. Although each stated fact was tested ten times and carried out with controls, Deuss does not commit himself more than to say that it is not known how far the tea ferment or ferments are necessary for the production of black tea.

The salient points of these investigations are that no pure preparation of the enzyme or ferment has yet been prepared, and that although an aqueous solution of the most pure product obtained reacted neutral, it was not free from tannin. The presence of the tannin makes it very difficult to investigate the action of the enzyme, and if the tannin is removed by precipitation with lead acetate the enzyme solution becomes inactive. The enzyme, which contains nitrogen, sulphur, phosphorus, manganese, magnesium, potassium, iron and albumin loses its solubility in water when dried and kept in a desiccator for a long time. The reaction between the enzyme and a guiacum solution is very slow between 32°F. and 50°F., strongest at 113°F. and slow again at 169° to 190°F., while boiling for 1, 2 or 3 hours renders the enzyme inactive.

Deuss' paper is a fair account of the facts known concerning the oxidising enzymes found in the tea leaf, so that the true position appears to be:—Oxidising enzymes are present in the tea leaf, and one of the reactions which takes place during tea fermentation is an oxidative change, but what rôle the enzymes play in the oxidative change or what effect the various processes of manufacture such as withering have on the enzyme in the tea leaf is not known.

Although Mann's work on the enzymes and the effect of withering on the concentration of these organic catalysts in the leaf has been criticised rather severely it is not suggested thereby that the quality or the nature of the tea produced is not affected by the manner of withering. On the contrary the evidence gleaned from practice supports the contention that the period of withering is undoubtedly very important. At the present moment, however, it is difficult to apportion the effect produced between the various factors concerned, such as a difference in the quality of the fresh leaf itself, moisture content of the fresh leaf, actual moisture content of the withered leaf, temperature conditions during withering and the duration of any period of high temperature. Again the diversity of opinions concerning withering may be due to a possibility that, with certain kinds of leaf and at particular seasons, better results may be obtained by extending or shortening the withering period so that the idea of a best time for withering cannot be upheld, and he who dogmatises on such questions is very liable to fall into error.

(*To be concluded*).