

TEA FERMENTATION—PART V.

D. I. EVANS.

THE MICRO-ORGANIC THEORY OF TEA FERMENTATION.

Bernard (*Meded. van het Proefstation voor Thee*, 1909) ascribes the first suggestion of the micro-organic theory of tea fermentation in 1891 to Kozai and then states that Wahgel looked upon the production of flavour as being due to special bacteria (1903). At this time, however, the investigators referred to in the previous article were busy investigating the enzymes of the tea leaf and the general opinion inclined to the belief that tea fermentation was due to an enzyme or ferment, though this theory remained unproved. On the other hand the idea that micro-organisms were concerned had not been disproved, although this appeared to be unlikely. The chief objections to the micro-organic theory were the following:—

(a) Fermentation takes place so quickly that it was suggested micro-organisms could not possibly take part in the process, in spite of the fact that they could be demonstrated as present on the leaf. The protagonists of the micro-organic theory, however, very rightly pointed out that the yeasts and bacteria in the fresh leaf could multiply very rapidly during the period of withering, and thus be present on the withered leaf in sufficient amount to effect the changes observed. It is to be regretted, however, that although the earlier workers on the yeasts and bacteria of tea leaf all state that these increase during withering and fermentation, they failed to produce any data in support of this. Tunstall (*Quart. Jour. I. T. A.*, 1923, IV.) says "there was a steady increase of the number of yeast cells present as the manufacture proceeded until the maximum was reached when fermentation was completed." There are however no data given in support of this and it is surprising, if the yeasts were responsible for fermentation, that this steady increase did not show some marked acceleration at the moment the leaf was crushed and the yeast enabled to utilise its proper medium. This question had already been carefully investigated by Bosscha and Brzeskowsky (*Med. v. h. Proefst. v. Thee*, 1916, XLVII) but their figures, which will be referred to later, do not show any great increase in the number of yeast colonies on the leaf during withering.

(b) The second objection to the micro-organic theory of tea fermentation was the claim that rolled leaf could ferment in the presence of chloroform vapour, which prevents the action of any micro-organisms. Although the weight of evidence appears to confirm this, there is a slight doubt, however, whether anybody has ever produced properly fermented tea in the presence of chloroform vapour or not.

In spite of these objections it was maintained that certain phenomena observed in practice could only be explained on the basis of the presence of active micro-organisms. Typical instances of these peculiarities are given by Bernard (*ibid*). Thus, it was stated that a slow fermenting leaf could be made to ferment quicker by inoculation with a small quantity of the quicker fermenting leaf. Several instances of this are referred to, such as the acceleration of

the fermentation at Tanara and Wanasoea as a result of mixing the slow fermenting leaf of these factories with leaf from Malabar where the fermentation was very rapid. After describing these particular instances Bernard describes his own experiments conducted to study the micro-organisms which could be separated from tea juice. He succeeded in isolating a yeast-like organism which appeared to be always present on the tea leaf, and which did not at least exert an unfavourable action on the fermenting tea, even if it were not responsible for the fermentation. At the same time it was found that certain bacteria were associated with the leaf and that, if these were allowed to multiply to any extent, then the leaf developed bad odours and became sticky. Finally, Bernard pointed out that the work in the factory must be carried out under as clean conditions as possible, and suggested further experiments which should be carried out in this particular field.

Deuss and Brzeskowsky (*Med. v.h. Proef. v. Thee*, 1916, XLV) report their experiments on tea fermentation at Kertasari, where the fermentation was very slow. The nature of these experiments can be seen from the way they were conducted:—

- (a) Kertasari leaf was fermented at Kertasari and Malabar.
- (b) Malabar leaf was fermented at Kertasari and Malabar.
- (c) Kertasari leaf was mixed with Malabar leaf for rolling.
- (d) Malabar leaf was mixed with Kertasari leaf for rolling.
- (e) Kertasari fresh leaf was manufactured at Malabar.
- (f) Malabar fresh leaf was manufactured at Kertasari.

These experiments were carefully watched by several people who are reported to be of the same opinion as to the results obtained. The experiments showed that mixing quickly fermenting leaf from Malabar with slow fermenting leaf from Kertasari had no effect on the rate of fermentation, whether conducted at Kertasari or Malabar. It was found, however, that the temperature of the surroundings was a very important factor in deciding the rate of the fermentation, and that the temperature was too low at Kertasari. This could be improved by fermenting the tea near the dryer, and the period of fermentation was reduced in this way from 6 hours to 3 hours. This fact eventually led Deuss to conduct the investigations on the effects of temperature on fermentation already described in a previous article. These particular experiments, carried out at Kertasari and Malabar, appear to have been subjected to a good deal of criticism by the advocates of the micro-organic theory of tea fermentation, but Deuss' later experiments concerning temperature of fermentation supplied more evidence in support of the contention that temperature differences were sufficient to account for the differences observed in the period of fermentation in the two estates.

Bosscha and Brzeskowsky (*Med. v.h. Proef. v. Thee*, 1916, No. XLVII) considered that the previous experiments did not decide definitely whether tea fermentation was due to an oxidase or to micro-organisms and that further work was necessary, since it was very important to determine whether aseptic condition should be maintained in manufacture or not. Further, they pointed out the necessity for determining the proper temperature, aeration, and thickness of spreading of the leaf that is necessary when experiments are being carried out if these variable factors are to be eliminated. The best material for experimentation would be plants grown under sterile conditions, but they considered this too laborious and expensive, and they thus turned their attention to ways and means of sterilising the leaf without changing its nature.

The first thing these authors did was to determine what organisms could be found in association with the tea leaf, and this necessitated a preliminary investigation of the most suitable media for the culture of the organisms. They describe the various media used and show that organic nitrogen in the form of peptones is necessary for their proper growth. A full description is given of their method of preparing the cultures from fresh leaf or leaf sap, followed by an account of the organisms isolated.

They found a variety of fungi, yeasts, and bacteria on the tea leaf, as reported by previous workers, but complain of the difficulty of working as a result of the mixtures which always developed on their plates. However the micro-organisms could be divided into 3 primary groups (1) Fungi (2) Yeasts and (3) Bacteria.

(1) *Fungi*.—The fungi, represented by different types such as *Aspergillus*, *Penicillium* and *Mucor* were found in profusion. When grown on a sterilised tea decoction they invariably produced a dark-brown colouration and a disagreeable smell. They also decomposed tannin solutions with the production of gallic acid, but if a tea decoction was used instead of a tannin solution then gallic acid was not produced, which indicates a difference in constitution between ordinary tannin and tea tannin. *Dematium* which lies between the fungi and the yeasts, was also found to be present, but this growth was not very prolific on a tea decoction.

(2) *Yeasts*.—By using a meat-agar culture they were able to isolate the yeasts almost free of bacteria, and found the same yeasts as had been previously described by Staub and Bernard. The number of yeast colonies did not show any great increase during withering, although they increased sharply during fermentation, as will be seen from the data they give:—

Fresh leaf	...	10-20 colonies.
Withered leaf	...	20-30 "
Fermented leaf	...	70-80 "
Pekoe after picking	...	0-5 "

Hot tea from the driers seemed to be free of yeasts, but picked up a few in the process of handling. They studied the action of these yeasts on starch paste and found that they contained a starch decomposing enzyme, and that the yeasts produced a brown colouration of a tea decoction, but this was not accompanied by a disagreeable odour as in the case of the fungi.

(3) *Bacteria*—Several kinds of bacteria were found and these are described. Without entering into a description of these organisms, the salient point to be noted from their work is that the bacteria also turned a green decoction of tea to a dark-brown colour, with the production of an objectionable smell resembling overfermented tea. The investigators inclined to the belief that the bacteria never benefit the fermentation but on the other hand corrupt the tea as a result of their activity. In this connection they also prepared enzymes from the micro-organisms isolated but none of these exhibited an oxidase or peroxidase reaction, so that, even if the micro-organisms do play a part in fermentation, it is not as the result of the formation of a peroxidase.

Sterilisation of the Tea Leaf.—Tea leaves were sterilised by being dipped in boiling water and then left hanging in steam. The leaf was rendered germ free by this process, but the nature of the leaf was so changed that no fermentation could take place. A brown colouration of the leaf could be produced by inoculation with fungi and yeasts or by the addition of oxidants like permanganate or chromic acid, but it was not the colour of properly fermented leaf and in no case was the tea aroma produced. Sterilisation by means of various chemicals was then investigated and it was found that the addition of formalin altered the composition of the leaf so that fermentation was prevented. Chloroform did not seem to affect the fermentation and no difference could be detected in chloroform-treated tea from non-treated tea. Hydrogen peroxide and corrosive sublimate in dilute solutions did not affect the normal fermentation but in greater concentrations this fermentation was stopped altogether. Teas prepared after treatment with sublimate of mercury or peroxide gave identical infusions and liquors as untreated tea, but this statement must be accepted with caution for it is modified by saying that the peroxide tea was not quite so fine and had a somewhat fishy taste.

When the authors were satisfied that good tea could be made from leaf treated with dilute peroxide or corrosive sublimate solutions they set out to discover what effect this concentration of antiseptic had on the micro-organisms. It was found that treatment of the leaf in this way rendered it free of any micro-organisms and this method was adopted to sterilise the leaf used in their experiments.

These experiments were followed by a series of experiments to determine the temperature necessary for a normal fermentation. They failed to produce any fermentation in leaf maintained in an autoclave at 100°C (212°F) and then tried at 50°C (122°F) but no fermentation could be detected. On testing out the leaf at different temperature levels varying by 1°C they experienced the first normal fermentation at 43.43.5°C (109.110.3°F). They regarded this temperature as a critical temperature for tea fermentation, as any slight increase above this figure gave a yellow

liquor and green infusion. Some of the bacteria were found to develop rapidly at 45°C (113°F) and a good growth of all the micro-organisms was obtained in tea treated at these high temperatures. The interruption of the fermentation at this temperature is not therefore in keeping with the idea that it is due to the presence of these organisms, but agrees with the conception that the enzyme is inactivated at this particular temperature. Although they considered tea fermented at 43°C (109.4°F) was normally fermented, it was yet inferior in the quality of the liquor and aroma to that fermented at lower temperatures.

The course of the fermentation was followed in their subsequent investigations by means of temperature determinations of the fermenting leaf, since they were able to satisfy themselves that the course of the fermentation could be followed in this way, and that the cut leaf used in their experiments did not differ in its conduct from normally crushed leaf. 800 gms. of crushed leaf was fermented in a specially constructed box to minimise the loss of heat from the fermenting leaf, and the following observations were made:—

- (1) Leaf temperature.
- (2) Yeast and bacteria counts.
- (3) Samples were dried and tasted for fermentation.

The temperature of the leaf went up at first to 29°C (84.2°F) and at this point plenty of yeasts and bacteria could be determined in the leaf material, but the yeasts predominated. The best tea was produced from the sample dried at this point in the temperature curve, the time taken to reach this temperature being 3-4 hours. The temperature of the leaf dropped after this, and later again increased to 34-35°C (93-95°F). When the temperature dropped and then increased a change took place in the micro-organisms isolated, since the yeasts appeared to diminish and the bacteria increased rapidly at this stage. Eventually the surface of the leaf became covered with a thick matt of fungi. The aroma of the fermented and dried leaf diminished as the temperature receded from the first maximum of 29°C (84.2°F) but the colour of the liquor and infusion were still satisfactory. When the temperature started to increase the second time, and the moulds developed, the liquors became black and the infusions a dark-brown, while the objectionable smell of overfermented tea developed.

By studying the leaf in this way they came to the conclusion that the presence of the yeasts did not interfere with the proper development of aroma, colour of the liquor, or infusion, and that tea fermented in their presence tasted normal.

The presence of bacteria produced the smell of overfermented tea, dark-brown liquors and infusion, and gave the tea a tarry taste.

Development of moulds produced a mouldy smell, light to yellow liquors, black infusions and a decidedly objectionable taste.

These experiments were then carried out with sterilised leaf, and although an initial rise in the temperature of the leaf occurred, as in the case of the non-sterilised leaf, showing that normal fermentation was proceeding in the absence of micro-organisms, the second rise in temperature could not be observed for several days or until fresh moulds entered the leaf and developed. Under certain conditions it was found possible to maintain the leaf sterile to the end of the experiment, and thus eliminate the second rise of temperature entirely. At the same time, the quality of the samples of tea prepared from the leaf sterilised by means of chloroform and mercury sublimate was equal to the ordinary make. By sterilising the leaf before it was fermented it was claimed that the fermented leaf could be kept in this condition for several days without deterioration, whereas the normal tea was overfermented at the end of the first day. A normal fermentation of tea thus appears to be possible under conditions which exclude the possibility of any micro-organic action and, when this sterile leaf is fermented, the ordinary signs of overfermentation is not produced in the leaf even after prolonged fermentation.

The next point to be investigated was the development of carbon dioxide by the fermenting leaf. Similar curves were traced for the rate of development of carbon dioxide as for the rise in temperature of the leaf. The carbon dioxide production reached a maximum soon after the start of the experiment, and then fell in the same way as the temperature of the leaf did. Carbon dioxide was also produced by sterilised leaf, but after the point of maximum production was reached the amount of carbon dioxide produced diminished and eventually attained a point where none was produced at all. When infection by means of spores took place the production of carbon dioxide again occurred very rapidly. Fermentation of tea thus results in the production of carbon dioxide but this carbon dioxide is not the result of the growth of micro-organisms since it is developed during the fermentation of completely sterilised leaf. When the fermentation is completed the development of carbon dioxide also ceases unless the tea becomes infected with moulds when it develops an overfermented taste and carbon dioxide production again becomes rapid.

As a result of these experiments it was concluded that the process of tea fermentation was independent of the growth of micro-organisms on the leaf, and that cleanliness above all things should be observed during the manufacture of tea. Further, tea fermentation is accompanied by the production of heat and the development

of carbon dioxide, but these phenomena occur in the same way in the absence of micro-organisms. Tea fermentation must therefore depend on the action of a ferment in the leaf or on an oxidation of the components of the tea by the oxygen of the air, but it was not possible to decide between these two possibilities as a result of the investigations just described.

Although some of the methods adopted in these investigations may be open to criticism, it must be admitted that the array of evidence collected by observations on the number of organisms present, the temperature changes in fermenting leaf, the production of carbon dioxide, and the quality of made tea, is all against the idea that bacteria or fungi play any beneficial part in tea fermentation. On the contrary, they invariably corrupt the tea, but this charge could not be brought against the yeasts present, although there was nothing to show that these were in any way necessary for a satisfactory fermentation.

Tunstall (*Q. J. I. T. A.*, 1923, 4) published a short note on the work done by him concerning the micro-organisms associated with tea fermentation. He decided to disregard the conclusions arrived at in Java, although it is difficult to understand his reasons for doing this. Tunstall reports that complete sterilisation of the leaf with chemicals always rendered the leaf incapable of further fermentation, and his experiments were carried out with unsterilised leaf. Startling results are described by the author who claims to have reproduced the aroma characteristic of fermenting leaf belonging to one particular factory in another factory by inoculation with the yeast produced in the first factory. He also claimed that it was possible to change the flavour of tea by inoculation with the proper yeast, and that the yeasts present when the best quality tea is made are more active than those found on the leaf at other times of the year. It is pointed out, however, that the organisms found on the fermenting floors, rollers, etc., are usually undesirable, and that some of the bacteria isolated gave characteristic taints to tea. One of these taints resembled the effect produced by overfiring.

It is impossible to judge the value of this contribution since no data as to the technique of the methods employed for the investigations are given and the actual experiments carried out are not described. The work was done at least seven years ago but no further details have been published and there does not appear to be any great development in the production of special yeasts for the inoculation of tea in North India. In this connection it may be pointed out that Benton (*Q. J. I. T. A.*, 1930, 4), says that "The main fermentation process is controlled by enzymes of the leaf, and is independent of outside biological influences."

In conclusion, it may be said that yeasts, moulds and bacteria do exist on the tea leaf and that these may multiply during the manufacture of tea. It is also possible to produce fermentation in completely sterilised leaf so that the micro-organisms are not essential for the chemical change. It is not very certain, however, whether this fermentation is a normal one or not. On the other hand, it is clear that a rapid development of the micro-organisms can take place at higher temperatures than allow of any fermentation of the leaf at all. Although no ill-effect could be ascribed to the presence of yeasts, any development of moulds and bacteria resulted in dark or yellow liquors, accompanied by characteristic smells such as overfermented tea, and it can be definitely stated that fermentation conditions which cause the development of moulds or bacteria will result in poor teas. It is, therefore, essential to have clean conditions for fermentation and rolling, and fermenting rooms should be kept sweet and fresh if taints are to be avoided. The remarkable point in the researches described is the discovery that tea leaf fermented after sterilisation could be kept for several days without showing any sign of overfermentation, even at the end of the period.

This is a very important discovery if it can be definitely proved that a normal tea can be produced with sterilised leaf.
