THE CHEMICAL COMPOSITION OF FRESH TEA FLUSH AS AFFECTED BY CLONE AND CLIMATE

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Introduction

The chemical composition of the tea leaf determines the usefulness of the tea plant (*Camellia sinensis* (L.), agriculturally, and is, in fact, the foundation of the tea industry. The very fact that we can make tea from the tea plant and not from other plants proves this point.

On the practical side, such agricultural practices as plucking and manuring are carried out in such a way as to obtain leaf with the best possible chemical composition. That is, plucking to two leaves and a bud is the accepted standard on almost every tea estate in the world and this is so primarily because it is this part of the tea plant which is richest in those chemical constituents which are desirable from the point of view of tea making. Manuring is done in such a way as to promote the balanced growth of the tea plant. The word balanced is used here to indicate only growth which ensues without appreciable loss in quality. One strives for this goal by supplying fertilizer in such forms and at such rates that the production of *all* cellular constituents is enhanced at approximately the same rate that net plant growth is enhanced. This latter requirement is of the utmost importance in the making of high quality tea and more will be said about this later.

This brings us to a consideration of the potential of tea leaf for making tea. When the tea leaf is plucked it has a certain potential for making tea. This potential appears to be made up in part by the physical condition of the leaf, *i.e.* its degree of toughness or fragility, but the major factor in determining this potential is the chemical composition of the leaf at the time of plucking. We can elaborate this point with a consideration of the metabolic reactions taking place in the tea leaves before and after plucking. These metabolic reactions are of many diverse kinds but they can be divided into two general types called anabolic and catabolic. Anabolic reactions are those in which complex organic compounds such as sugars, flavanols, amino acids, etc., are built up and catabolic reactions are those in which they are broken down. Both processes proceed simultaneously in the tea leaf attached to the plant but the anabolic reactions predominate. In this case there is a net synthesis of organic compounds and growth results. On the other hand, once the leaf is plucked the anabolic reactions practically cease while the catabolic reactions continue resulting in a continuous loss of organic matter from the time of leaf plucking. Some of the catabolic processes are desirable from the point of view of tea making but the fact remains that once the leaf is plucked the amount of raw materials with which the tea maker can work are fixed. Therefore, the potential of that leaf to make tea is fixed.

Careful handling and proper manufacture will tend to realize the potential of fresh leaf while poor handling and manufacturing techniques or adverse manufaoturing conditions will result in a tea inferior to that which was possible. In order to improve our understanding of the potential of fresh tea leaf it is necessary to define potential in chemical terms. To this end an investigation into the chemical composition of fresh tea leaf as it relates to the quality of the tea made from this leaf has been initiated at the Tea Research Institute. This is a report of the results of this nvestigation to date with a discussion of the possible relation of the chemical composition of fresh tea leaf to its potential as regards tea making.

Detailed comparison of two Clones of widely Differing Quality Potential over a Period of Eight Months

The initial investigation involved the sampling of the two clones TRI 740 and TRI 777 over an eight month period between 27 March and 2 December, 1963. These clones were selected for this purpose because one was of low quality potential (TRI 740 is classified as a "C" grade clone according to Keegel, 1959) and one was of high quality potential (TRI 777 is classified as an "A" grade clone according to Keegel, 1959). It was hoped that comparison of two such clones would help to reveal the biochemical basis of quality in the differences found. That is, since the clone TRI 777 exemplifies quality, its chemical composition and/or properties should be near optimal in relation to quality in tea whereas the chemical composition and/or properties of the clone TRI 740, which exemplifies a clone with little or no quality, should not be optimal. From this one can infer that chemical quantities which are the same in the two clones probably are not involved in determining quality whereas those which differ markedly are likely to be involved.

The two clones used in this investigation were also chosen because they had been planted at the same time (May, 1952), they were located in adjacent plots so that cultural and climatic effects would be minimized, and they had been pruned at the same time (December, 1962).

These plots were plucked regularly on an average ten-day plucking round. Samples were taken from each pluck for chemical analysis and the bulk of the leaf was used for the preparation of sample teas by the Technology Division. The sample teas were made in order to ascertain that the "good quality clone" was in fact better than the "poor quality clone". All sample teas were evaluated by four commercial tea tasters in Colombo. The tasters' reports on the teas made confirmed with a high degree of significance ($P < 0.01$) that clone TRI 777 made a better quality tea than clone TRI 740. The Technologist's summary of the tasters' evaluations is shown in Table 1.

TABLE 1.—*Average Marks given by Colombo Tasters to Twenty Teas made from Clones TRI 740 and TRI 777 over the Period 27 March to 14 October, 1963.* (*Table supplied by E. L. Keegel, Technologist*).

Characteristic	Clone	Taster A	Taster B	Taster C	Taster D	Totals
Infusion	777	6.21 n.s.	6.14*	6.93**	6.00**	25.28**
	740	5.64	5.21	4.97	4.50	20.14
Colour	777	8.43**	9.29**	7.57**	8.07**	33.36**
	740	6.36	6.71	5.93	4.93	23.93
Strength	777	5.86 n.s.	6.64**	6.36**	7.57**	26.43**
	740	5.29	6.00	5.36	5.07	21.72
Quality	777	4.21	4.50	7.21**	6.14**	22.06**
	740	4.86*	5.07 n.s.	5.00	4.64	19.57
Valuation	777	Rs. 2.37	Rs. 2.38**	Rs. 2.13**	Rs. 2.50**	Rs. 9.38**
	740	Rs. 2.45n.s.	Rs. 2.25	Rs. 1.83	Rs. 2.10	Rs. 8.63

n.s. = no significant difference.
* = significantly greater at 5% level (P = .05)
** = significantly greater at 1% level (P = .01).

Twelve chemical quantities were determined on samples from as many of the 25 plucks as was possible. The averages of the results of the chemical analyses are shown in Table 2. Statistical analysis of these results, also shown in Table 2, indicated that the high quality clone TRI 777 contained significantly higher levels of total nitrogen, caffeine, protein, total soluble solids, total soluble nitrogen, and total flavanols, but lower levels of ash and crude fats than the poor quality clone TRI 740.

TABLE 2.—*Average Level of Twelve Chemical Quantities in Fresh Tea Flush from Clones TRI 740 and TRI 777 over an Eight Month Period* (25 *plucks were carried out between* 27 *March and* 2 *December,* 1963).

Chemical Quantity Measured	No. of Pluckings Sampled	CLONE				Level of Significance of Differences
		TRI 740		TRI 777		
		% FRESH WEIGHT				
Moisture	25	78.00	(76.2–80.2)*	78.2	(76.5–80.2)	None
		% DRY WEIGHT				
Ash	25	5.35	(4.90–5.77)	4.81	(4.32–5.17)	1%
Total nitrogen	25	4.69	(4.27–5.65)	4.93	(4.44–5.48)	1%
Caffeine	25	3.13	(2.83–3.95)	3.63	(3.23–4.00)	1%
Protein	25	23.7	(21.3–28.5)	24.3	(21.3–27.3)	1%
Pectin	25	5.42	(3.63–6.42)	5.22	(3.23–6.42)	Non
Total soluble solids	25	42.2	(32.5–47.1)	43.3	(31.2–46.9)	1%
Total soluble nitrogen	25	1.71	(1.45–2.27)	2.05	(1.71–2.44)	1%
Total oxidizable matter	25	26.2	(17.0–30.8)	26.5	(14.9–30.7)	None
Crude fats	10	1.38	(1.16–2.03)	1.26	(1.08–1.73)	2%
Crude fibre	12	10.5	(9.9–11.6)	10.3	(9.6–11.6)	None
Total flavanols	25	26.0	(18.0–36.0)	27.8	(20.0–33.8)	1%

*Brackets enclose minimum and maximum values found.

Of particular interest was the finding that all the nitrogenous quantities determined (total nitrogen, caffeine, protein, and total soluble nitrogen) were present at higher levels in the high quality clone and the differences were all highly significant. This might at first seem to be contradictory to previous findings (*cf.* Ramasamy, 1963, and Keegel, 1959a and 1963) which have indicated that nitrogen is deleterious to quality, but we think that there is a simple explanation. First of all, we must recognize that excessive nitrogen by itself appears to be detrimental to quality as has been shown by many trials. But the results of the investigation reported here suggest that nitrogen is not detrimental in itself; in fact it may be beneficial. The important consideration would seem to be the chemical balance within the tea plant. An exposition of the effect of nitrogen upon the chemical balance in plant tissues was put forth in 1918 by Kraus and Kraybill which, with some modification, seems to be relevant here. They pointed out that high levels of nitrogen (N) coupled with low levels of carbohydrates** (c) in plant tissues results in rapid but spindly growth; this represents an imbalance of cellular constituents. Low levels of nitrogen (n) coupled with high levels of carbohydrates (C) results in slow coarse growth; this represents an imbalance also. Low levels of nitrogen (n) and low levels of carbohydrates (c) results in slow normal growth while high levels of nitrogen (N) and high levels of carbohydrates (C) results in vigorous normal growth; both conditions lead to balanced growth. Notice that it is the balance of nitrogen to carbohydrates which is important for normal growth. These generalizations have found wide application in horticulture and it is our contention that they apply to tea as well. Furthermore, we suspect that it is necessary to have our flush produced under balanced growth conditions in order to produce the best teas possible.

**The term carbohydrates will be used here to indicate true carbohydrates plus some other non-nitrogenous compounds, especially the flavanols.

Assuming for the present that our contentions and suspicions are correct, we see from the above discussion that we can get the desired results, *i.e.* balanced growth of our tea plants, at either low levels of nitrogen (n/c) or at high levels of nitrogen (N/C) remembering, of course, that the respective carbohydrate levels must be manipulated accordingly. However, we can only get high yields with vigorous plants, *i.e.* with high levels of nitrogen, and, therefore, we are only really interested in the latter case. But as we raise the levels of nitrogen available to the plant we must also raise the levels of the factors contributing to the levels of carbohydrates and related compounds or loose quality. If this is true then additional nitrogen applications will have to be accompanied by other changes in planting practices in order to preserve the quality of our tea. For instance, fertilizers other than nitrogen may be required, some of which may not be required at all with todays levels of nitrogen fertilization. Changes in plucking frequency, planting distances, use of shade, etc. may also be necessary.

It is relevant to this discussion to point out that all considerations of quality must revolve around the "quality potential" of the starting material. It is doubtful whether poor clones or poor jats could ever make good tea no matter what is done to the plants in the field or to the flush from these plants in the factory. We must see to it that we plant good material in the field and then apply the above mentioned principles so as to preserve its inherent qualities at all cropping levels.

The results of measurements of the levels of the enzymes polyphenol oxidase and pectin esterase are summarized in Table 3. The level of polyphenol oxidase was markedly higher in the high quality clone TRI 777 than in the low quality clone TRI 740. This accounts for the fact that clone TRI 777 is a very good fermenter (and has very good colour) while clone TRI 740 is a rather poor fermenter. The importance of this enzyme in tea manufacture cannot be overemphasized because it is the agent responsible for the oxidation of the tea leaf flavanols during fermentation. We simply cannot make black tea without it. However, evidence has recently been obtained which indicates that the level of this enzyme within the leaf is not always the factor which determines the rate of fermentation (Sanderson, 1963b). This matter is under further investigation.

TABLE 3.—*Average Level of Activity of Two Enzymes in Fresh Tea Flush from Clones TRI 740 and TRI 777 over an Eight Month Period* (25 *plucks were carried out between* 27 *March and* 2 *December*, 1963).

Enzyme	No. of Pluckings Analyzed	CLONE		Level of Significance of Difference
		TRI 740	TRI 777	
		ENZYME UNITS/G ACETONE POWDER		
Polyphenol Oxidase	24	71.4 (31.3–171.1)*	164.3 (89.9–376.0)	1%
Pectin Esterase	16	6.54 (1.65–20.45)	6.00 (1.25–16.29)	None

*Brackets enclose minimum and maximum values found.

Testing of the Above Findings with Eleven Clones

As a beginning towards determining which of the chemical differences listed in Tables 2 and 3 are casually related to quality and which are only fortuitous, eleven clones representing all four quality classes, (Keegel, 1959b, 1962b and 1963) and two unclassified clones have been compared for five of the relevant chemical quantities (ash, total flavanols, caffeine, total nitrogen and polyphenol oxidase activity). All clones were sampled simultaneously on three different dates. A summary of these results is shown in Table 4.

TABLE 4 (a–e). *Averages of Five Chemical Quantities in Eleven Clones Sampled on Three Dates*

The brackets enclose clones whose means *did not* differ significantly. Therefore, only clones not enclosed by a particular bracket are significantly different: 5% level of significance.

The sampling dates were 17/9/63, 10/12/63 and 17/12/63. With every quantity measured the mean level on at least one date was significantly different from the others; 1% level of significance.

TABLE 4 a—*Ash*

Rank	Clone (Classification)	Ave. % Dry Wt.
1.	(C) PA. 22	6.19
2.	(C) TRI. 2026	5.66
3.	(A_1) DT. 1	5.64
4.	(B) KEN. 16/3	5.63
5.	(?) CY. 9	5.59
6.	(B) TRI. 2025	5.56
7.	(B) UH. 9/3	5.48
8.	(A_2) TRI. 2024	5.44
9.	(A_2) TK. 48	5.44
10.	(?) CV. 5.B.1	5.22
11.	(A_1) TRI. 777	5.04

TABLE 4 b - TOTAL FLAVANOLS

Rank	Clone (Classification)	Ave. % Dry Wt.
1.	(B) UH. 9/3	24.0
2.	(B) TRI. 2025	21.2
3.	(?) CV.5.B.1	20.4
4.	(A_2) TRI. 2024	20.4
5.	(A_1) DT. 1	19.6
6.	(C) PA. 22	19.2
7.	(A_1) TRI. 777	18.0
8.	(C) TRI. 2026	17.8
9.	(B) KEN. 16/3	17.8
10.	(A_2) TK. 48	17.6
11.	(?) CY. 9	17.2

TABLE 4 c - TOTAL NITROGEN

Rank	Clone (Classification)	Ave. % Dry Wt.
1.	(C) TRI. 2026	4.83
2.	(A_2) TRI. 2024	4.73
3.	(?) CY. 9	4.69
4.	(A_2) TK. 48	4.69
5.	(B) TRI. 2025	4.65
6.	(C) PA. 22	4.60
7.	(A_1) TRI. 777	4.58
8.	(?) CV.5.B.1	4.48
9.	(B) KEN. 16/3	4.44
10.	(B) UH. 9/3	4.43
11.	(A_1) DT. 1	4.19

TABLE 4 d - CAFFEINE

Rank	Clone (Classification)	Ave. % Dry. Wt.
1.	(A_1) TRI. 777	3.55
2.	(?) CY. 9	3.45
3.	(B) UH. 9/3	3.33
4.	(A_1) DT. 1	3.26
5.	(A_2) TRI. 2024	3.19
6.	(B) TRI. 2025	3.16
7.	(?) CV. 5.B.1	3.12
8.	(C) TRI. 2026	3.11
9.	(C) PA. 22	2.93
10.	(B) KEN. 16/3	2.58
11.	(A_2) TK. 48	2.41

TABLE 4 e - POLYPHENOL OXIDASE ACTIVITY

Rank	Clone (Classification)		Ave. Activity Units
1.	(A_1)	TRI. 777	99
2.	(A_2)	TRI. 2024	78
3.	(B)	UH. 9/3	65
4.	(B)	TRI. 2025	65
5.	(A_1)	DT. 1	56
6.	(?)	CY. 9	55
7.	(B)	KEN. 16/3	52
8.	(C)	TRI. 2025	51
9.	(C)	PA. 22	51
10.	(A_2)	TK. 48	43
11.	(?)	CV. 5.B.1	29

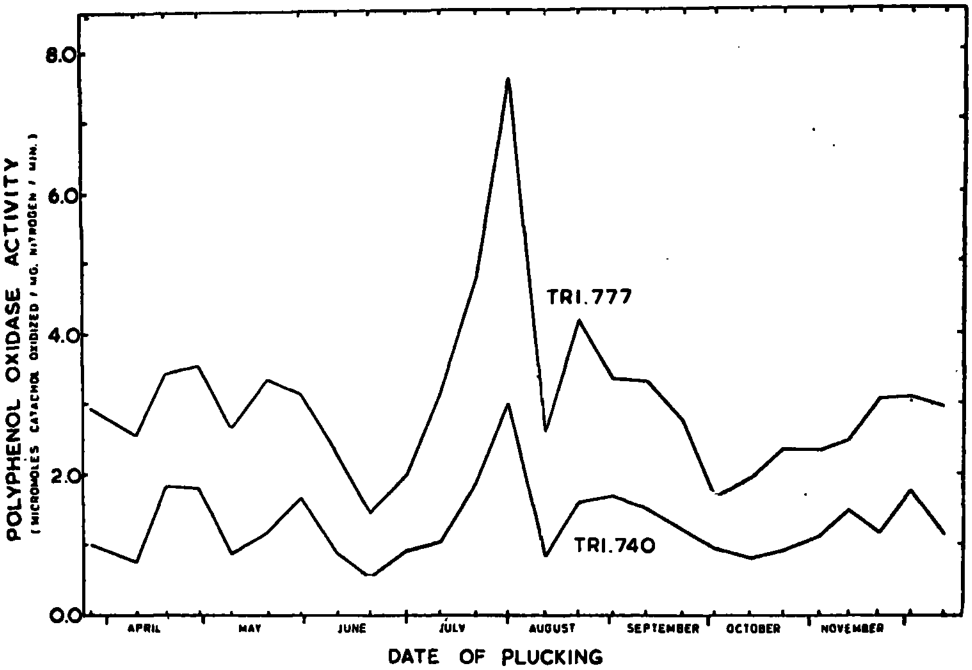
In Table 4 the clones have been arranged in descending order with respect to the level of the chemical quantity measured. This was done to enable one to notice at a glance which of the chemical quantities are related to quality. That is, if there is a real relationship between any chemical quantity and quality, the clones should arrange themselves in such a table in order of their decreasing or increasing quality potential depending on whether that particular chemical quantity is positively of negatively related to quality. On the other hand, where there is a tendency to random distribution of clones according to quality in such a table there is little likelihood of a relation existing between quality and that chemical quantity. All three cases may be seen in Table 4. Ash content is negatively related to quality (Table 4a), total flavanol content and total nitrogen content are unrelated to quality (Table 4b,c) caffeine appears to be weakly positively related to quality (Table 4d) and polyphenol oxidase activity is positively related to quality (Table 4e).

To qualify the above statements, it should be pointed out that in no case was a perfect relationship found. The clone DT 1 with its excellent quality potential (Keegel, 1963) but high ash content and only moderate polyphenol oxidase activity is noteworthy as an example of a clone which does not follow the rules. The implication is that no one chemical quantity will ever be found which will by itself give a sure indication of the quality potential of a clone. Quality is determined by a number of interacting factors. Nevertheless, the above generalizations should prove helpful in our pursuit of the chemical explanation of quality and the anomolous clones should provide most interesting investigational material.

The Seasonal Changes in the Chemical Composition of the Fresh Tea Flush

The comparative study of the clones TRI 740 and TRI 777 afforded a good opportunity to follow the seasonal changes in the chemical quantities being measured. Many of the changes were considerable over the eight month period as can be seen in Tables 2 and 3 (the variation in the values found is indicated by the figures in brackets in Tables 2 and 3 which represent minimum and maximum values found), Characteristic was the variation shown by the level of activity of the enzyme poly-phenol oxidase in the fresh flush. The activity of this enzyme is plotted against date of plucking in Figure 1. Examination of this figure shows that sampling in studies such as this is of extreme importance. The level of polyphenol oxidase activity in clone TRI 777 was always from two to three times higher than in clone TRI 740. Yet if one had only sampled these clones once on different dates and had inadvertently sampled clone TRI 777 on 20th June and clone TRI 740 on 1st August exactly the opposite conclusions would have been arrived at. The situation also holds to a lesser extent for the other chemical quantities measured as examination of Tables 2 and 3 will reveal.

FIGURE 1. CHANGES IN POLYPHENOL OXIDASE ACTIVITY IN FRESH TEA FLUSH FROM CLONES TRI.740 AND TRI.777 OVER AN EIGHT MONTH PERIOD - APRIL THROUGH NOVEMBER, 1963.



It is thought that these seasonal fluctuations are responses by the tea plant to changing climatic conditions but no correlation has been found between them and the weather data available at the Tea Research Institute. It is hoped that progress on this problem can be made in the near future.

Attempts to correlate the fluctuations in the composition of fresh leaf with the evaluation of the tea made from this leaf by the tea tasters have not been successful so far. We hope that with the addition of a tea taster to our staff some progress in this direction can be made.

Evans (1929) showed over thirty years ago that there were marked seasonal variations of total soluble solids, tannin, total soluble nitrogen and total nitrogen in fresh tea leaf but little practical use has been made of this information. However, these findings suggest that we are only getting the best out of a small portion of our fresh leaf. That is, the tea maker of today develops a manufacturing program which gives him the best possible results over the year, and then he adheres to this program with very little variation month after month. Yet if his leaf is varying markedly in composition every day, it would seem reasonable that it might be profitable to vary his manufacturing program day by day also.

In practice, however, there are three important obstacles which must be overcome before we can hope to implement such a radical departure from current manufacturing procedures as a daily varying manufacturing program. First, we must determine what changes the various chemical constituents of the fresh tea leaf undergo during tea manufacture and what effect, if any, they have upon the final product. Next we must determine what manufacturing conditions are necessary to make the most out of leaf of all types. Then we must furnish the tea maker with rapid easy to use methods for determining the status of his leaf as he receives it to enable him to plan his program for each days leaf.

We are a long way from being able to tailor make manufacturing programs but the problems are not insurmountable. With improved cultural practices, such as planting of clonal tea and controlled manuring, and with new manufacturing techniques and machines (cf. Keegel, 1962a, Sanderson, 1963a, Trinick, 1963a and 1963b, and Weragoda, 1964) it should become easier to implement such practices in the near future.

Conclusion

Our findings to date convince us that the tea plant is a very dynamic one. We need to know why the chemistry of our tea plant varies from day to day in order to be able to control it as much as possible and so that we can understand the characteristics of our raw material better on a day to day basis. Eventually we want to be able to take full advantage of the potential of our fresh leaf every day of the year.

Unavoidably, the foregoing duscussion contains many conjectures and much speculation. We hope that by the time of our next conference some of this guess work will have been given a firm scientific foundation.

Acknowledgement

The co-operation of E. L. Keegel, Technologist, in these investigations has been invaluable.

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ANSWER TO QUESTIONS RAISED ON DR SANDERSON'S PAPER

Questions :—**1.** Is there any effect on the quality of tea produced by increased nitrogen application? Dr K.T. Hasan

2. It has been stated that manuring over 120 lbs nitrogen per acre tends to depress quality. Is it worth while getting quantity by manuring heavily at the expense of quality? Anonymous.

3. What are the dangers and difficulties you anticipate at high levels of nitrogen, applied to high yielding tea? I am thinking of high grown yields of well over 2000 lbs and nitrogen applications of well over 200 lbs of nitrogen per acre per annum. Manager, High Forest.

Answer:—Most of the points raised by the above questions are answered in my paper. However, for clarity I will briefly outline our existing knowledge on these points here and now. In the first place, it has been found in Assam that nitrogen manuring when done alone, without the addition of other fertilizers, at levels of over 120 lb acre/annum will under their conditions bring about a loss of quality. Keegel has published results on his investigations in Ceylon (Technologists' Annual Report for 1957 and 1963) which tend to support this. In Ceylon where nitrogen applications are accompanied by potash and phosphate applications we think that it is possible to go to higher levels of nitrogen manuring without losing appreciably as regards quality. Further-more, as I brought out in my paper, there is good reason to think that we need not lose quality even at very high levels of nitrogen manuring, say over 200 lb nitrogen/acre/annum, provided the enhanced growth caused by the extra nitrogen is 'balanced' growth. We know very little about how to ensure 'balanced' growth at high levels of nitrogen manuring at the present time, but we can suggest the following factors for consideration in seeking a solution:

1. Use of clones which can utilize high levels of nitrogen without losing quality.

2. In manuring, keeping a proper balance between nitrogen and other nutrients.

3. Modification of agronomic factors; for example, length of plucking round, planting distances, use of shade and others.

4. Modification of factories to ensure adequate room and equipment to handle the increased crops.

The paper that we have heard from Mr. A.D. Neale refers to the economic aspects of these questions. It would appear that some estates have decided that it pays to go for yield at the risk of losing some or all quality. It is our contention, however, that it is both economic and far sighted to do everything possible to retain or even improve the quality of our tea. Not only is a tea with quality worth more today than one without it, but in the future as world competition becomes greater and greater there is a good possibility that teas without quality will be entirely unsaleable.