CHAPTER 6
BIOCHEMISTRY

6. Biochemistry

6.1 Introduction

Identifying and defining quality of tea cultivars is important in the breeding and use of tea cultivars. The biochemistry research programme was focused on developing improved methods for identifying and defining quality in tea. This was approached in the following ways:

- Development of models of quality
- Develop chemical and sensory techniques for monitoring quality at different stages.
- Identify critical steps in the chemical pathway leading to the formation of colour and flavour in tea
- Develop chemical methods for screening breeders' material for quality

The main programme was implemented at the University of Pretoria (see publications list). However, one major component of the research programme conducted at TRF Malawi concerned an investigation into the critical steps in the chemical pathway leading to the formation of colour and flavour in tea. This chapter provides a summary of the work conducted by Temple (1999).

6.2 Thearubigins of black tea

While historically much attention has been paid by biochemists to theaflavins, responsible for briskness in tea, surprisingly little is known about thearubigins (TRs). They contribute 10 – 20% by weight of black tea and may account for 75% of the total depth of colour. The term was first used to describe a varied group of substances thought to be breakdown products of theaflavins and has subsequently become the description of soluble products for fermentation which are not theaflavins. Clearly, much remains to be known of the major components of made tea, given the importance of colour in the marketing of regional teas. New techniques were applied to the study of thearubigins, with particular emphasis on their development as influenced by steps in tea manufacture.

Leaf handling

A series of articles have been published on leaf handling studies (Mashingaidze and Tomlins, 1997). These concentrated on various aspects of losses likely to occur from plucking to arrival at the factory, especially in relation to temperature rise and exposure, but they did not explain the change in biochemical terms.

Chromatographic profiles using HPLC techniques indicated changes in the thearubigin (TR) fraction after exposure of leaf to heat for long periods; the magnitude of change in liquor profile implies that the handling of leaf prior to arrival at the factory is perhaps more important to quality than withering of drying. Given this observation, it is essential that treatment of leaf after plucking must be accurately recorded in all manufacturing trials.

Withering

While the various withering treatments used in trials at TRF had little effect on the commercial value of the teas produced, there were distinct biochemical differences, which were not detectable by standard colorimetric techniques but using the size exclusion HPLC technique to separate the thearubigins by size. Overnight withering at ambient

temperature promoted the development of caffeine - precipitatable thearubigins and the creaming properties of the liquor. These phenomena are of interest to those selling clear chilled tea beverages, where low creaming is desirable.

Fermentation

Time

Commercially, PC 108 is fermented for between 45 and 50 minutes according to ambient temperature, to yield the desirable "brisk tea". Longer fermentation gives rise to "soft" and "flat" liquors. Despite increasing colour there is a decline in the parameters of briskness and strength. The caffeine-precipitatable thearubigins are shown to be products of fermentation rather than plant metabolites and thus their levels in liquors can be manipulated during manufacture.

Aeration

A comparison was made between well aerated and restricted, rather than excluded, aeration. Under normal circumstances oxygen enters the dhool during maceration and is also dissolved in cell sap; air is also found in the interstitial spaces of the leaf structure so exclusion does not arise.

Restriction of air retarded fermentation, particularly the yielded theaflavin 3.3digallate, which is associated with greater astringency. It is assumed that the early fermentation utilizes oxygen dissolved in the dhool and, when that is exhausted, fermentation is limited. With longer fermentation time, and with more aeration there was a reduction in total TRs, implying the consumption of these highly coloured molecules in the generation of TR.

Temperature

The study did not isolate temperature specifically, so that fermentation would also be affected by changes in enzyme activity and oxygen solubility owing to temperature. Elevated temperature reduced the total theaflavins; oxygen depletion occurs at elevated temperatures due to the reduced solubility of oxygen and rapid assimilation as reaction rates rise. The study indicated that elevated temperature promotes alternative reaction pathways. The parabolic curves for total TF development and decay are more acute at higher temperature, showing that the duration of fermentation is more critical at elaborate temperature. High temperature does not necessarily inhibit quality as long as attention is paid to timing.

Drying

A coppery liquor is very desirable in the trade, whilst the dry tea particles are required to be black. Colour of liquor was highest when tea was dried at 80 – 120°C and above this range a burnt taint was detected. Teas dried at the higher temperatures also contained less caffeine, which is probably a result of sublimation. If a fluid bed dryer is well controlled, genotype and fermentation period are greater determinants of pigment patterns than the drying process itself, and the temperature profile during drying should be adjusted to avoid burning of the tea. Further work was required to determine drying conditions promoting stability or good storage properties.

6.3 Prediction of quality from fresh leaf analysis

A study was on-going to establish a parameter of fresh tea leaf that correlates with final black tea quality (Wright et al., 2000). Twenty each of VP cultivars rated as relatively good and poor quality were selected from the breeding programme at TRF (CA). The flavan-3-ol profile of the fresh leaves was analysed by capillary electrophoresis while total theaflavin (TF) content was determined using flavognost

method, and were correlated with total scores and valuations from two tea brokers with regression analysis carried out.

The total TF content of the leaf correlated very well with the value of the tea. Of all the parameters determined for the fresh leaf, the highest correlation value was obtained with – epicatechin (r = 0.65). This may facilitate the early selection of good quality cultivars in the future.

6.4 Publications

1. Apostolides, Z., Balentine, D.A., Harbowy, M.E, Hara. Y and Weisburger, J.H. (1997). Inhibition of PhIP mutagenicity by catechins, and by theaflavins and gallate esters, **389**, 167-172.
2. Apostolides, Z. (1999). Health properties of tea – opportunities and threats. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **135**, 15-17.
3. Apostolides, Z. (2001). The Anti-oxidant content of a cup of tea. World Tea Convention, Nairobi, Kenya, October 2001.
4. Aucamp, J. Gaspar, A. Hara, Y. and Apostolides, Z (1997). Inhibition of Xanthine Oxidase by catechins from Tea (Camelia sinensis). *Anticancer research*, **17**, 4381-4386.
5. Aucamp, J.P. (2000). The Inhiibition of Xanthine Oxidase with polyphenols off tea ,'(*Camellia sinensis*). M.Sc. Thesis, University of Pretoria, pp.160 (unpublished).
6. Aucamp, J.P., Hara, Y. and Apostolides, Z. (2000). Simultaneous analysis of tea catechins, caffeine, gallic acid, theanine and ascorbic acid by micellar electrokinetic capillary chromatography. *Journal of Chromatography* **A876**, 235-242.
7. du Toit R., Volsteedt Y. and Apostolides, Z. (2001). Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. *Toxicology* **166**, 63-69.
8. Selematsela, M. Gaspar, A., Becker R and Apostolides, Z. (2001). A Mouse model of AIDS for testing AZT in a combination therapy with tea polyphenols. International O-Cha (tea) culture and science conference, Shizoka, Japan, October 2001.
9. Wright, L.P. (1996). Biochemical Markers for the Identification of Tea Clones. MSc. thesis, University of Pretoria, 98 pp. (unpublished).
10. Wright, L.P. Mphangwe, N.I.K., Nyirenda, H.E. and Apostolides, Z. (2000). Analysis of caffeine and flavan-3-ol composition in the fresh leaf of *Camellia sinensis* for predicting the quality of the black tea produced in Central and Southern Africa. *Journal of the Science of Food and Agriculture* **80**, 1823 – 1830.
11. Wright, L.P., Aucamp, J.P. and Apostolides, Z. (2001). Analysis of black tea theaflavins by non-aqueous capillary electrophoresis. *Journal of Chromatography* **919**, 205-213.
12. Wright, L.P., Mphangwe, N.I.K., Nyirenda, H.E. and Apostolides, Z. (2001). Analysis of theaflavin composition in black tea (Camellia sinensis) for predicting the quality of tea produced in Central and Southern Africa. *Journal of the Science of Food and Agriculture* (in press).
13. Temple, C.M. (1999). Thearubigins of Black Tea: Manufacturing –based studies. Ph.D. Thesis, University of Surrey, 208 pp. (unpublished).

6. Other Publications

1. de Jong, J. (1997a). Calculation of the tractor and equipment operating costs based on the litres of fuel used for an operation on a per hectare

basis. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **127**, 15-21.
2. de Jong, J. (1997b). Manual conveying of water from source to the watering are within the nursery when watering young tea. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **127**, 15-18.
3. de Jong, J. (1997c).Aspects of labour management. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **128**, 9-12.
4. de Jong, J. (1997d). Fertilizing and top-dressing by hand. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **128**, 12-15.
5. de Jong, J. (1998a). Irrigation and irrigation management. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **129**, 13-19.
6. de Jong, J. (1998b).Mechanical harvesting of tea. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **129**, 19-25.
7. de Jong, J. (1998c).Work planning. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **130**, 20-39.
8. de Jong, J. (2000).Productivity in Tea Production, *Tea Research Foundation (Central Africa).*
9. Martin, P.J. (1999). Main grade tea prices at Limbe auctions, 1996 to 1999. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **135**, 13-14.
10. Martin, P.J. and Nachanje, Y.H. (1999). Recent TRF support to the Malawi smallholder sector. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **132**, 3-8.
11. Mputeni, P.S. (1998a). Economic model of replanting. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **129**, 25-28.
12. Mputeni, P.S. (1998B). Reducing the payback period. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **130**, 16-19.
13. Mputeni, P.S. (1999).Demand analysis of the market for tea facing the Malawi producers. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **136,** 3-6.
14. von Lucttichau, C and L. (2000). The TRF training programme. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **140**, 17-18.
15. von Luttichau, C. and Dankeni, P.Y.P. (2001). An update on TRF(CA)'s training programme. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **143**, 38-39.
16. Whittle, A.M. (2000). Smallholder expansion plan. *Tea Research Foundation (Central Africa) Quarterly Newsletter* **137**, 22-23.