



The Evaluation of Volatile Quality Factors in Black Tea

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FOREWORD

A fundamental problem for any food industry, particularly one which deals with minimal processing of a natural commodity such as black tea, is the relationship between perceived quality and the presence of natural flavour compounds in the product.

This study has attempted to determine a relationship between an objective semi-quantitative measure of the aroma compounds and a subjective organoleptic one. Characterisation of organic extractives was also a significant part of the research.

The project identified some of the important extractable organic compounds in black tea, although a definitive relationship between the level of these compounds and organoleptic appraisal was not clearly established.

The characterisation of the organic extractives also led to the development of a terpene index and chemical “fingerprints”.

The study has implications for the Australian tea industry. For example, the project’s methodology could be used to assess the flavour quality of black tea and tea manufacturers could use terpene indices and chemical profiles to grade teas, verify the quality of pre-shipment samples, monitor pre and post processing deterioration of tea, evaluate the effects of climatic and agronomic variation on tea quality, and source suitable material from other countries and suppliers.

This report is a valuable addition to the Corporation’s New Plant Products program which facilitates the development of new industries based on plants or plant products that have commercial potential for Australia.

Peter Core

Managing Director

Rural Industries Research and Development Corporation

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EXECUTIVE SUMMARY

The aroma of different black teas is influenced by a number of factors which include the particular variety, the degree of growth prior to harvesting, and the particular agronomic variables under which the tea has been grown, together with the particular method of processing employed.

The majority of aroma compounds are formed as a result of processing, and are formed from amino acids, fatty acids and glycosides.

Although tea quality is evaluated on a number of parameters, an attractive aroma is essential for quality tea. Indeed, the composition and concentration of aroma compounds has been shown to play a vital role in the valuation and/or pricing of tea.

In this project, simultaneous steam distillation extraction (SDE, water/hexane) was used to extract the volatile compounds present in black tea grades from three countries (Sri Lanka, India and Indonesia), and a blended tea produced from them.

The concentrated hexane extract (rotary evaporation) was subjected to gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analysis to both quantify (internal standard: methyl undecanoate), and identify the individual volatile compounds.

Sensory evaluation was performed by a panel of three regular tea tasters, and scores were based on infusion colour, brightness, aroma, flavour strength, astringency and overall balance, on a scale of 0 to 10 for each parameter.

The concentrations of some 35 compounds within the hexane SDE extract were tabulated for each batch of each particular tea grade, and considerable variation was found both within and between different grades.

A terpene index was calculated for all tea samples using the ratio of the levels of linalools (linalool and linalool oxides) to that of linalools and geraniols (geraniol and geranial); and a plot of the total concentration of these terpenes versus terpene index allowed some discrimination of the various tea grades. Significant difference was noted between teas manufactured by different methods (crush, tear, curl (CTC) and orthodox), and from differing countries of origin.

Determined correlation coefficients indicated some relationship between terpene levels and organoleptic score when orthodox teas were used. However, no clear relationship seemed to exist between terpene index/levels of terpenes for CTC teas, and organoleptic score.

Principal component analysis using a statistical program (systat) was used upon all compounds and two subsets, in an attempt to discriminate between grades. The greatest percentage of variation between samples was accounted for using three principle components and a subset comprised of the compounds used in the terpene index. However, it was still not possible to separate all grades of tea into specific clusters; the greatest problem being with the Sri Lankan teas produced by the same manufacturing process.

Chemical fingerprints were prepared for each grade of tea by using eleven compounds selected on the basis of concentration and variability. Using such fingerprints, it was largely possible to distinguish between grades. The majority of batches of the very similar Sri Lankan 535 and 532 orthodox teas could be discriminated by this technique, although analysis indicated that whether these teas could be justifiably distinguished into two different grades was questionable.

It is thus possible to build a database and identify teas on the basis of the levels of specific compounds, using one or more of the aforementioned techniques. The data obtained would suggest that there is a relationship between the concentration of such compounds and the perceived quality of the tea. It is suggested that the techniques discussed could be used to match pre-shipment samples, source new material and monitor deterioration or variability in black tea quality.

1.0 INTRODUCTION

Black tea is one of the most widely consumed beverages in the world. Various kinds of tea are consumed, but almost all of them are produced from the same species of plant, *Camellia sinensis*. Teas are classified into three major categories according to the manufacturing process: unfermented green tea, partially fermented oolong tea, and fully fermented black tea.

Note that the term 'fermentation' is a misnomer, as no microbial action is involved in the fermentation. Fermentation in this case is the result of enzymic action and exposure to atmospheric oxygen.

Although tea quality has been evaluated on the colour, aroma and taste of tea, an attractive aroma is essential for quality tea. The chemical nature of tea aroma, the mechanisms of aroma formation during tea leaf processing, and the objective estimation of finished tea aroma are all subjects which have long been the focus of interest of many researchers (Bokuchava and Skobeleva, 1986).

The aroma of different teas is influenced by a number of factors which include the particular variety, the degree of growth prior to harvesting, the particular agronomic variables under which the tea has been grown; together with the particular method of processing employed.

Consumer acceptability of tea is largely dependent upon the flavour of the product. Flavour can be broadly divided into taste and aroma. The volatile components comprise the aroma, whereas the non-volatile components are responsible for taste. However, aroma is considered to have the most impact on the flavour of food products, the sense of smell being much more highly developed than the sense of taste (Robinson and Owuor, 1992), although the perception limit for different compounds does vary significantly.

Many volatile compounds have been identified in tea, and new compounds continue to be found. A number of the compounds identified to date is given in Appendix 4 (adapted from Willson and Clifford, 1992). Interestingly, the synthetic pathways for the formation of the majority of these compounds have not been fully elucidated, and are awaiting further investigation. However, compounds may be identified as either primary or secondary plant metabolites (Sanderson and Graham, 1973). Primary metabolites are those synthesised and present in fresh leaf, whereas secondary products are produced during processing .

Primary metabolites are mostly alcohols and include hexanol, linalool plus its oxides, nerol, geraniol and nerolidol. The concentration of these compounds changes during processing and may either increase, due to direct production, or apparently decrease, due to the formation of non-volatile glycosides. The majority of aroma compounds are formed as a result of processing, and are formed from amino acids, fatty acids, carotenoids and glycosides (Yamanishi, 1981). These aroma compounds are secondary metabolites, although some of these compounds could technically be classified as either primary or secondary, as they may continue to be formed during processing.

Aroma compounds may also be categorised into one of two groups, namely, those that are thought to be deleterious to tea quality when present at higher concentrations, and those the presence of which is considered advantageous. These groups have been denoted as group I and group II respectively. Such classification has been based upon retention time during analysis or odour characteristics (grassy or fruity). The ratio of group II to I has been previously used to rank teas in order of flavour quality (Owour and Langat 1988), as has the ratio of terpenoid to non-terpenoid compounds (Mahanta and Baruah, 1989).

Early planters found that the best quality tea was produced from the actively growing bud. Tea produced from leaves further from the tip showed a gradual deterioration in quality. However, a heavier crop can be obtained by removing the larger, lower quality leaves. Harvesting the first two leaves together with the bud proved to be the best compromise. Figure 1 illustrates the components of a typical tea shoot.

The harvesting operation is known as '*plucking*', as this describes the action of harvesting manually. There are variations of the material harvested, plucking two leaves or less and a bud is known as '*fine*' and more than two

and a bud as '*coarse*'. Fine plucking produces a higher quality tea containing more monoterpenoids and less non-terpenoids than coarse plucking (Mahanta and Baruah, 1988). Coarse plucking is used when yield is more important than quality. Apart from the two and a bud, there will always be overmature, immature, dormant (banjhi) and damaged leaf.

The length of time between consecutive occasions when a field is plucked should remain fairly steady when weather conditions are reasonably constant. The interval between pluckings is known as the '*plucking round*'. Rounds may be as short as 4 or 5 days, and as long as a month during adverse conditions. All the teas used in this investigation were manually plucked, although the plucking standard was unknown. One would presume that between two leaves and a bud, to four leaves and a bud would be a fairly typical standard for these teas.

After harvesting, the levels of amino acids in the fresh leaf increases due to catabolic reactions, due for the most part to peptidases (Sanderson and Roberts, 1964). However, during processing, levels of amino acids fall, accompanied by the formation of aldehydes. In this manner, phenylalanine is deaminated to form phenylacetaldehyde. Aldehydes may be oxidised to form carboxylic acids, or reduced to the corresponding alcohol (Figure 2). Such reactions are catalysed by polyphenol oxidase in the presence of oxygen and catechins (Saijo and Takeo, 1970). Catechins are oxidised to ortho-quinones, a proportion of which are transformed into theaflavins, thearubigins and theaflavic acids, which give black tea its characteristic colour and taste. The remainder of the ortho-quinones interact with amino acids, carotenes and plant lipids resulting in the formation of aroma compounds.

Figure 1: Tea shoot showing scale and foliage leaves, plucking levels and normal *'two and a bud'* crop (reproduced from Willson and Clifford, 1992)

Figure 2: The formation of aldehydes, alcohols and carboxylic acids from amino acids (reproduced from Willson and Clifford, 1992)

Other investigations have revealed that in addition to the enzymic pathway of aroma formation, a thermal route also exists (Popov, 1966).

The majority of plant lipid is composed of free fatty acids, especially linolenic, and their esters, although the fatty acid profile will change with variety/clone and agronomic conditions (Selvendran *et al.*, 1978). Levels of fatty acids change during tea processing, unsaturated fatty acids degrading to form aroma compounds. Linoleic and linolenic acids produce hexenal and trans-2-hexenal respectively (Hatanaka *et al.*, 1987). Alcohol dehydrogenase is responsible for the production of hexanol from hexenal (Hatanaka *et al.*, 1974). In fact, the majority of straight chain aroma compounds are thought to be derived from lipid degradation (Figure 3).

Figure 3: The production of C₆ volatile flavour compounds from linoleic and linolenic acids (reproduced from Willson and Clifford, 1992)

The levels of carotenoid pigments fall during tea processing, with the concomitant formation of various aroma compounds such as α and β -ionones, and linalool (Figure 4). Oxidative, enzymic reactions during withering, and pyrolytic reactions during firing, have been shown to be responsible (Sanderson and Graham, 1973).

The production of the various aroma compounds occurs during the stages of manufacture, which can be divided into withering, leaf disruption, fermentation and firing.

The extent of withering has been shown to directly affect the levels of volatile compounds. In particular, the higher proportion of linalool and its oxides in hard withered teas, and the lower levels of hexenal, may be one of the reasons why such teas are more fragrant. Indeed, it has been established that linalool and its oxides, along with methyl salicylate, play an important role in the flowery flavour of black tea, while hexanal constitutes to the grassy flavour (Takeo, 1984).

The volatile flavour components are lower in non-fermented tea than fermented tea. Linalool oxides are found in the essential oil extracted from fermented leaves, but not in the homogenates of fresh leaves. Rapid oxidation of polyphenols appear to hamper volatile flavour compound formation in tea leaves (Hazarika and Mahanta, 1983). Lowering of fermentation temperature may therefore result in greater production. Thus, both duration and temperature of fermentation should be controlled to ensure optimum production of flavour compounds. The enzymic oxidations in fermentation proceed maximally at 28 °C, although a temperature range of between 24 to 28 °C is thought to be optimal.

Figure 4: Scheme for oxidative degradation of carotenes during tea fermentation showing the postulated black tea aroma constituents derived by this reaction (Sanderson, 1975)

Upon firing or drying, a significant amount of the volatiles are lost and there will be a proportional change in the level of the various compounds, the assumption being that the more volatile components are lost preferentially. Vorontsov (1939), found that during firing of the fermented leaf, which is performed at 95 - 100 °C, about 75 - 80% of the essential oil is lost. The initial temperature of firing is also important, and must be high enough to inhibit enzyme activity, otherwise this may lead to deterioration.

Seasonal variation in tea aroma and quality may well be due to variation in atmospheric temperatures. The majority of work in this area has been undertaken by Wickremasinghe (Wickremasinghe, 1974, 1978). This authors work has noted that there are two seasons in Sri Lanka when the best quality teas are produced. These

are January/February in Dimbula, and July/August in Uva districts. These seasons are characterised by cool temperatures (20 °C), dry cloudless days, and cool nights (6 - 10 °C). Under these conditions, plucking intervals are roughly two weeks, and lead to the production of a high quality flavoury tea. Lower temperatures may assist by leading to intermediate rates of fermentation, thus facilitating reaction sequences leading to volatile compound formation (Hazarika *et al.*, 1984). However, it appears from Wickremasinghe's work that the important factor is the rate of carbon dioxide fixation, which in turn affects the rate of intra and extracellular chloroplast reactions. The same phenomenon has been observed in the Darjeeling mountains in India (Howard, 1978).

The aroma of CTC black tea is said to be inferior to that of orthodox black tea, because of the high levels of undesirable carbonyl compounds such as trans-2-hexenal and the low concentration of desirable aroma compounds such as linalool and geraniol. In the CTC manufacturing process, oxidation occurs quickly, leading to an imbalance in the oxidative, hydrolytic and esterification reactions that ensue. The liberation of monoterpene alcohols is favoured by anaerobic conditions. Takeo (1984), stated that the less fragrant nature of CTC teas may be assigned to the lower amounts of essential volatile compounds, especially linalool and its oxides, together with methyl salicylate. Interestingly, higher levels of polyphenols, in particular theaflavins, in CTC as compared to orthodox tea, has been associated with inhibition of hydrolytic enzymes responsible for the production of linalool, its oxides, and methyl salicylate (Takeo and Mahanta, 1983a).

The caffeine content of the tea shoot is highest in the terminal bud and first leaf, where it may range from 4 to 5%. In the second leaf it may be 3%, and in the stalk, up to 1.5%. Caffeine levels appear to play no part in the chemical changes taking place during manufacture, and presumably volatile compound formation. However, caffeine does play a part in the formation of cream or precipitate, seen when a tea infusion cools, which is a mixture of caffeine, theaflavin and thearubigins (Agarwal, 1989).

One of the principal methods for separating volatile compounds from foods is that of steam distillation, frequently followed by extraction with an organic solvent. A simple method of performing both operations simultaneously, was published by Likens and Nickerson in 1964. Advantages of using their extraction head is that desired substances are concentrated thousands of fold in a single operation. Such chemical procedures may help solve a food quality problem that centers on the lack of an objective method for the verification of tea flavour quality.

Within Australia, tea consumption per capita is fairly high on the world scale. Current total consumption of tea has remained fairly steady over the past twenty years, at about 20,000 tonnes per annum. About 95% of this is imported. There are currently approximately twelve commercial tea growing operations in Australia; ten in north Queensland and two in northern New South Wales. The total commercial area being estimated at 750 hectares.

Madura Tea Estates have some twenty hectares of mature tea plantation near Condong in northern New South Wales. Already claiming nine percent of the Queensland market and three percent for the rest of Australia, the company has expanded rapidly in the past few years. The company is actively involved in product development (Ford, 1994), and has contributed significantly to this investigation.

A general economic principle in today's global economy is one of optimising quality and hence value of a product, albeit food or manufactured goods. A higher quality product may demand a higher market price, although this will obviously depend on the overall quality of goods on the market. A fundamental problem for any food industry, particularly one which deals with minimal processing of a natural commodity, is the relationship of perceived quality to the presence of natural flavour compounds in the product. As far as tea is concerned, the composition and concentration of aroma compounds has been shown to play a vital role in its valuation (Yamanishi *et al.*, 1966, 1968a,b; Wickremasinghe *et al.*, 1973; Owuor *et al.*, 1988).

Amongst the aroma compounds, terpenes are of particular significance. Such compounds are widespread in plants as constituents of essential oils. Many terpenes are hydrocarbons, but oxygen containing compounds such as alcohols, aldehydes and ketones (terpenoids) are also found. Their building block is the hydrocarbon isoprene, $\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}=\text{CH}_2$.

Terpene hydrocarbons therefore have molecular formulas $(\text{C}_5\text{H}_8)_n$, they are classified according to the number of isoprene units:

monoterpenes 2 isoprene units eg pinene, nerol, citral and limonene

sesquiterpenes 3 isoprene units eg nerolidol, farnesol
diterpenes 4 isoprene units eg phytol
triterpenes 6 isoprene unit eg squalene
tetraterpenes 8 isoprene unit eg carotene

The concentration and ratio of terpenes to both other terpenes and non-terpenes, is of particular significance to tea quality. Indeed, several quality indices have been based upon such an assumption. In this investigation, the concentrations of two terpenes, linalool and geraniol and their derivatives are of particular interest.

1.1 Tea Processing

An understanding of the operations involved in tea processing is necessary for an appreciation of the significance of each stage with regard to the quality of the final tea.

The basic operations involved in processing the tea are:

- (i) Withering
- (ii) Cutting & rolling
- (iii) Fermentation
- (iv) Drying
- (v) Sorting

(i) Withering: The withering stage has two purposes. It allows certain chemical changes to occur, and a reduction in the moisture content from 75-80% by weight, down to 55-70%, depending on the subsequent process to be employed. Throughout the process, the temperature of the leaf should be maintained at around ambient levels, otherwise faster moisture removal would be offset by adverse chemical changes. Withering time is usually in the range of 12-16 hours. During withering, a certain amount of the solid matter, as well as moisture, is lost from the leaf. This is due to the loss of carbon dioxide by respiration, and can account for 3-4% of the dry weight content of the incoming leaf. The relationship between the process of withering and the aroma characteristics of black tea were examined by Mahanta and Baruah, (1989); Owour *et al.*, (1987c, 1989); and Takeo, (1984). These studies confirm the importance of the role of withering in the tea manufacturing process.

(ii) Cutting & rolling: The basic requirements of this stage are size reduction together with a degree of cell damage which permits the exposure of new surfaces to air, in the subsequent fermentation stage. The type of machinery used at the leaf disruption stage is often used to denote the processing line as a whole. The principal machines employed in the industry are:

a. the traditional rolling machine (the orthodox process).

b. the CTC (crush, tear, curl) machine which uses contra-rotating rollers.

The CTC machine became especially popular with the development of the tea bag market, as it produces smaller leaf particles compared with older processes.

Each of the cutting or rolling methods operate at different optimum moisture levels which in turn affects the design of the withering system, which in turn affects the design of the drying system.

The act of rolling or cutting generates mechanical heat, and the effect of leaf disruption on leaf temperature is different for the various cutting machines. Because the leaf temperature has a vital effect on the fermentation step, the leaf temperature rise on cutting must be allowed for in the design of the system.

It is known that the aroma characteristics of orthodox and CTC teas are different and that the flavour of CTC is generally inferior to that of orthodox tea. A comparison of black tea aromas of orthodox and CTC tea and of black teas made from different varieties was made by Takeo and Mahanta (1983a, b); and the effects of maceration method on the chemical composition and quality of black tea was examined by Owour and Othieno (1989).

In plain teas where colour, strength, briskness and pungency are of major importance the industry in the main is changing to CTC (Willson and Clifford 1992). This is illustrated in the dilemma facing the Sri Lankan tea industry, as detailed by Ilangakoon (1994).

One kilogram of orthodox tea will only produce 250 cups of tea, whereas one kilogram of CTC of a similar grade may produce some 400 cups. The brewing time of CTC tea is also much reduced.

(iii) Fermentation: Following the leaf disruption stage, the leaf is allowed to contact the surrounding air for a period which may be between 40 minutes and 3 hours, depending on the process used and the temperature. During this period, the temperature of the leaf mass tends to rise due to the heat given out by the biochemical reactions which occur. Steps must be taken to control the temperature rise, otherwise the quality of the product may suffer due to unwanted reactions proceeding too quickly.

(iv) Drying: Tea is dried by exposing the moist fermented leaf particles to a stream of heated air. Some of the flavour characteristics of the final product are developed as the leaf heats up during the drying process. Eventually the leaf temperature and moisture levels bring about the enzyme destruction or inactivation necessary for preservation of the product. Tea normally enters the drier with 60-72% moisture, depending on the cutting process used, and is dried down to 2.5-3.5% moisture.

(v) Sorting: Dried tea leaving the drier consists of a mixture of different particle sizes and includes a percentage of stalk and fibre. The latter can depend on the original plucking standard in use and the climatic conditions during growth. The sorting equipment therefore consists of stalk and fibre removal apparatus, combined with machinery for separating the different sized tea particles into even sized portions. This is usually achieved by passing the teas over a series of screens, and as the leaf moves along the various conveyers in the sorting room, it passes close between or beneath electrostatically charged rollers, which attract the fibre and stalk preferentially.

With regard to the sorting process, there is no standard which stipulates the particle size range for each grade of tea, therefore, the traditional grade names are no more than an indication of leaf size (Table 1).

Table 1: Traditional tea grades
(Adapted from Willson & Clifford, 1992)

<i>Grades</i>	<i>Full name</i>	<i>Abbreviation</i>	<i>Source</i>
Whole leaf grades	Golden Flowery Orange Pekoe	GFOP	Orthodox manufacture only
	Flowery Orange Pekoe	FOP	
	Orange Pekoe	OP	
Brokens	Flowery Broken Orange Pekoe	FBOP	Products of both orthodox and CTC manufacture
	Broken Orange Pekoe	BOP	
	Broken Pekoe	BP	
Fannings	Broken Orange Pekoe Fannings	BOPF	Major products from CTC manufacture
	Orange Fannings	OF	
	Pekoe Fannings	PF	
Dusts	Pekoe Dust	PD	Predominantly CTC
	Residual Dust	RD	

2.0 OBJECTIVE

To characterise the extractable organic substances in black tea and to develop a reproducible chemical procedure for the objective assessment of flavour quality, by identifying a relationship between organoleptic appraisal and the ratio of certain extractable organic substances.

3.0 METHODOLOGY

This study has attempted to determine a relationship between an objective semi-quantitative measure of the aroma compounds and a subjective organoleptic evaluation. The work has involved the use of advanced analytical instrumentation in the Analytical Chromatography Laboratory of the Department of Food Science and Technology.

Characterisation of organic extractives is significant to the development of a quality index. It is anticipated that this methodology may be used by the Australian tea industry for the objective assessment of the flavour quality of black tea, either with or without the present entirely subjective organoleptic method.

Black tea samples were donated by the Madura tea company and represented tea(s) of different origins, harvested at intervals of the growing season. Madura receive some 12-15 deliveries of black tea over a one year period. This tea is comprised of produce from differing countries of origin and many plantations, with varying agronomic/climatic conditions. Only limited information is available on the tea purchased (grade, country of origin), however, it will have been appraised by tasters in the country of origin, and deemed to be of a certain quality.

The 12-15 deliveries of tea are used to produce some 20-40 batches of blended tea. This blended tea will contain teas of different sources, depending upon the appraisal by staff at Madura. However, its composition is predominantly orthodox manufactured teas. Samples consisted of 250 g packages of the blended tea, as supplied to retail outlets.

The extraction of volatile compounds was achieved by simultaneous steam distillation (SDE) and solvent extraction (hexane), using a Likens and Nickerson apparatus, modified as described by Schultz *et al.*, ((1977) Figure 5).

3.1 Extraction Procedure

50 grams of black tea, and one litre of distilled water were added to a two litre round bottomed flask connected to the right hand side of the apparatus. 1 mL of hexane containing 500 ug of methyl undecanoate internal standard was also added prior to the commencement of heating. 50 mL of A.R. grade hexane was contained in a 100 mL round bottomed flask connected to the left hand side of the apparatus.

The hexane was heated so as to ensure that it refluxed prior to the water, thus minimising the condensation of water into the hexane. Extraction was deemed as commencing when both hexane and water were condensing into the U bend of the apparatus. Extraction was allowed to continue for two hours, and was deemed to cease when the power to the electrothermals was switched off.

Following extraction and cooling of the apparatus, hexane contained in the U bend was returned to the 100 mL round bottomed flask by tilting the apparatus, care being taken to ensure that no water entered along with the hexane.

The volume of hexane contained in the 100 mL round bottomed flask was then reduced to approximately 5 mL by rotary evaporation at 30 °C. The concentrated extract was made to volume in a 5 mL volumetric flask, using either washings from the flask to increase volume, or exposure to a stream of nitrogen gas to reduce volume.

Approximately 1.5 mL of extract was kept in a sample vial for direct injection into the Perkin Elmer Gas Chromatograph. A Gas Chromatograph-Mass Spectrometry (GCMS) sample was prepared by blowing down the remainder of the sample to approximately 200 uL with nitrogen gas. This further concentration was necessary due to the lower sensitivity of the Mass Spectrometry detector. Duplicate extractions were produced for all tea samples.

3.2 Component Identification

The identification and quantification by gas chromatography of the organic extractives was a major component of the research. The analysis utilised a Hewlett Packard 5890 series II gas chromatograph, coupled to a Hewlett Packard 5970 quadrupole mass selective detector; and a Perkin Elmer Autosystem gas chromatograph, fitted with a standard flame ionisation detector.

The column in both machines was a BPX50 (50 metre length), manufactured by Scientific Glass Engineering.

Injected samples were subjected to a temperature program as detailed in Table 2.

Table 2: Temperature program for gas chromatography work

Temperature	Hold
50 °C	1 minute
Ramp 1: 3 °C / min to 250 °C	3 minutes
Ramp 2: 20 °C / min to 290 °C	43 minutes

Compound identification initially involved computer matching of the mass spectra of the volatiles against a mass spectral library. This analysis was followed by the use of retention indices, in comparison with authentic compounds (where such reference compounds were available). Kovat indices were also used.

3.3 Madura Tea Estates: Premium Blend Tea

3.3.1 Imported Tea Components

Deliveries of imported tea used in the production of the *Premium Blend Tea* were received at roughly monthly intervals. Subsequent to arrival at Condong, the tea may have been stored at a warehouse in Sydney, and

transported to Condong as production required. Warehouse storage at both Sydney and Condong were at ambient temperatures and humidities, away from direct sunlight.

The 'grade' of leaf used in the production of the premium blend was ostensibly Broken Orange Pekoe, with a bulk density of approximately 400 g/L. However, the bulk density was subject to some variation (see notes on tea grades in section 1.1).

The origin and grade of the components of *Premium Blend Tea* are as tabulated in Table 3. Each tea was produced by one of two manufacturing method, either crush, tear, curl (CTC), or by the orthodox method (see notes in section 1.1).

Table 3: Components of *Premium Blend Tea*

Origin and grade	Manufacturing method	Comments by Madura Tea
Sri Lankan 531	CTC	Consistent colour and strength
532	Orthodox	Variable quality
533	Orthodox	Variable quality
535	Orthodox	Variable quality(expensive)
Indian 1 (N. Indian)	CTC	Consistent quality
Indian 2 (S. Indian)	Orthodox	Consistent quality
Indonesian VR7	Orthodox	Consistent quality

The above deliveries were received for the most part in multiple paper-walled bags with a foil liner. Each bag contained approximately 50 kg of tea.

The sampling regime detailed in Table 4 was applied to the deliveries. Sampled bags were selected using a random number table.

Table 4: Sampling regime for imported teas

No. of bags in delivery	No. sampled
2 - 5	2
6 - 10	3
11 - 20	4
21 - 30	5
31 - 40	6
41 - 50	7
51 - 60	8

One kilogram of mixed samples was required. Thus, if five bags/chests were to be sampled, then 5 x 200 g was removed, pooled and mixed*.

Prior to mixing, the samples were sieved, to remove dust. This sieving mimicked the screening used on the individual teas prior to blending.

**The mixing method used combined the samples in a polyethylene bag which was shaken for 15 seconds. Approximately two hundred grams of the resulting sieved and mixed sample were stored in a 500 mL glass jar sealed with an air tight metal lid. A further two hundred grams of the sample were kept for organoleptic evaluation by the tasting panel. All stored tea samples were kept in sealed glass jars, stored in a cool dry location, away from bright light.*

The tasting panel consisted of three persons who evaluated each tea independently, using the scorecard shown in Appendix 3).

3.3.2 Blended Product

A combination of the imported teas detailed above were blended to produce *Premium Blend Tea*. The exact formulation of the blends were decided by Madura personnel after an evaluation of the individual teas and an initial blend produced.

The actual number of production runs produced monthly was somewhat variable depending upon demand for the product. During the project period (October to July 1997) some 9 production runs were sampled.

Each production run comprised up to twenty consecutive 350 kg batches, each batch containing the same composition of individual teas. The batches were sampled on one occasion, half-way through the packaging of the batch at the middle of the production run. Three consecutive 250 g packages were removed from the production line. Each set of three sample packages were pooled and mixed as indicated previously. Two 200 g sub-samples were taken. One sub-sample was used for chemical analysis and stored at -18 °C, and the second was used for immediate organoleptic evaluation. Both sub-samples were contained in airtight glass jars, sealed with metal lids.

3.3.3 Organoleptic Evaluation

2 grams of tea were subjected to a four minute infusion with 130 mL of boiling water.

The resulting sample was independently evaluated by three experienced tea tasters using a **Tea Scorecard** (Appendix 3). Personal opinions could be exchanged only subsequent to the completion of the scorecards.

Each imported tea was evaluated alongside the other teas that it was blended with. The final infusion was evaluated; this meant that the panellist would not be able to identify the tea from the appearance of the tea leaves.

Samples of manufactured blended tea were evaluated alongside a reference sample determined to be of good quality. This was the only sample identified as such, and acts as a frame of reference for evaluation. The remaining samples were assigned a number by a person who was not undertaking the sensory evaluation. The panellists were not told the identity of the individual teas until the conclusion of the evaluation.

Tea samples were subjected to the same extraction procedure and analytical methodology. Each 200 g sample received was sub-sampled and subjected to duplicate extraction and analysis.

4.0 DETAILED RESULTS

4.1 Determination of Optimum Extraction Time

In order to determine optimum extraction time, a sample of Sri Lankan orthodox black tea (532(10384)) was subjected to duplicate SDE extraction at 1, 2, 4 and 6 hour periods.

A selection of compounds was made in order to cover a range of volatility. This was achieved by reference to retention times using gas chromatography, and the anticipated importance of these compounds to tea flavour quality. The concentration of these compounds was determined against the internal standard methyl undecanoate, 500 ug of which was added to the tea infusion prior to extraction. The final hexane volume was 5 mL, 1 uL of which was injected into the GC, this would thus yield a peak equivalent to 100 ng, assuming a recovery of 100%.

The average concentrations of these compounds is listed in Table 5, and represented graphically in Figure 6 (see page 32).

Table 5: Average concentration of selected tea compounds (ng/uL extract)

Compound/Time	1 hour	2 hours	4 hours	6 hours
trans-2-hexenal	137.2	136.1	149.6	147.6
benzaldehyde	8.5	10.5	9.8	8.3
phenylacetaldehyde	67.7	83.3	112.6	120.9
linalool	67.9	81.2	83.9	76.6
methyl salicylate	38.4	45.5	44.6	38.7
geraniol	25	29.9	32.8	29.6
trans- α -ionone	2.3	3.8	2.9	3.5
β -ionone	7.9	8.5	9.8	9.3

4.2 Recovery of Internal Standard (Methyl Undecanoate)

In order to determine the recovery rate of the internal standard, a stock solution of 0.1 g of methyl undecanoate was prepared in 10 mL of A.R. grade hexane. From this stock, a 1:10 dilution was prepared. 1 mL of this dilution was made to 5 mL volume, and 1 uL was injected consecutively into the GC, and represented the unextracted standard (column A in Table 6).

1 mL of the same solution was subjected to 2 hour SDE extraction, the 50 mL of hexane used in the procedure being reduced to a final volume of 5mL by rotary evaporation. Consecutive injections of 1 uL of this extract represents the extracted standard (column B in Table 6).

A comparison of the mean area for each of the procedures resulted in a determined recovery for the internal standard of 96 %.

Area A: 500 ug Methyl Undecanoate in 5 mL Hexane
Consecutive injections of 1uL

Area B: B: 500 ug Methyl Undecanoate added prior to extraction (final solvent volume 5 mL)
Consecutive injections of 1 uL

Table 6: Determination of recovery of internal standard

Injection	A(area)	B(area)
#1	21259	21234
#2	21982	22190
#3	23326	21172
#4	20686	21788
#5	25970	21436
#6	23650	23657
Mean	22813	21913
sd	1927	935
cv	8.4	4.3

Percentage recovery = $21913 / 22813 \times 100 = 96 \%$

4.3 Recovery of Extracted Compounds

In order to determine the efficiency of the extraction procedure for some of the compounds of interest, a chemical cocktail was prepared using pure standards of compounds present in the black tea samples.

For the unextracted samples, 1 mL of the cocktail was diluted to 5 mL. Both the initial cocktail preparation and the dilutions used hexane as the solvent.

For the extracted samples, 1 mL of the cocktail was added to 1 L of distilled water. The mixture was subjected to SDE extraction for 2 hours using 50 mL hexane as the extracting solvent. The extracting solvent was concentrated to 5 mL volume using rotary evaporation.

Extracts from six consecutive extractions were analysed, and the concentration of individual compounds quantified against the internal standard methyl undecanoate.

Example calculation:

$$\text{Recovery of } \beta\text{-ionone} = \frac{\text{mean area of extracted samples}}{\text{mean area of unextracted samples}} = \frac{10626}{12936} \times 100 \% = 82.1 \%$$

The cocktail also permitted an opportunity to test the comparative efficiency of two Likens and Nickerson distillation heads that were used in the study. Both of these distillation heads were prepared by the glass-blowing workshop in the Chemistry Department of the University.

The cocktail mixture was extracted three times with each extraction head, numbers 1 - 3 representing one unit, and numbers 4-6 a second unit. An examination of the compound gas chromatogram areas (Table 7) reveals that the mean area for samples 4 - 6 are higher than those for samples 1 - 3. It was endeavoured to produce a comparable rate of distillation in the two units, but due to the fact that the electrothermals were of different design (although comparable in heat output) this may not have been possible. Consequently, individual tea samples were extracted in duplicate using the same distillation head at the same heat settings.

This investigation also indicated that the average extraction efficiency for the internal standard, using both extraction heads was 81.9 %, somewhat lower than indicated in the previous section. This is presumed to be due primarily to variation in extraction efficiency between the two extraction units, as indicated above. However, the overall coefficient of variation for the extraction of the internal standard was found to be 19 % which was deemed to be acceptable. The data for this investigation is detailed in Table 7.

Table 7: Recovery of extracted compounds from *cocktail* mixture

EXTRACTED	Retention	SDE HEAD #1			mean	SDE HEAD #2			mean	MEAN	sd	cv	ext
	Time	#1	#2	#3	area#1	#4	#5	#6	area#2	area1&2	area		%
<i>hexanal</i>	8.2	2901	4073	4020	3665	4108	4691	5584	4794	4229	883	21	52.3
<i>trans-2-hexenal</i>	10.2	3107	3906	4311	3775	4249	5056	5352	4886	4330	807	19	36.8
<i>benzaldehyde</i>	15.5	1585	1969	2289	1948	2112	2402	3099	2538	2243	507	23	53.5
<i>2-pentyl furan</i>	16.2	1171	2619	885	1558	1843	2991	3446	2760	2159	1026	48	45.4
<i>limonene</i>	18.2	4520	10526	3184	6077	6589	11702	13560	10617	8347	4185	50	50.8
<i>phenyl acetaldehyde</i>	19.7	5865	7935	9131	7644	7985	8849	11700	9511	8577	1910	22	65.6
<i>linalool</i>	21.9	9613	12489	13715	11939	12970	13349	17354	14558	13248	2488	19	78.8
<i>methyl salicylate</i>	27.4	5165	6857	8180	6734	7428	7504	11166	8699	7717	1975	26	69.5
<i>β-cyclocitral</i>	28.6	5491	7338	7627	6819	7432	7713	9990	8378	7598	1433	19	79.8
<i>geraniol</i>	29.7	12093	14874	16270	14412	15329	15563	20590	17161	15786	2758	17	81.8
<i>indole</i>	33.0	632	851	677	720	444	608	1212	755	737	267	36	32.4
<i>methyl undecanoate*</i>	37.5	11831	15850	17138	14940	16023	16371	21381	17925	16432	3055	19	81.9
<i>trans-α-ionone</i>	38.1	7501	9860	10551	9304	10208	10058	13181	11149	10227	1812	18	83.3
<i>β-ionone</i>	40.7	7763	10226	10984	9658	10635	10401	13747	11594	10626	1912	18	82.1
UNEXTRACTED	g/100mL	#1	#2	#3	mean	#4	#5	#6	mean	MEAN	sd	cv	
<i>hexanal</i>	0.0269	8116	9060	7161	8112	8752	7864	7566	8061	8087	717	9	
<i>trans-2-hexenal</i>	0.0219	11917	12716	10515	11716	12664	11525	11342	11844	11780	841	7	
<i>benzaldehyde</i>	0.0254	4164	4520	3547	4077	4515	3919	4509	4314	4196	401	10	
<i>2-pentyl furan</i>	0.0210	4773	5194	4085	4684	4721	4291	5453	4822	4753	519	11	
<i>limonene</i>	0.0248	15987	17672	13930	15863	17802	15509	17720	17010	16437	1574	10	
<i>phenyl acetaldehyde</i>	0.0242	12336	14558	10295	12396	14584	12040	14660	13761	13079	1807	14	
<i>linalool</i>	0.0252	16345	18112	14087	16181	18269	15821	18233	17441	16811	1700	10	
<i>methyl salicylate</i>	0.0273	10639	12477	9231	10782	11957	10078	12268	11434	11109	1323	12	
<i>β-cyclocitral</i>	0.0196	9229	10304	7933	9155	10426	8913	10312	9884	9519	1004	11	
<i>geraniol</i>	0.0349	18869	21210	15974	18684	21042	17670	21069	19927	19306	2178	11	
<i>indole</i>	0.0064	1397	2488	1499	1795	3568	1054	3646	2756	2275	1137	50	
<i>methyl undecanoate*</i>	0.0317	19328	22058	15735	19040	22730	18548	21957	21078	20060	2692	13	
<i>trans-α-ionone</i>	0.0192	12001	13370	9928	11766	13504	11446	13406	12785	12276	1432	12	
<i>β-ionone</i>	0.0190	12526	14172	10423	12374	14295	12036	14163	13498	12936	1560	12	

* *internal standard*

4.4 Sampled Material

Imported tea samples are detailed in Table 8. This table includes the mean organoleptic score for each batch of tea, the mean terpene index and the mean level of terpenes in ug/g of tea. Note that each batch of tea was subject to duplicate SDE extraction and analysis. The concentrations of some 35 compounds was determined with reference to the internal standard methyl undecanoate.

Appendix 1 (Tables 1 to 7) lists:

- a) concentrations of individual compounds,
- b) a consolidated terpene value (ng/uL extract) based on linalool, linalool oxides, geraniol and geranial,
- c) the concentration of these terpenes per gram of tea, and
- d) a terpene index calculated thus:

$$\frac{\text{concentration of linalool and linalool oxides}}{\text{concentration of linalool, linalool oxides, geraniol and geranial}}$$

The same values pertaining to the *Premium Blend Tea* produced from the individual imported teas are detailed in Appendix 1, Table 8.

Note that compounds in these tables are expressed as ng/uL of extract. To convert such values to ug/g of tea, simply divide by 10*.

**The final extract volume was 5 mL, and tea samples were 50 g; 1 uL of extract was injected. Thus (ng component x 5000 uL/50 g)/1000 = ug/g tea.*

4.5 Tabulated Results

A summary of the mean concentration of terpenes per gram of tea, the standard deviation, and the coefficient of variation for all batches of a particular grade of tea, is given in Table 10.

Similar data for the terpene index and organoleptic score are given in Tables 11 and 12. Table 13 summarises mean values from Tables 10 - 12. Table 14 details lower and upper concentrations of selected volatile compounds in all black tea grades.

Tables 15 - 17 detail rotated loadings, calculated from principal component analysis using the 35 compounds evaluated, and two subsets. Tables 19 - 21 detail determined concentrations for selected compounds within the Sri Lankan orthodox tea grades. The discussion section reviews the data in all these tables.

Table 8: Mean organoleptic score, terpene indices, and terpenes (ug/g), for individual batches of all grades of imported teas.

Grade	Batch	Mean Organoleptic Score	Mean Terpene Index	Mean Terpenes (ug/g tea)	
531 (CTC)	10391	36.7	0.820	10.5	
	10408	40.0	0.729	11.4	
	10399	44.0	0.848	11.4	
	10415	44.0	0.769	12.1	
	10432	41.7	0.761	9.6	
	10422	37.3	0.763	9.2	
			correlation	0.1483	0.6215
532 (orthodox)	10384	35.7	0.809	18.2	
	10400	36.7	0.835	17.0	
	10409	39.0	0.839	21.5	
	10416	35.3	0.850	17.5	
	10423	34.0	0.797	17.4	
	10414	38.3	0.859	19.7	
			correlation	0.6661	0.8285
533 (orthodox)	10385	43.3	0.833	23.0	
	10401	44.0	0.757	21.8	
	10417	30.3	0.804	26.8	
	10424	41.7	0.835	24.4	
	10436	31.3	0.825	29.6	
			correlation	-0.1808	-0.9124
535 (orthodox)	10366	34.7	0.809	21.4	
	10374	39.7	0.828	17.4	
	10386	39.6	0.853	15.6	
	10402	38.3	0.751	18.0	
	10394	42.3	0.794	17.7	
	10418	30.3	0.818	22.8	
			correlation	-0.0722	-0.2900
Indian 1 (CTC)	11896	38.7	0.877	6.6	
	221196	39.0	0.868	8.0	
	4397	40.7	0.854	7.2	
	13397	35.3	0.875	6.7	
	20597	31.7	0.841	7.0	
	3697	38.0	0.841	6.4	
			correlation	0.3062	0.2148
Indian 2 (orthodox)	221196	40.3	0.885	29.4	
	15297	35.7	0.865	19.6	
	4397	33.7	0.854	15.2	
	20597	27.3	0.868	19.4	
	3697	41.7	0.846	13.8	
	10797	31.3	0.854	15.7	
		correlation	0.0398	0.2252	

Table 8 continued

Grade	Batch	Mean Organoleptic Score	Mean Terpene Index	Mean Terpenes (ug/g tea)
VR7 (orthodox)	1326	35.3	0.935	17.6
	1378	35.3	0.927	23.7
	1333	41.3	0.932	22.5
	1883	36.0	0.919	23.9

	1711	37.0	0.914	20.8
	1905	35.3	0.919	23.2
	1746	38.7	0.930	23.0
	1889	39.3	0.919	22.7
	1761	34.7	0.914	20.9
	1727	37.8	0.911	21.9
	1771	36.7	0.924	20.6
	1993	37.7	0.919	21.7
		correlation	0.2296	0.2352

Table 9: Mean organoleptic score, terpene index and terpenes (ug/g) for individual production runs of *Premium Blend Tea*

'Use-by' Date	Mean Organoleptic Score	Mean Terpene Index	Mean Terpenes (ug/g tea)
6/7/98	34.7	0.794	15.8
1/8/98	35.3	0.810	17.4
Aug 98	33.7	0.822	15.0
1/10/98	37.0	0.816	16.0
2/10/98	34.3	0.810	17.3
4/10/98	30.3	0.827	17.9
6/10/98	40.0	0.835	17.0
7/10/98	38.0	0.839	16.2
Feb 99	32.0	0.850	18.9
	correlation	0.0333	-0.3981

Table 10: Summary of terpene index, standard deviation and coefficient of variation for all samples of each grade of tea

Grade	Mean Terpene Index	SD	CV
531(CTC)	0.781	0.043	5
532	0.831	0.024	3
533	0.811	0.031	4
535	0.809	0.033	4
Indian 1(CTC)	0.859	0.018	2.1
Indian 2	0.862(*0.857)	0.014	1.6
VR7	0.922	0.009	1
Premium Blend	0.822	0.019	2

* value after omitting batch 221196

Table 11: Summary of terpenes (ug/g tea), standard deviation, and coefficient of variation for all samples of each grade of tea

Grade	Mean Terpenes (ug/g tea)	SD	CV
531(CTC)	10.7	1.1	11
532	18.5	1.7	9
533	25.1	3	12
535	18.8	2.6	14
Indian 1(CTC)	7	0.7	10
Indian 2	18.8(*16.7)	5.4	29
VR7	21.8	1.9	9
Premium Blend	16.8	1.2	7

* value after omitting batch 221196

Table 12: Summary of organoleptic score, standard deviation and coefficient of variation for all samples of each grade of tea

Grade	Mean Organoleptic Score	SD	CV
531(CTC)	40.6	3.2	7.9
532	36.5	1.9	5.2
533	38.1	6.7	17.7
535	37.5	4.3	12.7
Indian 1(CTC)	37.2	3.2	8.7
Indian 2	35.0	5.4	15.5
VR7	37.1	2.0	5.3
Premium Blend	35	3.0	8.5

Table 13: Summary of organoleptic score, terpene index and terpenes (ug/g tea) for all samples of each grade of tea

Grade	Mean Organoleptic Score	Mean Terpene Index	Mean Terpenes (ug/g tea)
531(CTC)	40.6	0.781	10.7
532	36.5	0.831	18.5
533	38.1	0.811	25.1
535	37.5	0.809	18.8
Indian 1(CTC)	37.2	0.859	7
Indian 2	35	0.862	18.8
VR7	37.1	0.922	21.8
Premium Blend	35	0.822	17.4

Table 14: Minimum maximum and range, for organoleptic appraisal, terpene index and terpene concentration, for all grades of tea examined

Grade	Organoleptic			Terpene Index			Terpenes (ug/g tea)		
	Min	Max	Range	Min	Max	Range	Min	Max	Range
531(CTC)	36.7	44	7.3	0.729	0.848	0.119	9.2	12.1	2.9
532	34	39	5	0.797	0.859	0.062	17	21.5	4.5
533	30.3	44	13.7	0.757	0.835	0.078	21.8	29.6	7.8
535	30.3	42.3	12	0.751	0.853	0.102	15.6	22.8	7.2
Indian 1(CTC)	31.7	40.7	9	0.84	0.877	0.037	6.4	8	1.6
Indian 2	27.3	41.7	14.4	0.846	0.885/ *0.868	0.039 *0.022	13.8	29.4 *19.6	15.6 *5.8
VR7	34.7	41.3	6.6	0.911	0.935	0.024	17.6	23.9	6.3
Premium Blend	30.3	40	9.7	0.794	0.85	0.056	15	18.9	3.9

* value after omitting batch 221196

Table 15: Rotated loadings for two and three principal component analysis using all 35 volatile compounds examined

Compound	Two Components		Three Components		
	1	2	1	2	3
hexanal	0.635	0.369	0.610	0.321	0.268
trans-2-hexenal	0.855	0.041	0.820	-0.019	0.246
hexanol	0.842	0.079	0.885	0.028	-0.038
benzaldehyde	0.039	0.596	0.098	0.595	-0.057
β -pinene	0.652	0.545	0.710	0.504	0.010
β -myrcene	0.101	0.220	0.022	0.203	0.365
2-pentyl furan	0.690	0.418	0.630	0.363	0.419
hexanoic acid	-0.092	0.623	-0.089	0.623	0.145
cis-2,4-heptadienal	0.289	0.486	0.213	0.455	0.449
limonene	0.206	0.540	0.251	0.527	0.001
cineole	0.459	0.416	0.432	0.380	0.267
phenylacetaldehyde	-0.387	0.477	-0.283	0.509	-0.309
linalool oxide I	0.938	0.116	0.887	0.048	0.336
linalool oxide II	0.940	0.067	0.909	0.001	0.247
linalool	0.902	0.055	0.925	-0.002	0.039
hotrienol	0.558	0.080	0.351	0.020	0.862
benzyl cyanide	0.407	-0.036	0.514	-0.051	-0.357
linalool oxide III	0.790	0.026	0.742	-0.031	0.285
terpinen-4-ol	0.098	0.204	0.186	0.205	0.262
methyl salicylate	0.915	0.019	0.922	-0.040	0.091
safranal	0.493	0.611	0.584	0.583	-0.117
β -cyclocitral	0.102	0.756	0.186	0.751	-0.097
neral	0.149	-0.002	0.001	-0.028	0.567
geraniol	0.458	-0.015	0.236	-0.068	0.882
geranial	0.252	0.822	0.240	0.797	0.293
cis- α -ionone	-0.033	0.680	0.087	0.689	-0.272
indole	0.789	-0.080	0.765	-0.134	0.166
hexanoic acid, 3-hexenyl ester	0.524	0.064	0.686	0.047	-0.523
damascenone	-0.127	0.112	-0.014	0.131	-0.408
β -caryophyllene	0.314	0.041	0.133	0.000	0.728
trans- α -ionone	-0.565	0.638	-0.505	0.675	-0.123
β -ionone	-0.420	0.857	-0.377	0.881	0.019
3-buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo hept-1-yl)	-0.134	0.914	-0.078	0.920	0.018
α -farnesene	0.499	-0.447	0.551	-0.470	-0.253
nerolidol	0.631	0.166	0.696	0.130	-0.119

Table 16: Rotated loadings for two and three principal component analysis using 11 volatile compounds

Compound	Two Components		Three Components		
	1	2	1	2	3
benzaldehyde	0.280	-0.422	0.041	-0.005	0.807
phenylacetaldehyde	-0.072	-0.694	-0.239	-0.388	0.674
linalool oxide I	0.885	0.418	0.860	0.465	0.044
linalool oxide II	0.893	0.385	0.899	0.385	-0.021
linalool	0.891	0.176	0.900	0.186	0.055
hotrienol	0.378	0.775	0.262	0.923	0.048
linalool oxide III	0.761	0.389	0.746	0.417	0.009
methyl salicylate	0.880	0.194	0.933	0.132	-0.064
geraniol	0.227	0.822	0.121	0.943	-0.022
geranial	0.466	-0.067	0.210	0.359	0.739
nerolidol	0.732	-0.207	0.780	-0.241	0.080

Table 17: Rotated loadings for two and three principal component analysis using 6 volatile compounds

Compounds	Two Components		Three Components		
	1	2	1	2	3
linalool oxide I	0.934	0.326	0.930	0.197	0.274
linalool oxide II	0.960	0.230	0.959	0.133	0.199
linalool	0.907	0.145	0.930	0.157	0.016
linalool oxide III	0.872	0.177	0.849	0.01	0.284
geraniol	0.331	0.668	0.235	0.150	0.953
geranial	0.077	0.882	0.151	0.976	0.138

Table 18: Lower and upper concentrations of selected volatile compounds in all black tea grades (ug/g tea)

Tea Grade	531		532		533		535		Indian 1		Indian 2		VR7		Blended	
	L	U	L	U	L	U	L	U	L	U	L	U	L	U	L	U
benzaldehyde	0.46	1.26	0.72	1.29	0.71	1.44	0.46	1.49	0.6	1.53U	0.78	1.26	0.51	1.68	0.85	1.5
phenylacetaldehyde	5.74	8.59	6.53	11.033	4.09	6.93	3.14	9.58	5.63	10.6	6.76	15.45	4.19	9.36	5.94	9.63
linalool oxide I	0.59	1.02	1.17	1.64	1.47	2.55	1.24	1.67	0.44	0.67	1.09	2.25	1.29	1.9	1.12	1.52
linalool oxide II	1.61	2.67	3.11	0.41	3.57	7.69	3.31	4.64	1.17	1.59	2.9	6.65	3.96	5.55	3.08	4.17
linalool	4.33	5.88	7.65	11.11	10.17	12.35	6.27	11.93	2.99	3.85	6.9	15.62	10.3	14.25	7.01	9.35
hotrienol	0.47	0.72	0.68	0.94	0.91	1.41	0.74	1.18	0.36	0.5	0.54	0.99	0.42	0.63	0.58	0.79
linalool oxide III	0.29	0.76	0.77	1.35	1.1	1.49	0.9	1.85	0.34	0.9	0.63	2.14	0.8	1.44	0.71	1.03
methyl salicylate	2.14	3.46	4.17	5.95	3.26	11.89	2.53	4.59	1.5	2.3	2.96	5.81	5.07	7.53	3.19	5.42
geraniol	1.34	2.81	1.74	3.33	3.33	4.54	1.77	3.87	0.48	0.69	1.24	2.71	0.98	1.41	2.08	2.91
geranial	0.14	0.47	0.24	0.95	0.31	0.94	0.05	0.69	0.17	0.67	0.53	0.96	0.13	0.82	0.43	0.68
nerolidol	0.3	1.16	0.55	1.84	0.36	2.33	0.27	1.54	0.31	1.33	0.3	1.52	1.08	1.89	0.37	1.56

Table 19: Concentrations of selected volatile compounds in each batch of Sri Lankan 532 orthodox black tea (ug/g tea)

Compound/Batch	10384	10400	10409	10416	10423	10414	Mean
benzaldehyde	0.90	0.96	1.22	0.75	0.85	1.02	0.95
phenylacetaldehyde	9.31	9.65	10.55	7.23	7.71	8.31	8.79
linalool oxide I	1.43	1.36	1.61	1.27	1.19	1.51	1.39
linalool oxide II	3.62	3.59	4.09	3.36	3.18	3.76	3.60
linalool	8.47	8.14	11.06	9.34	8.58	10.74	9.39
hotrienol	0.94	0.78	0.80	0.73	0.70	0.76	0.78
linalool oxide III	1.20	1.10	1.27	0.83	0.88	0.88	1.02
methyl salicylate	4.48	4.32	5.91	5.21	4.99	5.79	5.11
geraniol	3.24	2.21	2.57	1.81	2.75	1.91	2.41
geranial	0.25	0.59	0.90	0.81	0.77	0.86	0.70
nerolidol	1.19	0.90	1.78	1.37	1.30	1.47	1.33

Table 20: Concentrations of selected volatile compounds in each batch of Sri Lankan 533 orthodox black tea (ug/g tea)

Compound/Batch	10385	10401	10417	10424	103436	Mean
benzaldehyde	0.72	1.35	1.39	1.05	0.85	1.07
phenylacetaldehyde	6.88	6.80	5.33	4.65	4.11	5.55
linalool oxide I	1.82	1.48	2.10	1.66	2.58	1.93
linalool oxide II	5.16	3.58	5.92	5.19	7.69	5.51
linalool	10.67	10.18	12.09	12.34	12.19	11.49
hotrienol	1.06	1.01	1.14	0.92	1.39	1.10
linalool oxide III	1.48	1.26	1.43	1.18	1.97	1.46
methyl salicylate	5.40	3.27	4.84	4.80	11.74	6.01
geraniol	3.51	4.49	4.41	3.34	4.26	4.00
geranial	0.33	0.81	0.85	0.70	0.93	0.72
nerolidol	1.22	0.37	1.43	1.36	2.30	1.33

Table 21: Concentrations of selected volatile compounds in each batch of Sri Lankan 535 orthodox black tea (ug/g tea)

Compound/Batch	10366	10374	10386	10402	10394	10418	Mean
benzaldehyde	0.54	0.64	1.00	1.46	1.93	1.16	0.94
phenylacetaldehyde	4.67	3.44	9.01	5.12	5.33	5.22	5.89
linalool oxide I	1.66	1.51	1.42	1.27	2.22	1.63	1.5
linalool oxide II	4.45	4.32	3.69	3.27	3.52	4.27	4.03
linalool	9.89	7.13	7.08	8.05	4.64	11.8	8.40
hotrienol	0.98	0.77	0.86	0.97	2.05	0.88	0.93
linalool oxide III	1.33	1.46	1.10	0.97	2.42	0.91	1.27
methyl salicylate	4.36	4.45	4.41	2.56	3.24	4.14	3.91
geraniol	3.81	2.81	1.79	3.83	3.00	3.57	3.14
geranial	0.28	0.19	0.50	0.67	1.81	0.57	0.47
nerolidol	1.48	1.26	0.72	0.59	2.14	1.27	1.03

5.0 DISCUSSION OF RESULTS

An examination of the effect of SDE extraction time upon recovery of selected compounds (Figure 6), indicates that artefacts may be introduced by extended distillation time. For those components evaluated, phenylacetaldehyde in particular, showed a considerable increase in concentration with time. This is presumably due to its formation from the amino acid phenylalanine. For the majority of compounds, extracted levels plateau then decrease, either due to small losses from the apparatus, or thermal degradation/oxidation. A two hour extraction time was chosen to minimise the possibility of such artefact formation, and yet still permit a high rate of recovery.

In order to determine the actual recovery rate of specific compounds, a 'cocktail' was prepared. Ideally, the concentration of compounds in the cocktail should have reflected the levels typically found within the samples. Compounds were weighed directly into a volumetric flask and due to the small quantities involved, this was not always possible. However, all compounds were within one order of magnitude of the typical concentrations determined by sample extraction.

Table 7 lists the calculated recovery rates for a number of compounds. These compounds were selected to represent the range of component volatility encountered, thus, hexanal and β -ionone were examples of the earlier and later eluting compounds on the GC chromatogram. Recovery rates varied from 32.8 % for indole to 83.3 % for trans- α -ionone.

One point that should be noted here, is that the cocktail compounds were directly dissolved in hexane, whilst in a tea sample these compounds would have to be stripped from the tea leaves initially. The affinity of compounds for other components within the tea leaves is unknown.

Tables 1 – 8 in Appendix 1, list the concentrations of selected components within the hexane SDE extract for each batch of a particular tea grade. Note that each batch sample was extracted in duplicate. Components were selected on the basis that they were either present at a reasonably high concentration and/or identifiable by use of the GC-MS library and reference compounds.

Retention times and Kovat indices, as well as mass spectra, were used to identify each component. Retention times and Kovat indices for each compound for both gas chromatographs (Perkin Elmer and Hewlett Packard) are given in Table 7, Appendix 1.

Figure 7 is a representative chromatogram obtained for Indonesian VR7 tea using a Perkin Elmer Autosystem Gas Chromatograph.

Although cis and trans forms of some components were identified, for example, 2,4-heptadienal and nerolidol, there were considerable differences in the concentration of such isomers. As a result of this, only the isomer present in the greater concentration was quantified. The first two linalool oxides are presumed to be the cis and trans furanoid forms respectively. Only one of the pyranoid forms of linalool oxide could be identified, whether this was the cis or trans form is unknown.

The peak immediately after linalool was identified as hotrienol (3,7-dimethyl-1,5,7-octa-trein-3-ol) using mass spectra from literature sources (Eberhardt and Pfannhauser, 1985).

Interestingly, it would appear that both the cis and trans forms of α -ionone are present in all the black tea samples. Although the chemical standard failed to reveal the presence of the two

isomers, two peaks identified as α -ionone were detected by GC-MS, at 33 and 40 minutes, for all black tea samples. Investigation of the literature (Enzell and Wahlberg, 1986) indicated that cyclization and extrusion of a methyl group prior to a retro-Diels Alder reaction, leads to the generation of abundant ions of m/z 177, 149 and 69. This was evident in the examination of the mass spectra, leading to the conclusion that the cis isomer has the lower retention time.

All of the compounds identified have been previously cited in literature as being present in black tea (Appendix 4).

Due to the fact that the extractable levels of the majority of compounds identified was highly variable, caution should be taken when interpreting the results. The perception limits of these compounds may differ widely, and some compounds may therefore have a greater impact than their concentration would otherwise suggest.

The mean, standard deviation, and coefficient of variation (cv) for each component within all of the batches for a particular grade of tea are listed. It will be noted that there is considerable variation in the cv for the majority of these compounds, even allowing for some 10% variation for both extraction and injection. It would seem that the majority of this is due to variation in both agronomic and processing conditions. However, part of the problem, at least for the more volatile early eluting components, would seem to be due to the rotary evaporation procedure used to concentrate the initial hexane extracts. Some compounds, in particular hexanal and trans-2-hexenal were preferentially lost during this procedure. Despite lowering the waterbath temperature from 40 °C to 30 °C significant differences between duplicate extracts were still apparent. Due to this fact, the ratio of group I to group II compounds was not evaluated as a means of determining tea quality.

The content of both linalools and geraniols have been shown to vary widely within and between different clonal teas when they are subjected to different manufacturing and agronomic treatments, and when clonal teas are obtained from different geographical regions (Owuor, 1989). However, every variety/clone has a specific terpene index, which varies only with plucking standard. This index has been shown to be a reliable taxonomic statistic for differentiating clonal teas (Owuor *et al.*, 1987b). The terpene index of pure variety *sinensis* is near zero and that of pure variety *assamica* is almost at 1 (Figure 8).

$$\text{Terpene Index} = \frac{\text{concentration of linalool and linalool oxides}}{\text{concentration of linalool, linalool oxides, geraniol and geranial}}$$

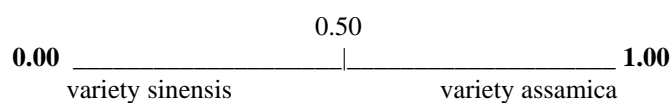


Figure 8: Terpene Index (modified from Yamanishi, 1992)

A terpene index was calculated for each of the tea samples evaluated. This index was calculated as the sum of the concentrations due to linalools to the sum of the concentrations due to linalools and geraniols. Note that

only three of the four oxides of linalool were identified on the chromatogram, a peak attributable to the fourth oxide of linalool could not be located. Significant peaks due to geraniol and geranial were identified, although no geranic acid could be located. A neral peak was identified, but due to the concentration of this compound being less than 1 ng/uL extract it was omitted from the index.

Table 8 lists each imported tea samples that were used in this study. Individual batch numbers are listed under the heading for the respective tea grade. The organoleptic evaluations for each tea grade are detailed, together with the mean terpene indices and mean terpene concentration (ug/g tea) for the duplicate extractions of each sampled batch. The terpene concentration was calculated using the same compounds used in the determination of the terpene index itself ie. linalool, linalool oxides, geraniol and geranial. Table 9 lists the same data for the *Premium Blend Tea* which was produced from the individual imported teas. Here, a 'use-by' date was used to identify the individual batches.

Despite the observation that most compounds evaluated show a cv well in excess of 20%, there were a number of compounds that showed variation close to 20%. These compounds included the linalool, geraniol and their derivatives.

Terpene indices for all grades of tea are listed in Table 10. The Sri Lankan CTC (531) tea had the lowest mean terpene index of all the grades. Interestingly, it is substantially lower than the Sri Lankan orthodox teas, indicating that the processing method has affected the terpene index. However, when one examines the Indian teas, there is very little difference between the terpene index for the CTC and orthodox grades. The Indonesian VR7 tea exhibited the highest terpene index, together with the lowest coefficient of variation, suggesting that the Indonesian tea is genetically close to variety *assamica* and subject to the most controlled harvesting and processing.

Sri Lankan 533 showed the highest mean concentration of terpenes for the orthodox teas, followed by the Indonesian VR7. As anticipated, the CTC teas have the lowest terpene levels, the Indian material containing less than the Sri Lankan. For the organoleptic appraisal, the greatest score cv occurred in the Sri Lankan 533, 535 and Indian 2 tea grades. These same teas also had the highest cv for terpene concentration, although not in the same sequence.

In order to establish if a relationship existed between the organoleptic score and either the terpene index, or the concentration of terpenes for samples within each grade of tea, the correlation coefficient was calculated. The respective values are detailed in Tables 8 and 9.

Note that the mean value for terpene index and terpene concentration was the average value for each pair of duplicate samples examined. The organoleptic score was the average value assigned by the three appraisers.

The determined correlations varied substantially, and no clear cut relationship could be established between these attributes. Both positive and negative correlations were evident.

In order to effectively increase the data used, the mean concentration of terpenes for each grade of tea (Table10), and the mean terpene index (Table 11), were plotted against the mean organoleptic score for each grade of tea ((Table 12) Figures 9 and 10). When the graph of

mean terpene concentration versus mean organoleptic score (Figure 10) was examined, it was apparent that the points representing the two CTC teas were somewhat removed from the other data points. Consequently, the graph was replotted, omitting these two points (Figure 11), and an improved correlation of 0.7381 was obtained. Thus, it may be the case that a relationship exists between the concentration of terpenes and organoleptic score, although it appears less likely that such a relationship exists between terpene index and organoleptic score.

The next step used in the analysis of the data was to plot the concentration of terpenes (ug/g tea) used in the calculation of the terpene index, against the terpene index itself. Thus, Figure 12 shows that for Sri Lankan 531 and Indian CTC teas, the points representing each sample analysed are distinctly clustered for each tea. Thus both teas may be characterised as occupying a distinct area of the graph. Similarly, for Figure 13, Indonesian and Indian orthodox teas are distinct. The one Indian tea batch occupying a position at the top of the graph would possibly be a first flush tea sample. Figure 14 shows the three types of Sri Lankan orthodox teas. Clustering of these tea types is not as distinct, although 533 has the highest average concentrations of terpenes, and 532 has a slightly higher average terpene index.

Figure 15 shows data for all the orthodox teas examined. It is quite apparent from this and the other graphs that such plots may be used to discriminate between teas produced with different leaf disruption steps and from differing countries of origin. However, where several tea grades are produced using the same methodology in the same country of origin, as in the case of Sri Lankan orthodox teas (Figure 14), it may be impossible to distinguish between them.

Figure 16 shows a plot of terpene index versus concentration of terpenes for the blended tea produced from the individual teas. Clustering is once again apparent, and indicates that blended tea production achieved a fairly consistent product quality as far as both terpene levels and terpene index were concerned.

Note that in Figures 12 to 16, crush, tear, curl (CTC) teas were plotted separately from orthodox manufactured teas. This was because the orthodox teas typically contained much higher levels of terpenes compared to CTC teas; a consequence of the manufacturing process. That this is in fact the case is readily confirmed by

examination of the levels of components for CTC and orthodox grades of tea listed in Appendix 1. Figures 17 and 18 are plots of the mean levels of a selection of compounds found in Sri Lankan and Indian black teas. Both Sri Lankan and Indian black teas show significant differences between the two leaf disruption methods, such variation being independent of the country of origin.

It seems logical to establish the upper and lower limits for both terpenes and terpene index for each grade of tea and the idea of an acceptable range is a logical extension. Table 14 lists the minimum, maximum and range, for both terpene index and terpene concentration in all grades of tea examined. The data indicates that the lowest levels, and the smallest range for actual concentration of terpenes, occurs in the CTC teas, as expected.

It is worth commenting on the actual grading of the teas concerned, particularly the Sri Lankan orthodox teas. Sri Lankan 532 and 535 would appear to be fairly similar, although Table 14 indicates that the 535 is slightly more variable in terms of both terpene index and actual concentration of terpenes. 533 would appear to be superior to both of these grades. Interestingly, 535 is the most expensive of these teas. These observations suggest that

535 could be replaced by 532 to produce a comparable tea, or 533 to produce a superior tea; with lower production costs.

Of all the imported teas, the Indonesian VR7 is the most consistent in terms of terpene index with a coefficient of variation of 1% only. This would imply that control with regard to plucking standard and processing is the most rigorous, unlike the Sri Lankan CTC tea which exhibits a variation of between 3 and 5%.

The Indian orthodox tea has a large range for the concentration of terpenes, this is partly due to the inclusion of batch 221196. I would anticipate that this batch was produced from the first flush of tea growth. The first flush is deemed to be of higher quality and noted for its high volatile content, although its presence would tend to indicate poor blending. Removing this one batch from the data results in a more typical terpene concentration compared to the other orthodox teas.

The data in Appendix 1 was also subjected to principal component analysis (PCA) using a multivariate statistical program (systat). The purpose of this was to attempt to pick out patterns of chemical composition from the data.

The aim of principal component analysis is reduction in the X matrix. Using this method, a new X matrix with fewer variables than the original X matrix is produced. However, the new matrix should contain as much variance as possible from the old matrix. Principal component analysis can only be performed if a correlation among variables in the original matrix exists. The first extracted variable (principal component) in the new matrix represents the largest amount of explained variance. The percentage of explained variance decreases from the first to the second to the third principal component.

Multivariate methods, such as PCA, extract information from the given data and produce a model that attempts to describe reality. However, a combination of experimental and model errors may be introduced that cannot be distinguished. Perhaps another disadvantage is deciding the optimum number of extracted compounds, both in terms of the raw data and when using multivariate analysis.

For the first of three ‘analyses’, all 35 compounds were utilised. The first subset contained eleven compounds which were selected on the following basis:

(a) they were present at an average level of greater than or equal to 4.9 ng/uL (0.49 ug/g tea) in the concentrated hexane extract (over all tea grades)

(b) they showed a coefficient of variation in excess of 25%, over all tea grades.

The second subset consisted of the six compounds which were used in the determination of the terpene index. These compounds were also included in the first subset.

Two and three dimensional graphs were produced for each set and subset as a result of using two and three extracted principal components in the data abstraction. For each set/subset of data, the percent of variation accounted for by the number of principal components is shown in Table 22. The rotated loadings for each compound within each set and subset and for two and three principal component analysis are shown in Tables 15, 16 and 17. These values indicate the relative contribution of each compound to the separation process. The higher the number (negative or positive) the greater the contribution.

Table 22: Percent variation accounted for by one, two and three principal components

No. of Principal Components	1	2	3	TOTAL
All compounds	29.995	19.127	-	49.122
All compounds	29.068	18.81	12.613	60.491
Subset 1 (11 compounds)	43.252	22.966	-	66.218
Subset 1	41.649	24.275	15.193	81.117
Subset 2 (6 compounds)	58.202	23.927	-	82.129
Subset 2	57.478	17.600	18.720	93.798

Figures 19 and 20 represent principal component scores for the complete set of 35 compounds. Examination of these graphs, especially the three dimensional graph (Figure 20) reveal some clustering of grades. Certainly, the Indonesian VR7 teas are distinct, and the Indian 1 and Sri Lankan CTC teas, although not distinct, occupy the same general area of the graph. The Sri Lankan orthodox teas and the Indian 2 occupy the same general area, but cannot be specifically tied to one location.

Figures 21 and 22 represent principal component scores for the first subset. In these graphs the separation of VR7 is more apparent, and the Sri Lankan CTC tea is more distinct from the Indian CTC tea. However, the remaining grades are as before, somewhat nebulous.

Figures 23 and 24 represent the final subset, those terpenes used in the terpene index discussed earlier. In the two dimensional graph, the separation of the CTC teas appears to have deteriorated, although the separation of the Sri Lankan orthodox teas seems to be improving.

The three dimensional graph (Figure 24) shows the best separation of all six graphs. The VR7, Indian, and Sri Lankan CTC teas all occupy distinct locations. The Indian orthodox appears to occupy a plane towards the centre of the plot. Of the Sri Lankan orthodox teas, the 532 has the most compact cluster, the 533 next, and the 535 the least structure.

It would appear that principal component analysis may be used to distinguish country of origin and method of processing, but, in this instance, and for the compounds that have been selected, it fails to discriminate between teas with the same country of origin and method of processing.

A simpler technique to differentiate tea grades and give some indication of quality would be of more direct use. Another possibility is to identify a chemical ‘*fingerprint*’ for each individual grade.

Using the same compounds as in PCA subset 1, a series of graphs were plotted using the upper and lower values for the 11 compounds for each tea grade (Figures 25 – 32). A known or unknown sample of tea could be analysed and the concentrations of the eleven components determined. Simply by overlaying a similarly scaled plot onto the individual grade plots, the identity of the tea could be quickly established. The terpene index and concentration of terpenes may also be calculated, and used to give an indication of the quality within the grade.

Examination of the fingerprints for the Sri Lankan orthodox teas revealed that with the exception of 532 and 535, each grade of tea could be differentiated (Table 23). In the case of 532 and 535, one batch could not be distinguished on this basis. One obvious answer to this problem is that perhaps there is insufficient difference between these two grades to really justify grading them separately. Examination of Figures 26 and 28 reveals only minor differences.

The fact that 535 is the most expensive grade, and very similar to 532, would appear to justify its removal from *Premium Blend Tea*. Surely, any grading must be verifiable in terms of the chemistry of its components. Comparison of the fingerprints of 532 and 533 reveal that with the exception of phenylacetaldehyde, all compounds are present at lower average levels in 532. If the harvested tea used to produce 532 is similar to that used for 533, then it may well be that a change in processing has led to the differences between these two grades. Certainly, the higher levels of phenylacetaldehyde would seem to indicate greater oxidation of the tea, perhaps due to an extended withering or fermentation time.

Table 23: Use of chemical ‘fingerprints’ to differentiate Sri Lankan orthodox black tea grades

Tea Grade I	Tea Grade II	Compound(s) used to differentiate grades
532	533	phenylacetaldehyde
532	535	methyl salicylate / phenylacetaldehyde*
533	535	linalool / linalool oxide II

* batch 532(10384) cannot be discriminated

One point that is of importance is the relative freshness of the tea when it is received at the factory. The VR7 teas are known to be harvested from one large plantation and shipped almost immediately after processing. However, the Indian and particularly the Sri Lankan teas are subject to an unknown storage time, due in the most part to the fact that the tea is gathered from a number of individual plantations.

It is known that a certain amount of product deterioration takes place during transit, accompanied by moisture pick up. In recent years the old familiar wooden tea chest has been replaced by laminated paper sacks with a foil liner. However, the new packaging does not guarantee zero product deterioration, and several different reactions may occur. Figure 33 illustrates the extent of these reactions, which varies depending on the moisture content of the product. The optimal moisture level would appear to be $4 \pm 1\%$.

Investigations by Springett *et al.* (1994), revealed that the use of packing regimes which exclude air significantly reduce the oxidative deterioration of black tea. Results also indicated the potential of using GC profiling and canonical variate analysis to discriminate teas stored for different times and under different packaging conditions.

Thus conditions during packing, storage, and indeed transportation, may mean the tea incurs periods of exposure to elevated temperatures and/or humidities, which may well affect the quality of the tea prior to its arrival at Madura. This may mean that a particular grade will exhibit a wider variation in the level of volatile compounds than would otherwise be the case if storage time and conditions were more controlled. A comparison between the pre-shipment sample and a sample taken on delivery may well indicate that such a problem exists.

Figure 33: The effect of moisture upon deteriorative reactions which occur in black tea (reproduced from Willson and Clifford, 1992)

Ideally, a buyer should have access to all details pertaining to the growing, harvesting, processing, storage and transportation of tea that is purchased, but this is unlikely to be the case, particularly where tea is purchased at auction.

The Australian Food Standards Code pertaining to tea (Q1), stipulates that:

Tea -

(I) shall contain not less than-

- (A) 300 g/kg of water-soluble extract;
- (B) 30 g/kg of water soluble ash;

(ii) may contain flavourings;

(iii) shall not contain -

- (A) more than 80 g/kg of total ash;
- (B) exhausted or partly exhausted leaves;
- (C) foreign matter.

However, no distinction is made between green, oolong and black tea and apart from the vague point in section (iii)B no stipulation is made on the levels of volatile compounds. Literature states that black tea may contain up to 0.2% of essential oil (Howard, 1978). Quantifying the yield of volatile components by dividing total chromatogram peak area by the area for a known quantity of internal standard gave a level of 0.007 % w/w volatiles for CTC tea (531:10391) and 0.012 % w/w for orthodox tea (532: 10409). The standard could be improved by either stating a minimum percentage of extractable organic substances; perhaps 0.005% for CTC tea and 0.01% for orthodox teas. The relative proportion of major compound(s) to other minor components could be used to check for possible adulteration of material.

6.0 IMPLICATIONS

This investigation has succeeded in identifying some of the important extractable organic compounds in black tea, although a definitive relationship between the level of these compounds and organoleptic appraisal was not clearly established. The fact that the non-volatile components of black tea also have a bearing on perceived quality is one complicating factor, as is the primary objective of any tasting panel. For example, are the panellists evaluating on the basis of their own preferences, or the perceived preferences of the 'typical' tea consumer.

With regard to the techniques used to characterise the individual teas, the use of terpene indices and terpene concentration appear to have some use, as acceptable ranges can be easily established and monitored. Such a technique would appear to be at least as useful as the more complicated statistical techniques using multivariate analysis. However, the incorporation of other components into principal component analysis may enhance its use.

The most useful tool would appear to be one based upon a number of volatile '*fingerprint*' compounds. The 11 compounds used in this report permitted the identification of particular tea grades as well as the determination of a terpene index and an indication of terpene concentration. In the one instance where the fingerprint technique failed to discriminate, it was doubtful if the two teas could be justifiably separated into two different grades. Such a procedure would be fairly simple to undertake and yield valuable information.

As far as the extraction methodology is concerned I would suggest that the technique could be improved by using either fractional distillation (at atmospheric or under vacuum), or the use of a Kaderna-Danish volatile trap to minimise volatile losses. This modification would permit the quantification of the group I compounds that are generally considered to be deleterious to tea quality.

As previously noted, the aroma complex has been shown to vary with the country of origin, variety and agronomic practices. All tea samples used in this study were the *assamica* variety of plant, although the precise genetic history was unknown.

7.0 RECOMMENDATIONS

Tea manufacturers could use the described methodology to establish an acceptable range of variation in respect of tea volatile components. In this manner teas could be graded, and the quality of any blend produced controlled to a much finer degree. However, in addition to ensuring that material meets the prescribed criteria for a particular grading, this methodology could also be used to:

- a) verify if pre-shipment samples match delivered material
- b) monitor pre-processing deterioration of imported tea due to storage abuse prior to and during transportation (see (a))
- c) monitor variability in tea quality over a growing season, due to either climatic/agronomic variability, or plucking standard. The particular plucking standard used could be modified to produce tea of the required quality and the effect of seasonal variation could be compensated for by altering the time between pluckings
- d) monitor deterioration of tea following packing and distribution to retail outlets
- e) source quality material from other countries/suppliers.

In conclusion, I would recommend that the Australian tea industry takes steps to monitor the quality of both domestically grown and imported tea. Such monitoring will by necessity include the evaluation of volatile components. Investigation will give producers a greater insight into the effect of variation in agronomic and manufacturing practices upon tea quality, as well as the deterioration of their product prior and subsequent to packaging and distribution.

8.0 INTELLECTUAL PROPERTY

The intellectual property associated with this project is procedural, and no protection is considered necessary. The outcomes of this project, as stated in the conclusion, are of significance to the Australian tea industry, and their adoption would facilitate greater control over imported material.

9.0 COMMUNICATIONS STRATEGY

Madura Tea Estates, as a participating tea industry member, will be directly aware of the outcomes of this project; the project will of course be particularly pertinent to them, as they were directly involved in it. One advantage of direct contact with the industry is that it will facilitate the communication and adoption of the findings of this study.

APPENDICES

