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Developing an index of quality for Australian tea

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Foreword

Although the production of black tea is currently small in Australia, compared to some of the main tea producing countries, the availability of suitable land and increasing experience of cultivation and processing should permit the rapid development of a viable tea industry. The tea industry in Australia has significant potential for import replacement. Current production supplies only 5-10 % of current consumption in Australia. Quality has been recognised as a very important factor in maintaining the competitiveness of Australian grown and made tea. The Australian tea industry needs data to assist with improving tea quality. As the health benefits of drinking tea become more firmly established the Australian tea industry also needs data to assist with effectively promoting its tea as overseas tea companies are already doing with their tea.

The production line and relevant technology for the processing of black tea in Australia have been modified from those currently employed in other tea producing countries. Thus, it is necessary to understand the changes in chemical composition occurring during the growing and processing of Australian tea, if quality improvements in tea are to be achieved. However, no published data are available relating the chemical composition, including phenolic compounds, to the growing and processing of Australian tea. Therefore, an investigation into the types and contents of flavonoids and other polyphenols in Australian grown tea at various growing seasons and made tea during different processing steps was carried out to provide the Australian tea industry with information to assist with maximising the quality of its black tea. Areas where quality could be improved were also identified.

This project was funded from RIRDC Core Funds which are provided by the Australian Government.

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Simon Hearn
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Abbreviations

Abbreviations of units

cm	centimetre(s)
°C	degree Celsius
°E	degree East longitude
g	gram(s)
h	hour(s)
ha	hectare(s)
kg	kilogram(s)
km	kilometre(s)
kPa	kilopascal(s)
L	litre(s)
m	metre(s)
mAU	milli absorbance units
mg	milligram(s)
mL	millilitre(s)
mm	millimetre(s)
min	minute(s)
nm	nanometre(s)
°N	degree North latitude
°S	degree South latitude
% (v/v)	percentage volume for volume
°W	degree West longitude
% (w/w)	percentage weight for weight
t	tonne(s)
μL	microlitre(s)
μm	micrometre(s)
UV/VIS	ultraviolet/visible

Other abbreviations*

ANOVA	analysis of variance
C	catechin
CA	chlorogenic acid
CCS	combined catechins/catechin gallates
CG	catechin gallate
CGS	total catechin gallates
CHD	coronary heart disease
CS	total catechins
CTC	crush(ing)-tear(ing)-curl(ing)
Cu	copper
CV	coefficient of variance
DNA	deoxyribonucleic acid
EC	epicatechin
ECG	epicatechin gallate
ECDG	epicatechin digallate
EGC	epigallocatechin
EGCG	epigallocatechin gallate
EGCDG	epigallocatechindigallate
ETFA	epitheaflavic acid
ETFAG	epitheaflavic acid 3-gallate
FBD	fluidised bed drying
Fe	iron
GA	gallic acid
GATE	Glen Allyn Tea Estates
GC	gallocatechin
GCG	gallocatechin gallate
GCMS	gas liquid chromatography mass spectrometry
HCl	hydrochloric acid
HIV	human immunodeficiency virus
H_2O_2	hydrogen peroxide
HPLC	high performance liquid chromatography
ISO	International Standard Organization
K3RG	kaempferol 3-rhamnosylglucoside.
KG	kaempferol glycoside
LSD	least significant difference
LTP	Lawrie Tea Processor
N-P-K	nitrogen-phosphorus-potassium
PDA	photodiode array
PPO	polyphenol(ic) oxidase
Q3G	quercetin 3-glucoside
Q3RG	quercetin 3-rhamnosylglucoside
QG	quercetin glycoside
SD	standard deviation
TCG/TC	total catechin gallates/total catechins
TF	theaflavin
TF3G	theaflavin 3-gallate
TF3′G	theaflavin 3′-gallate
TFDG	theaflavin 3,3′-gallate
THG	theogallin
TPS	total phenolic compounds
TR	thearubigin(s)
UK	United Kingdom
US	United States (of America)
VFC	volatile flavour compound(s)

*Abbreviations for the black tea processing steps are attached in Appendix 5.2.

Executive summary

An initial investigation into the quality of teas available in Australian supermarkets was conducted. Samples of both green and black leaf tea and teabags were randomly collected from supermarkets, including Australian teas. Moisture, water extraction, total polyphenols (PPs), theaflavin, thearubigen and theabrownin were measured in the samples. The aim was to provide an overall profile of the quality of teas available in Australia.

Phenolic compounds constitute 50 % to 70 % of tea water extracts and are thus considered the main quality parameters for teas. Theaflavin (TF), thearubigin (TR) and theabrownin (TB) are the main polyphenols that determine the quality of black tea. These compounds were measured in 56 samples of leaf teas and teabags available in Australian supermarkets. The variability in TF, ranging from 0.29 % to 1.25 % for black teabags and 0.32 % to 1.10 % for black leaf tea indicates a quality difference among the teas studied. Low TF suggests the teas were over-fermented and/or stored for long periods. The solubility of TR and TB from teabags ranged from 82 % to 92 % indicating that the permeability of teabags was variable. Total polyphenols in black tea leaf were between 14 % and 20 %, with an average of 17 %. This was slightly lower than the total polyphenols detected in black teabags, which ranged from 13 % to 27 % with an average of 18 %. Total polyphenols in the green teas studied ranged from 15 % to 34 % with an average of 23 %. This shows quality differences in both green and black teas. The solubility of total polyphenols from teabags could be a useful quality index of the filter paper used for the teabags. Results from this chemical analysis suggest that phenolic compounds could be used for the quality control of both Australian grown and imported teas.

The aim of the next part of the study was to investigate in detail flavonoids and other polyphenols in Australian grown and made tea. Phenolic compounds in fresh tea leaves collected from a tea farm in North Queensland were analysed. This field study was conducted on different commercial harvests from April 2000 to May 2001, using both hand plucking and mechanical harvesting to collect the fresh tea leaves in order to determine seasonal variations. The analysis of Australian made tea was conducted on the samples collected off the processing line of a black tea processing factory in North Queensland, at three month interval from April 2000 to January 2001, so the process could be evaluated in terms of its effects on tea flavonoids. Prior to this study, no published research has been conducted on the flavonoids and other polyphenols of Australian tea.

Fresh tea leaves, consisting of one apical bud and two adjunct leaves, were hand plucked just before each mechanical harvest during the sampling period. The samples were packed in dry ice and delivered to the laboratory by overnight transport and stored in a freezer at –80 ˚C before analysis. The phenolic compounds were extracted with methanol using a method developed and optimised for this study, and were analysed using an HPLC with photodiode array detection. Four catechins, six catechin gallates, five flavonoid glycosides, and seven phenolic acids were identified and quantified. The main flavonoids found in the fresh tea leaves were epigallocatechin gallate (EGCG), epicatechin gallate (ECG) and epigallocatechin (EGC).

The major findings of the analysis of the fresh tea leaves from the field are that there were lower levels of catechins gallates in the tea leaves harvested in the cooler months of July to September 2000 (EGCG, 92.94 mg/g; ECG, 33.41 mg/g; total catechin gallates, 132.61 mg/g), and higher levels in the tea leaves harvested during the warmer months of November 2000 to February 2001 (EGCG, 112.37 mg/g; ECG, 37.13 mg/g; total catechin gallates, 159.34 mg/g). For the catechin levels in the harvested tea samples, higher and constant levels were found in those harvested in the cooler months (EGC, 50.50 mg/g; total catechins 89.67 mg/g) and lower levels were found in those harvested in the warmer months (EGC, 44.86 mg/g; total catechins, 79.26 mg/g). On comparing the hand plucked samples with the mechanically harvested tea leaves, it was found that the levels of catechins and catechin gallates were found to be lower in the mechanically harvested leaves probably due to more mature leaves being in these samples than in the hand plucked ones.

The samples from the processing line were collected during three sessions of black tea processing in April, July, and October 2000, and January 2001. The in-line samples were collected at each of the main processing steps from the initial field green leaves to the final black tea. The samples were also packed in dry ice and delivered to the laboratory by overnight transport and stored in a freezer at –80 ˚C before analysis. The phenolic compounds in these samples were extracted using methanol and aqueous methanol, and analysed using a HPLC with photodiode array detection. Catechins, catechin gallates and theaflavins were the main flavonoids quantified for the in-line samples.

Analysis of the results for the in-line tea samples shows that the main decreases in the individual and total catechins and catechin gallates due to oxidation occurred at the early stages of the fermentation period. Correspondingly, formation of individual and the total theaflavins occurred at the early stages of the fermentation period, suggesting the individual theaflavins are oxidation products of the catechins, catechin gallates and other phenolic acids such as gallic acid. The only seasonal variation that can be explained is that higher levels of the catechin gallates in the fresh leaves in January 2001 corresponded with higher levels of the oxidation products, theaflavins, in the resulting black tea. However, other seasonal variations in the formation of theaflavins and reduction in the levels of the catechins and their gallates showed no regular patterns throughout the black tea processing. This could be due to factors associated with the tea processing.

It can be concluded that tea leaves harvested in the warmer months, such as January, when processed, would contain the highest level of theaflavins, and produce a correspondingly higher quality black tea. The ratio (ECG+EGCG)/EGC could be used to measure seasonal variation in the levels of flavonoids in green tea leaves in the field and thus for monitoring the best time of the year to harvest tea leaves to produce quality black tea. As the ratio is similar in both mechanically harvested and hand plucked green tea leaves, the more easily obtained mechanically harvested leaves can be analysed for the ratio, which can be used as a quality index for the processed black tea.

In the part of the study above, the theaflavin levels peaked well before the designated end of fermentation indicating that the tea was overfermented. This highlighted the need to develop an on-line method of determining optimum fermentation, ie an on-line method for measuring theaflavin content during fermentation.

The first step involved assessing the methods using in other countries for measuring theaflavin content. Most of these methods are based on extracting the theaflavins and thearubigens and measuring by spectophotometric analysis. For these trials the dried tea samples collected during fermentation and after final drying were analysed for theaflavins, thearubigins, soluble solids, and total colour. The methods were assessed for their ease of use and adaptability for on-line use as well as their ability to distinguish between levels of theaflavins. None of the methods were simple enough to fit the criteria for adaptation to the factory situation. Whilst the measurements of theaflavins is relatively simple, the need to extract the theaflavins make the tests more complex requiring space, equipment and skilled technical support not available in the factory situation.

Next the feasibility of using colour as measure of theaflavin content was investigated. The drawback found here was that as the theaflavin content peaked during fermentation, total colour and thearubigens continued to rise. So it was not possible to separate the contribution of theaflavins to colour using these methods. However some recent work suggested that the colour contribution of theaflavins could be separated from thearubigen contribution using a colour meter such as the Hunter Lab or Minolta colour meter. These instruments measure 4 different components of colour and it appears that theaflavins can be positively correlated with some of these. Also the Minolta colour meter is a hand held instrument making it ideal for on-line use. More information is first required on thearubigen fractions before futher work can progress on this. Some preliminary analytical gel-permeation chromatography work has been undertaken.

1 Introduction

1.1 Tea flavonoids

Australian tea is mainly black tea, which is produced using a fermentation procedure as part of tea processing. The Australian tea industry supplies 5-10 % of the total quantity of tea consumed in Australia, but the majority is still sourced from developing countries such as Malaysia and Indonesia (Chudleigh, 1999). Production in the Australian tea industry is approximately 1 300 t per annum (International Tea Committee, 2001), whereas annual consumption is estimated to be about 17 000 t of black tea (Chudleigh, 1999). Quality has been recognised as a very important factor in maintaining the profile of the Australian grown and made tea.

The main difference in the processing of black tea between Australia and other countries is that the major tea producer in Australia operates its processing factory with "low labour, high mechanisation and high throughput", while other countries use a labour-intensive production model (Benson, 2000-2002). There is no traditional withering (the initial step of black tea processing) facility associated with the Australia factories but there are bins for the storage of green leaves prior to processing. The production line and relevant technology for the processing of black tea have been modified from those currently employed in other tea producing countries. Thus, it is necessary to understand the changes in chemical composition occurring during the growing and processing of Australian tea, if quality improvements in tea are to be achieved. However, no published data are available relating the chemical composition, including phenolic compounds, to the growing and processing of Australian tea. Therefore, an investigation into the types and contents of flavonoids and other polyphenols in Australian grown tea at various growing seasons and made tea during different processing steps would assist with maximising the quality of the black tea.

Tea is one of the principal food sources of flavonoids in the human diet because of its higher level of consumption combined with its relatively higher flavonoid content (Ho *et al*., 1994; Hollman and Arts, 2000). Flavonoids occuring in green tea are mainly flavan-3-ols, which constitute 90 % (w/w) of the phenolic compounds in the leaves (Lakenbrink *et al*., 2000). Flavonoids in black tea are mainly theaflavins and thearubigins (Harbowy and Balentine 1997), the complex oxidation products of green tea phenolic compounds. Thearubigins, the structures of which have not yet been elucidated, are a group of polymerised oxidation products of tea catechins and their gallates (Roberts, 1962; Bailey *et al*., 1991; Lakenbrink *et al*., 2000). The main tea phenolic compounds are catechins and their gallates, which were previously called tea tannins (Bokuchava and Skobeleva, 1969), and also referred to as tea polyphenols (Harbowy and Balentine, 1997; Roberts, 1962). These polyphenols account for 20-35 % (w/w) of the dry tea (Sanderson, 1972; Xiao, 1994), thus representing a significant proportion of the tea constituents.

Phenolic compounds have long been regarded as one of the principal quality parameters or indicators of tea (Roberts and Smith, 1961; Deb and Ullah, 1968; Ellis and Cloughley, 1981; Owuor, 1982; Davies, 1983; Ding *et al*., 1992; Obanda *et al*., 1992, 1997). Theaflavins in black tea have been used to assess market value (Hilton and Ellis, 1972; Sanderson *et al*., 1976; Owuor *et al*., 1986ad), clonal variations (Roberts and Fernando, 1981; Owuor and Obanda, 1995a), and seasonal quality variations (Hilton *et al*., 1973; Malec and Vigo, 1988). In addition to theaflavins in black tea, and catechins and the gallates in green tea, total phenolic compounds are considered to be quality factors (Yao *et al*. 1992, 1993) and dictate the price of the tea (McDowell *et al*., 1991; Owuor and Obanda, 1995a).

Some phenolic compounds, such as gallic acid, the catechins and their gallates, and quinic acid derivatives do not influence the colour of black tea as theaflavins that impart colour to the tea liquor (Bailey *et al*, 1990). This is because these phenolic compounds can only absorb light in the ultraviolet (UV) range. However, these phenolic compounds may influence the taste and other liquor characters of the tea. For example, chlorogenic acid, one of the quinic acids in tea, has been linked with astringency (Haslam, 1989). Additionally, all phenolic compounds are capable of forming hydrogen bonds related to a complexation, which was referred to as "cream down" in black tea liquor (Roberts,

1962). The "cream down" of black tea is related to the quality of tea since it may impart colour, flavour or mouthfeel to the liquor (Roberts, 1962; Eden, 1976; Bailey *et al*., 1990). It has been found that the whole profile of phenolic compounds in black tea is closely associated with its price and sensory characteristics (Takeo and Oosawa, 1976). Thus, analysis of phenolic compounds in tea is important for the determination and objective description of tea quality.

1.2 Aim and objectives of this project

A study of the flavonoids and other polyphenols in Australian tea was undertaken. This involved a number of experiments, with the following objectives:

1. To determine quality profiles of teas on Australian market and how Australian grown teas compare with other teas.
2. To determine the phenolic profiles and their seasonal changes for fresh green tea leaves grown in Australia.
3. To identify the changes in the phenolic profiles of tea during the processing of green leaves to black tea.
4. To compare the phenolic compositions of Australian grown and made tea to the teas produced in other countries.
5. To develop an online method for monitoring and optimising fermentation of Australian grown and made black tea.

2 Literature review

2.1 Flavonoids

2.1.1 Occurrence and chemistry of flavonoids

Flavonoids are a group of polyphenolic compounds, diverse in chemical structure and characteristics. They occur in virtually all plant parts, particularly the photosynthesising plant cells, and are an integral part of both human and animal diets (Cook and Samman, 1996; Bravo, 1998). As plant phytochemicals, flavonoids cannot be synthesised by humans and animals (Harborne, 1967, 1988; Harborne *et al*., 1975). Flavonoids found in animals are considered to originate from plants upon which the animals feed rather than being biosynthesised *in situ* (Harborne, 1967). About 4000 individual compounds belonging to this class are already known (Harborne, 1988), with the actual number of flavonoids possibly being closer to 5000 (Cadenas and Packer, 1996). The distribution of flavonoids in plants suggests there is a strong tendency for taxonomically related plants to produce similar types of flavonoids (Markham, 1982).

Naturally occurring flavonoids are generally classified into six classes according to their chemical structures (Peterson and Dwyer, 1998), including flavanones, flavones, isoflavonoids, flavans (flavanols), anthocyanins and flavonols. These flavonoids vary in their structural characteristics around the heterocyclic oxygen ring, forming a unique carbon skeleton C_6-C_3-C_6 (Figure 2.1). This structure can have various numbers of hydroxyl substitutions and other functional groups attached to form many different types of flavonoids (Harborne, 1967, 1988). Chalcones, rich in fruits such as apples, are usually classified as yellow flavonoids because of a similar C_6-C_3-C_6 skeleton and properties (Harborne and Turner, 1984). Flavonoids occur as both aglycones and glycosides. In general, all flavonoids are derivatives of the 2-phenylchromone parent compound composed of three phenolic rings referred to as the A, B and C rings, all of which contain varying levels of hydroxylation and methoxylation (Clifford and Cuppett, 1997). The biochemical activities of flavonoids and their metabolites depend on their chemical structures and the relative orientation of various moieties on the molecule.

Studies on the chromatography of flavonoids have shown that most flavones and flavonols exhibit two major absorption bands: Band I 320-385 nm represents the B ring absorption, and Band II 250-285 nm represents the A ring absorption (Rice-Evans, *et al*., 1995, 1996; Cook and Samman, 1996; Hall and Cuppett, 1996). Functional groups attached to the flavonoid skeleton may cause a shift of absorption, such as from 367 nm in kaempferol with 3,5,7,4′ hydroxyl groups, to 371 nm in quercetin with 3,5,7,3′,4′ hydroxyl groups, and to 374 nm in myricetin with 3,5,7,3′,4′,5′ hydroxyl groups. The absence of a 3-hydroxyl group in flavones (which distinguishes flavones from flavonols) means that Band I flavones always absorb at a shorter wavelength (by 20-30 nm), such as the 337 nm for apigenin (5,7,4′), than the equivalent Band I flavonols (Rice-Evans, *et al*., 1995-1997). *O*-methylation and glycosylation produce hypochromic shifts. Flavanones have a saturated heterocyclic C ring, with the consequent lack of conjugation between the A and B rings being defined by their ultraviolet (UV) spectral characteristics and in their lower antioxidant activity (Rice-Evans, *et al*., 1995, 1996). Flavanones exhibit a very strong maximum absorption (Band II) between 270 and 295 nm, namely 288 nm (naringenin) and 285 nm (taxifolin) and only a shoulder for Band I at 326 and 327 nm. Band II appears as one peak (ca 270 nm) in compounds with a monosubstituted B ring, but as two peaks or one peak (ca 258 nm) with a shoulder (ca 272 nm) when a di-, tri-, or *o*-substituted B ring is present. The colour of the anthocyanins varies according to the number and position of the hydroxyl groups (Wollenweber and Dietz, 1981). Anthocyanins show distinctive Band I peaks in the 450-560 nm region due to the B ring hydroxyl cinnamoyl system and Band II peaks in the 240-280 nm region due to the A ring benzoyl system (Harborne, 1967, 1988; Harborne and Turner, 1984).

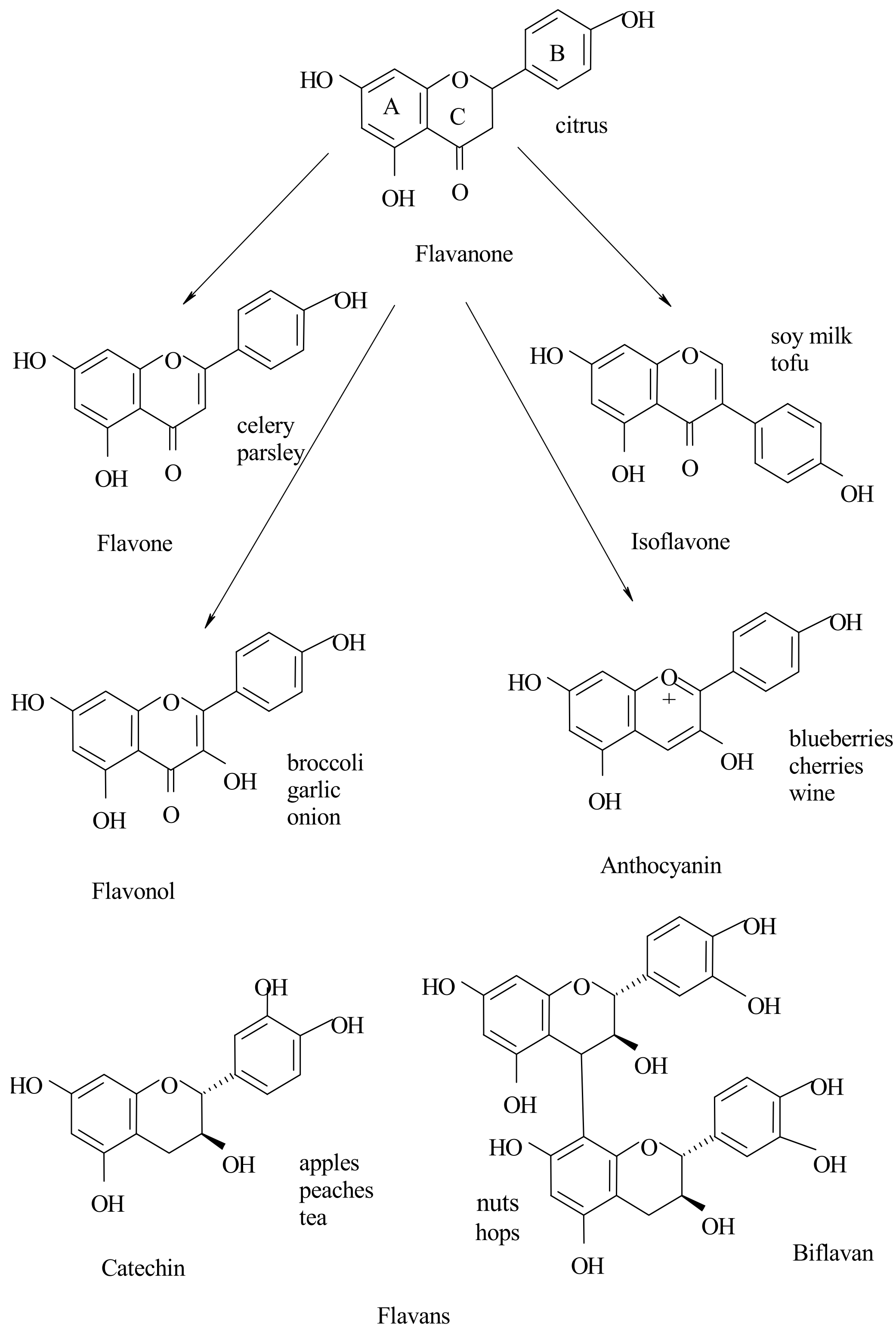


Figure 2.1 Structure and dietary occurrence of the main classes of flavonoids (arrows indicate biosynthetic path)
Source: adapted from Peterson and Dwyer, 1998.

The structure of flavonoids is the basis of many hypotheses about their physiological actions. Flavonoids are easily oxidised at the B ring which leads to opening of this ring at the oxygen atom (Havsteen, 1983). The high chemical reactivity of flavonoids is expressed in the binding affinity to biological polymers and heavy metal ions, and the ability to catalyse electron transport and to scavenge free radicals (Havsteen, 1983). Thus, flavonoids are a group of phenolic compounds that share some common structural features and physicochemical properties, which create interest in their biological effects.

Flavonoids in plants, such as anthocyanins, impart colour as pigments, and ensure pollination, fertilisation and seed dispersal by animals (Harborne, 1975, 1988; Mazza, 1997). These compounds act as a light screen against damaging UV radiation in young leaves, provide resistance to pathogens, and act as antioxidants, enzyme inhibitors and precursors of toxic substances. In addition, flavonoids may function as photosensitising and energy-transferring compounds, and take part in the control of plant growth and development in concert with plant hormones (Harborne, 1975, 1988). Flavonoids appear to be universal chemical tools that plants use to interact with their environment. Furthermore, these compounds have been implicated in defense against other plants, fungi, insects and bacteria; as regulators of interactions between beneficial fungi, herbivores, and insects; as plant hormones; and as important constituents of animal diets, both nutritionally and medically (Berhow, 1998). Consequently, scientists are showing increased interest in food flavonoids, due to their possible beneficial roles in human health as antioxidants, in the prevention of cancer and cardiovascular diseases and in the treatment and prevention of many other pathological disorders, such as gastric and duodenal ulcers, allergies, vascular fragility, and viral and bacterial infections (Bravo, 1998).

2.1.2 Biochemical systematics of flavonoids

Flavonoids have been regarded as important taxonomic markers in systematic studies since 1962 (Harborne, 1967). The reasons why flavonoids are preferred to most other low molecular weight constituents are: (1) they are universally distributed in vascular plants and show considerable structural diversity; (2) they are so chemically stable that they can be detected in herbarium tissue; and (3) they are easily and rapidly identified (Harborne, 1975, 1982, 1988; Harborne and Mabry, 1982; Harborne and Turner, 1984).

Distribution and biochemical evolution of flavonoids in the plant kingdom may indicate the evolution of plants. This evolving flavonoid pattern in higher plants has very useful properties (Harborne, 1982). Firstly, the pattern is reasonably consistent and representative within any given family. Secondly, the relative frequency of the occurrence of a given flavonoid in a family is often more important than the presence/absence of the flavonoid because large differences can be found in such frequencies. Thirdly, families can be placed into groups according to the major flavonoids present. However, no overall evolutionary trend in flavonoids has been found. These properties suggest that flavonoid profiles in plants or plant products may be useful in the study of botanical or geographical origins of foods such as honey and fruit jams (Garcia-Viguera *et al*., 1993, 1994, 1997a).

2.1.3 Dietary occurrence of flavonoids

2.1.3.1 Generic distribution in foods

Flavonoids are not present in animals but occur in all plant foods. Flavonoids occurring in foods are generally responsible for colour, taste, the protection of fats against oxidation, the destruction of vitamins, and the inactivation of enzymes (Swain, 1962). Flavanones occur predominantly in citrus while isoflavonoids occur in legumes (Huang *et al*., 1994). Flavones occur mainly in herbs (Ho *et al*. 1994); while anthocyanins and catechins are found in teas, fruits and vegetables (Ho *et al*. 1994; Huang *et al*., 1994); and flavonols occur in all fruits and vegetables (Peterson and Dwyer, 1998). Any foods containing natural flavours and colourings, or made from plants, may contain flavonoids. The dietary occurrence of flavonoids in foods is summarised in Figure 2.1.

Rich amounts of natural phenolic compounds are found in teas, fruits, and vegetables, while some amounts of polyphenols exist in red wine and coffee (Ho *et al*., 1992). The flavonoids found in citrus are flavanones, flavones and flavonols (Benavente-Garcia *et al*., 1997), while genistein has been found in citrus volatiles (Saleh *et al*., 1998) even though it is not normally considered a volatile. Fifteen anthocyanins and 10 flavonoids have been found in 34 French wines originating from 6 grape varieties and 3 growing areas (Etievant *et al*., 1988). There are very many anthocyanins found in 27 families of food plants. The worldwide annual consumption of anthocyanins from black grapes alone is estimated at 10 000 t (Clifford, 2000b).

2.1.3.2 Level of flavonoids in foods

The levels of individual and total flavonoids are influenced by genetic factors such as species, environmental conditions such as light, ripeness, and post-harvest treatments such as processing and storage (Bravo, 1998; Duthie *et al*., 2000). Although most fruits, chocolate, and some legumes contain catechins, the levels vary to a large extent from 4.5 mg/kg in kiwifruit to 610 mg/kg in black chocolate (Arts *et al*., 2000a). Here, catechin and epicatechin (EC) are the predominant catechins, whereas gallocatechin (GC), epigallocatechin (EGC) and epicatechin gallate (ECG) are detected only in certain foods. Tea is the only beverage that contains GC, EGC, ECG, and epigallocatechin gallate (EGCG), in addition to catechin and EC (Arts *et al*., 2000a).

Phenolic compounds have been assumed to influence wine flavour. Total phenolic contents of wines are approximately 200 mg gallic acid equivalent/L (Singleton and Noble, 1976). In addition, these researchers found that typical young red table wines have about 120 mg/L anthocyanins, 50 mg/L flavonols, 250 mg/L catechins, and 750 mg/L anthocyanogenic tannins. Green tea has the highest level of phenolic compounds amongst foods; up to 35 % of the dry matter (Bravo, 1998). Leaves of the sweet potato and onion plants also possess very high amounts of flavonoids (Chu *et al*., 2000). For sweet potato plant, the green leaves contain 185.01 mg/kg of flavonoids, and the purple leaves contain 426.82 mg/kg. The outer leaves of the onion plant contain 264.01 mg/kg flavonoids.

Levels of individual and total flavonoids vary according to the plant varieties and food types. Quercetin levels in the edible parts of vegetables are generally below 10 mg/kg (Hertog *et al*., 1992a, 1993a). In black tea infusions, quercetin ranges 10-25 mg/L, while kaempferol and myricetin are 7-17 mg/L and 2-5 mg/L, respectively. These variations in the flavonoid levels may be used to differentiate food types (Rouseff *et al*., 1987). Furthermore, preparation and processing of food may decrease flavonoid levels depending on the methods used (Garcia-Viguera *et al*., 1997b). For example, in a recent study, orange juices were found to contain 81-200 mg/L soluble flavanones (Gil-Izquierdo *et al*., 2001), while the content in the cloud was 206-644 mg/L, suggesting that the flavanones precipitated and presented in the cloud during processing and storage.

2.1.3.3 Dietary intake of flavonoids

Estimation of the average dietary intake of flavonoids is difficult. This is due to there being many types of flavonoids distributed extensively in various plants, and humans having diverse dietary styles. With this mind, the dietary intake of flavonoids has been estimated to vary from 23 to 1 000 mg/day (Peterson and Dwyer, 1998), while a study of the Western diet suggests an intake of 1 000 mg/day of mixed flavonoids (Clifford and Cuppett, 1997). The daily intake of anthocyanins alone has been shown to reach 215 mg in summer and 180 mg in winter in the USA (Clifford, 2000b). Although the dietary data on flavanones and dihydrochalcones are not available, these compounds may make a significant contribution to the daily intake of flavonoids (Tomás-Barberán and Clifford, 2000). Thus, the measurement of dietary intake of flavonoids depends entirely on the criteria of survey, the method used, and the reference compounds selected for analysis. Using only a few flavonoids to determine the dietary intake is not accurate enough and can lead to a serious underestimation of flavonoid contribution to the diet.

2.1.4 Flavonoids and health benefits

2.1.4.1 General benefits of consumption of flavonoid rich food

Food phenolic compounds, particularly flavonoids, are thought to play important roles in human health (Ho *et al*., 1992, 1994; Huang *et al*., 1992, 1994). *In vitro* and animal studies have demonstrated that flavonoids have antioxidant and anti-mutagenic activities (Peterson and Dwyer, 1998) and may thus reduce the risk of cardiovascular disease and stroke (Duthie *et al*., 2000). Isoflavonoids, such as phytoestrogens, have a wide range of hormonal and non-hormonal activities in animals or *in vitro* (Cassidy *et al*., 2000), suggesting potential human health benefits of diets rich in these compounds.

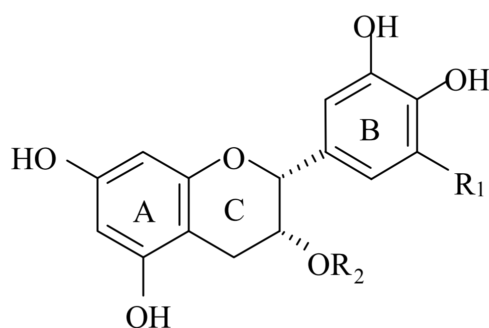
Flavonoids may act as antioxidants to inhibit free-radical mediated cytotoxicity and lipid peroxidation; as anti-proliferative agents to inhibit tumour growth; or as weak oestrogen agonists or antagonists to modulate endogenous hormone activity (Lyons-Wall and Samman, 1997). In these ways, flavonoids may confer protection against chronic diseases such as atherosclerosis and cancer, and assist in the management of menopausal symptoms. Thus, flavonoids have been referred to as semi-essential food components (Kuhnau, 1976).

Early studies have uncovered some properties of tea polyphenols related to human health (Chow and Kramer, 1990), including a capillary-strengthening property, an antioxidative property responsible for the radioprotective effect, and the antimicrobial property. Hara (1992) showed that the habit of tea drinking could prevent cardiovascular diseases, by increasing plasma antioxidant capacity in humans (Nakagawa *et al*., 1999; Duthie *et al*., 2000). Interestingly, tea polyphenols are rapidly absorbed after drinking with milk, and milk does not impair the bioavailability of polyphenols (Van het Hof *et al.*, 1998).

2.1.4.2 Flavonoid structures and antioxidant activities

Antioxidants can be defined as compounds that inhibit or delay but do not completely prevent oxidation (Clifford and Cuppett, 1997). They can be compounds capable of inhibiting oxygen-mediated oxidation of diverse substrates and/or inhibiting free radical chain-propagation reactions (Ho *et al*., 1994). There are generally two groups of antioxidants, synthetic and natural. The antioxidant mechanisms of the synthetic antioxidants are well established, but only the mechanisms of certain classes of natural ones such as tocopherols and carotenes have been determined. Clifford and Cuppett (1997) classified the antioxidant mechanisms of flavonoids into free radical chain-breaking, metal-chelating and singlet oxygen quenching, with the inhibition of enzymatic activity possibly being included. Further, Bors *et al*. (1996) suggested that the mechanisms might include the synergistic effects.

Flavonoid compounds with similar chemical structures exhibit comparable trends in antioxidant activity (Fukumoto and Mazza, 2000). This activity usually increases with an increase in the number of hydroxyl groups and a decrease in glycosylation. Flavonoids such as EC and ECG with a vicinal diphenol structure in the B ring and a saturated C ring exhibit the strongest effects (Figure 2.2) (Frankel *et al*., 1998).



Epicatechin (EC) $R_1 = R_2 = H$
Epicatechin gallate (ECG) $R_1 = H, R_2 = gallate$
Epigallocatechin (EGC) $R_1 = OH, R_2 = H$
Epigallocatechin gallate (EGCG) $R_1 = OH, R_2 = gallate$

Figure 2.2 Structure of EC, ECG, EGC and EGCG.

EGCG with two triphenol components in its structure, one from the B ring and one from the gallate attachment (Figure 2.2), has been found to strongly and dose-dependently inhibit histamine release from rat basophilic leukemia cells (Matsuo *et al*., 1997). EGC (in the B ring) and ECG (in the gallate attachment) with only one triphenol in the structure, moderately inhibit such release (Matsuo *et al*., 1997). The catechins, such as EC, with only a diphenolic structure in the B ring, do not produce such an effect. Similar findings were reported recently by Toschi *et al*. (2000). These results suggest that the triphenol structure plays an important role in the activities of tea polyphenols (Pannala *et al*., 2001).

2.1.4.3 Variation in scavenging and antioxidant properties

Flavonoids have been labeled as ‘high level’ antioxidants based on their abilities to scavenge free radicals and active oxygen species. However, research has shown that antioxidant activity varies among flavonoids (Auroma, 1997). Quercetin and 5,3′,4′-trihydroxyl-7-methoxyflavone show synergism when they are tested in a mixture, while the relative antioxidant effectiveness of tea polyphenols in an aqueous system has been found to be different from that tested in a lipid system (Auroma, 1997). This result indicates that the activity of flavonoids is influenced by environmental factors.

Salah *et al*. (1995) showed that the total antioxidative activity and the order of effectiveness of green tea polyphenols as radical scavengers is: ECG > EGCG > EGC > gallic acid > EC = catechin. The oxidation of low density lipoproteins is inhibited by catechin, EC, ECG and EGCG to a similar degree, but not as much as in the presence of EGC or gallic acid. Miller *et al*. (1996) showed that the relative antioxidative capacity of black tea polyphenols in both aqueous and lipophilic phases is theaflavin digallate (TFDG) > theaflavin 3′-gallate (TF3′G) = theaflavin 3-gallate (TF3G) > theaflavin (TF). Similar findings for black tea theaflavins were reported by Chen and Ho (1995) and Lin *et al*. (2000). These results suggest that variations of these activities of tea polyphenols may depend on the test conditions and the structures of the polyphenols. In addition, Amarowicz and Shahidi (1995) found that the efficacy of antioxidant activity of a reconstituted green tea polyphenol mixture was lower than that of the extracted crude mixture itself. This may indicate that non-catechin components in the mixture possessed their own antioxidant activity or acted synergistically with the catechins.

Recent comparison of different teas showed a wide difference in *in vitro* antioxidant power that was strongly correlated with total phenolic content (Benzie and Szeto, 1999). Green tea showed the

strongest activity, oolong tea the next, while black tea showed the least activity (Chen *et al*., 1996; Serafini *et al*., 1996; Gardner *et al*., 1998). Tea flavonoids are more effective scavengers of aqueous and lipophilic stable radicals than many other flavonoids and the antioxidant vitamins (Zhao *et al*., 1989; Wiseman *et al*., 1997). It has been reported that tea flavonoids scavenge radicals and protect low density lipoproteins from oxidation more effectively than established antioxidants (Zhu *et al*., 1999). In addition, tea flavonoids demonstrate *in vivo* protection in smokers and in rodents exposed to oxidative stress induced by radiation, chemicals or diet (Unilever, 1996).

2.1.4.4 Chelating capacity and redox potential

The chelating properties of flavonoids may be attributed to their antioxidant activities (Auroma and Cuppett, 1997). Most flavonoids chelate iron (Fe^{2+}), but there are large differences in the chelating capacity (Van Acker *et al*., 1996). For good scavenging activity, a catechol moiety on ring B is required. Chelation can raise the scavenging activity to the level of the most active scavengers, possibly by site-specific scavenging. Thus, antioxidative capacity of flavonoids increases as their Fe^{2+}-chelating activities increase. However, some of the chelating activity of flavonoids may adversely affect human health (Santos-Buelga and Scalbert, 2000). *In vitro*, animal, and human studies on the prevention of some chronic diseases have shown that proanthocyanidins might reduce the bioavailability of several minerals (e.g. iron).

Spectroscopic studies on theaflavins from black tea revealed that all these compounds chelate iron and copper (Miller *et al*., 1996). Another study showed that green tea extract markedly delayed lipid peroxidation in low density lipoproteins, with a dose-dependent pattern (Yokozawa and Dong, 1997). Copper chelation was recognised as one of the possible mechanisms of low density lipoprotein anti-peroxidation. An enhanced absorption in the visible region is observed in the case of the iron-digallate complex, but not with copper-digallate complex. For flavonoids, *ortho* 3′,4′-hydroxyl substitution in the B ring has been shown to be important for Cu^{2+}-chelating formation, thus influencing the antioxidant activity (Brown *et al*., 1998).

The antioxidative capacity of flavonoids is also associated with their redox potentials (Bors *et al*. 1996). In many instances, the reduction potentials of flavonoids, such as catechins of green tea (Jovanovic *et al*., 1995) and theaflavins of black tea (Jovanovic *et al*., 1997), are lower than that of vitamin E. Low reduction potentials and high rates of scavenging of biological oxidant-superoxide radical in neutral media (Unno *et al*., 2000) are indicative of high antioxidant potentials of tea catechins and theaflavins (Jovanovic *et al*., 1995-1998).

2.1.4.5 Metabolism and clinical effects

Flavonoids are absorbed by the gastrointestinal tracts of humans and animals, and are excreted either unchanged or as their metabolites in the urine and faeces (Cook and Samman, 1996). Colonic bacteria split the heterocyclic ring and degrade flavonoids to phenyl acids which may be absorbed, conjugated, and excreted or metabolised further by the bacteria (Peterson and Dwyer, 1998). Some flavonoid glycosides are rapidly deglycosylated by enzymes in human tissues whereas others may remain unchanged. The rate and extent of deglycosylation depends on the structure of the flavonoid and the position/nature of the sugar substitutions (Day *et al*., 1998). Measurement of plasma and urine antioxidant power after ingestion of green tea has shown that absorption of green tea antioxidants is rapid (Benzie *et al*., 1999). The antioxidants enter the systemic circulation soon after ingestion and cause a significant increase in plasma antioxidant status. Benzie *et al*. (1999) suggested that this increase might lower oxidative damage to the deoxyribonucleic acid (DNA) and thus decrease risk of cancer.

Flavonoids have profound effects on the function of immune and inflammatory cells (Middleton and Kandaswami, 1992). In animal studies, two EGCG methyl esters extracted from oolong tea significantly inhibited mice allergic reactions (Sano *et al*., 1999). Gaby (1998) suggested that quercetin may be of value in the treatment of asthma and be beneficial for diabetic and human immunodeficiency virus (HIV) infected patients. The flavonoid, baicalin, was recently shown to

possess anti-inflammatory and anti-HIV-1 activities (Li *et al*., 2000), by interfering with the interaction of the HIV-1 envelope proteins with chemokine co-receptors and blocking the HIV-1 entry to target cells.

2.1.4.6 Protective activities against heart disease

Possible protective effects of flavonoids against heart disease may be due to their ability to prevent the oxidation of low density lipoproteins to an atherogenic form, although anti-platelet aggregation activity and vasodilatory properties are also reported (Muldoon and Kritchevsky, 1996; Chen *et al*., 2000; Duthie *et al*., 2000; Santos-Buelga and Scalbert, 2000). Flavonoid intake may reduce the risk of death from coronary heart disease in women (Knekt *et al*., 1996; Duthie *et al*., 2000), postmenopausal women (Yochum *et al*., 2000ab) or elderly men (Hertog *et al*., 1993b, 1995). Differences in flavonoid intake in different countries may partly contribute to differences in coronary heart disease mortality across populations (Hertog *et al*., 1995).

The habitual intake of flavonoids from food sources such as tea may also protect against stroke (Keli *et al*., 1996), or lead to a lower risk of atherosclerosis and coronary heart disease (Weisburger, 1996; Tijburg *et al*., 1997; Duthie *et al*., 2000). This is because tea pigments can reduce blood coagulability, increase fibrinolysis, prevent platelet adhesion and aggregation, and decrease the cholesterol content in aortic walls *in vivo* (Lou *et al*., 1992). Green and black teas are able to protect against nitric oxide toxicity, which may relate the beneficial effects of flavonoid intake to the prevention of coronary heart disease (Paquay *et al*., 2000). In addition, consumption of quercetin may protect against cardiovascular disease (Hollman *et al*., 1997) by curing capillary fragility and inhibiting platelet aggregation (Gaby, 1998).

2.1.4.7 Cancer prevention

Animal studies and epidemiological data indicate that dietary factors play an important role in animal and human health, and in the development of certain diseases, including cancer. Fresh fruits and vegetables are rich in vitamins A, C, and E, β-carotene, flavonoids and other constituents that have been studied as cancer chemopreventive agents (Ho *et al*., 1994). Numerous cell culture and animal models indicate potent anti-carcinogenic activity by certain polyphenols mediated through a range of mechanisms (Duthie *et al*., 2000). Whether an antioxidant is an anti-carcinogenic agent may depend on its efficacy as an oxygen radical inactivator and inhibitor (Kuo, 1997). Location, concentration *in situ*, reaction kinetics (rate constants), energetics (redox potentials) and products (intermediate and final) contribute to the efficacy of an antioxidant.

Diets rich in radical scavengers would reduce the cancer-promoting action of some radicals (Sawa *et al*. 1999). Some flavonoids can modify enzymes and bind carcinogens to DNA, thus exerting an anti-carcinogenic effect (Siess *et al*. 1996). Quercetin is one of the most extensively studied flavonoids that possess this activity. Proanthocyanins may participate in the prevention of cancers by acting as reducing agents (Santos-Buelga and Scalbert, 2000). Studies by Siess *et al*. (1996) and Dragsted *et al*. (1997) showed that a small dose of flavonoids, while ineffective alone, provided an effect when used in combination at the equivalent concentration. Tea is a significant source of flavonoid antioxidants, with a suggested role in prevention of cancer (Oguni *et al*., 1992; Osawa *et al*., 1992; Balentine, 1997). Polyphenols present in green tea show cancer chemopreventive effects against tumor initiation (Gensler *et al*., 1996) and against promotion stages of multistage carcinogenesis in many animal tumor models (Conney *et al*., 1992; Katiyar *et al*., 1992ab; Khan *et al*., 1992; Wang *et al*., 1992; Yang and Wang, 1993; Mukhtar *et al*., 1994; Dreosti *et al*., 1997; Kivits *et al*., 1997; Landau and Yang, 1997). Green tea may protect against cancer by causing cell cycle arrest and inducing apoptosis (Ahmad *et al*., 1997), while black tea can produce an inhibitory effect on tumor promotion (Nakamura *et al*., 1992).

The inhibiting effects of tea components may meaningfully reduce the risk for several important types of cancer in the world (Cheng and Ho, 1988; Weisburger, 1992, 1996; Xu *et al*., 1992ab). Histopathological examination revealed that both green and black teas were able to inhibit tumor cell

proliferation in animal models (Chen, 1992; Chen *et al*., 1999). EGCG, EGC, and ECG inhibited soybean lipoxygenase, a carcinogen and tumor promoter, most effectively at lower doses (Ho *et al*., 1992), while polyphenols from oolong and black teas have displayed strong inhibitory effects in human cancer cells (Pan *et al*., 2000). However, more epidemiological data on the bioavailability, metabolism and intracellular location of polyphenols are required before recommending increasing polyphenol intake for the prevention or treatment of human cancer (Duthie *et al*., 2000).

2.2 Tea

2.2.1 Botany

The tea shrub is a perennial evergreen plant (Bokuchava and Skobeleva, 1969). It is classified in the *Theaceae* family and the *Camellia* species (*Camellia sinensis*, (L) O. Kuntze) (Hara *et al*., 1995; Jones, 1998). *Camellia sinensis* consists mainly of two varieties, *Camellia sinensis* variety *sinensis* and *Camellia sinensis* variety *assamica* (Hara *et al*., 1995; Jones, 1998). In nature, tea trees can attain a height of 20-30 m. Some trees more than 1500 years old are still thriving in their original forests of Yunnan Province in the southwestern China (Hara *et al*., 1995). Tea plants are grown in a wide range of latitudes in the world, from 45 °N (Russia) to 30 °S (South Africa), and longitudes from 150 °E (New Guinea) to 60 °W (Argentina). The plant is kept as an evergreen shrub by pruning. Only the apical bud and the first few leaves are plucked for tea processing. In tropical countries, tea leaves are harvested all year around. In temperate countries, harvesting is seasonal. There are many different kinds of products of different quality arising from different cultivation practices, growing conditions and processing methods (Bhatia and Ullah, 1962; Millin *et al*., 1987; Hara *et al*., 1995).

2.2.2 Types of tea

There are currently six main types of tea produced including black, green, white, yellow, oolong, and reprocessed teas (Hara *et al*., 1995). These types are converted into large range of tea products-there are over 300 kinds of reprocessed teas alone, of which some are the well-known scented teas, such as jasmine, and brick teas (Hara *et al*., 1995). White and yellow teas have been regarded as two subclasses of green tea by Harbowy and Balentine (1997). These two types of tea are different from green tea due to differences in variety, processing, geographical and traditional distributions (Lu, 1987).

There are hundreds of tea cultivars and an individual cultivar may only be suited for processing into one of the six types of tea. It depends not only on the chemical constituents of the tea, but also on its biological characteristics (Lu, 1987). For instances, tea cultivars suitable for processing into "Chunmee" or "Gunpowder" are unsuitable for black tea because of low polyphenol content. These tea cultivars are also unsuitable for making good quality oolong tea because their leaves are usually small, thin and light in colour. Good quality oolong tea usually requires cultivars with thick leaves, medium to dark leaf colour, and medium polyphenol content (Lu, 1987). Excellent reviews on the history, cultivation, processing, classification and/or health benefits of tea are available (Wilson and Clifford, 1992; Hara *et al*., 1995; Clydesdale, 1997; Harbowy and Balentine, 1997).

2.2.3 History of tea

Tea and Chinese history date back to the year 2737 B.C. when the Emperor Shen Nong discovered tea, according to the Chinese medical book, the Ben Chao, written during the Han Dynasty, circa 25 to 221 A.D. (Cheng and Sheng, 1981; Hara *et al*., 1995; Harbowy and Balentine, 1997). An ancient Chinese document published in 347 A.D. states that people in southwest China used teas for paying tribute to the Chinese emperors as early as 1066 B.C. The poetic work "Er Ya", published in 130 B.C., described the ecology of tea trees and tea drinking. In the essay "Tong Yue", written by a country landlord Wang Bao and published in 59 B.C., there is mention of the making and sale of tea (Cheng and Sheng, 1981; Hara *et al*., 1995). It showed that tea was commercially available in the local country or shire market, suggesting that tea processing, marketing and making into a drink became a routine part of life as early as 59 B.C. in southwest China. An excavation of the Western Han Tombs

built in 200 B.C. revealed that tea was one of the items included in the list of burial objects (Hara *et al.*, 1995). Thus, tea has been a commodity for at least 2200 years, not only for everyday life, but also for some ritual purposes. Tea was already a staple commodity, second only to salt, when Lu Yu (737-804 A.D.) published the book "Cha Jing" or "Tea Classics" in 780 A.D. The chapters include the origin, characteristics, names, and qualities of tea; plucking and processing; tea making and drinking; storage and plantations, etc. (Chow and Kramer, 1990). The "Tea Classics" demonstrates that tea was part of routine life of Chinese people at that time.

The probable centre of origin of tea was in southeast China near the source of the Irrawaddy River (Taylor and McDowell, 1993). From there it spread to the southern portion of China, parts of India, Burma, Thailand, Laos and Vietnam. From these main centres in southeast Asia, tea has spread into many tropical and subtropical countries. The spread of tea from China to other parts might have commenced as early as 221 B.C. (Hara *et al.*, 1995), with the migration of minority nationalities from China to Vietnam, Burma (Myanmar), Laos, and Thailand, resulting from incessant internal wars prevailing at that time. Methods of tea processing used in some mountainous areas of these countries today are similar to those employed in ancient China (Zhuang, 1989). During the 5th century, China already had a well-established tea trade with Turkey. Many buyers, especially from Iran, had trading posts in Luo Yang, the capital city of China at that time. From then on, China traded tea with Rome, Arabia, Iran, Afghanistan, Pakistan, Korea, and Japan, some of them on the famed Silk Road. In 805 A.D., tea seeds and cultivation were imported into Japan, and in 828 A.D. into Korea.

It has been suggested that tea was first introduced to Europe by a Portuguese Father in 1560 (Lu, 1987). However, other work suggested that the first tea reached Europe around 1610 on Dutch ships from Java (Chow and Kramer, 1990). The earliest records of tea in the West are contained in the travel notes made by an Arab traveler, Soliman, in 850 and in two books, "Chai Cattai" and "Navigation et Viaggi" by the Venetian writer Giambatista Ramasio in 1559 (Hara *et al.*, 1995). Thus, some literature suggested that tea was first brought to Europe in 1559 (Opie, 1992). The first recorded exports to various countries were to Russia in 1628, England in 1637, Indonesia in 1648, and to America in 1650. The first cultivation introduced to various countries was Indonesia in 1684, India in 1780, Russia in 1833, Sri Lanka in 1839, Malawi in 1875, Iran in 1900, Kenya in 1903, and Turkey and Argentina in 1924 (Chen, 1987). In the late 17th century, tea became a popular beverage served in numerous tea houses in London. From then on, growth of its popularity was rapid and today it is the most widely consumed beverage in the world.

2.2.4 International production and consumption

The principal teas produced and consumed in the world are black and green teas, with small amount of other types (Hara *et al.*, 1995). A summary of world tea production and consumption is shown in Table 2.1. The major producers of tea are India, China, Sri Lanka, and Kenya (International Tea Committee, 2001), while the major consumers are India, China, Turkey, and Japan. The largest per capita consumption is Ireland, followed by Qatar, UK, and Iraq.

During the 1990s, the world production and consumption of tea has increased steadily with occasional fluctuation in some years (Table 2.1). Thus, *Camellia sinensis* has become a very important agricultural and commercial product, with unique horticultural and processing methods (Harbowy and Balentine, 1997).

Table 2.1 World production, exports, imports, supply and absorption of tea (Adapted from International Tea Committee, 2001).

Year	Production	Export	Import[a]	Supply	Absorption	Surplus[b]
	(in thousand metric tonnes)					
1990	2 538	1 134	1 100	1 771	1 711	60
1991	2 581	1 077	1 093	1 764	1 724	40
1992	2 436	1 015	1 039	1 639	1 659	-20
1993	2 550	1 152	1 132	1 834	1 758	76
1994	2 518	1 031	1 051	1 761	1 718	43
1995	2 521	1 087	1 081	1 799	1 760	39
1996	2 643	1 126	1 145	1 887	1 857	30
1997	2 720	1 199	1 196	1 969	1 937	32
1998	2 970	1 291	1 233	2 154	2 012	142
1999	2 877	1 217	1 215	2.016	1 995	21
2000	2 940	1 321	1 227	2.112	2 031	81

[a] Imports adjusted for re-exports
[b] Surplus = Supply – Absorption. Supply indicates the capacity of tea supply to the world demands, whereas absorption indicates the actual absorption of tea from the market, which includes teas for consumption as a beverage and those used for other industrial purposes. A minus value for surplus indicates a shortfall in supply.

Black tea is the dominant tea product in the world. It accounts for approximately 80 % of the world tea production and 90 % of the world tea trade (Table 2.2).

Table 2.2 Proportion of world black tea production and trade (Adapted from International Tea Committee, 2001).

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
A %[1]	79.64	79.16	76.91	76.96	77.68	77.41	77.72	77.39	78.14	76.51
B %[2]	91.61	90.98	90.05	90.33	90.10	92.44	93.50	91.88	89.91	88.13

[1] A % = World production of black tea / World production of all teas
[2] B % = Total black tea / all teas traded in the world market

2.2.5 Australian tea

2.2.5.1 Botanical variety, use and history

The commercial variety of tea plant currently grown in Australia is *Camellia sinensis* var. a*ssamica* (Wood *et al*., 1994). This variety is a tree that can grow up to 20-30 m high in nature (Hara *et al*., 1995), but is usually pruned in hedges of about 1.5 m for commercial production in Australia. Harvesting usually involves removing an apical bud and the first few leaves using a mechanical harvester. The young shoots are usually withered (not in the Glen Allyn Tea Estates factory), processed, fermented, dried, and sorted to make black tea that is used as a popular inexpensive drink. Green tea, which is popular in Japan and China, represents only a small segment of the Australian tea market (Wood *et al*., 1994). Madura Tea Estates in north New South Wales has produced both green and black teas, whereas Nerada Tea Pty. Ltd. in North Queensland currently only produces black tea.

Sir Joseph Banks established a few tea bushes in Melbourne's Botanical Gardens in 1824, but Herbert Frederick Cutten planted the first tea on a large scale and is acknowledged as the father of the tea industry in Australia (Ford, 1994). The tea was planted along with coffee, fruits and citrus in 1884 by Cutten's family at Bingil Bay, 30 km south of Innisfail, in North Queensland (Taylor, 1982). The seeds were from Sri Lanka. However, the tea plantation was not successful and was destroyed by the 1890, 1911, and 1918 cyclones (Taylor, 1982; Ford, 1994). Some of the plants were able to survive and thrive in the dense rainforest. In 1980, the tea bushes covering about 1 ha were discovered growing wild in the rainforest near the original plantation site (Ford, 1994).

Interest in tea was reawakened by the Bureau of Tropical Agriculture when half an acre (ca 0.2 ha) of tea was established at the South Johnstone Research Station, South Johnstone, North Queensland in 1936 from seed originating from the Botanical Gardens at Kew, London, UK where it had been imported from Assam (Graham, 1956; Johns, 1998). This initial research was to examine the suitability of soil and climate for tea growth. Mr. Graham began trials in 1953 on this tea and his experiments paved the way for a critical appraisal of tea growing on a commercial scale in North Queensland. Commercial tea growing began in Johnstone, North Queensland, in the late 1950s (Hobman, 1973), when the annual value of tea imports exceeded £ 15 000 000 (Graham, 1956). Experiments done at the research station demonstrated that tea could be harvested mechanically and that this method of harvesting did not greatly impair quality (Graham, 1957).

In the late 1950s, Dr Maruff began planting tea in a small way at Malanda in North Queensland (Hobman and Nimmo, 1972). Forgotten for a number of years, tea was replanted in the 1960s by Dr Maruff and the plantation was expanded to 130 ha, with 32 ha of mature tea in 1970 (Taylor, 1982). The first tea produced on a commercial scale in Australia was made in 1970 (Hobman and Nimmo, 1972), when Nerada Tea Estates Pty. Ltd. was formed (Taylor, 1982). The first tea factory was constructed in Australia at Malanda in 1971 and started operating in 1972 (Taylor, 1982). However, Nerada Tea Estates Pty. Ltd. experienced serious financial problems stemming from several teething problems associated with harvester and the factory. The problems were exacerbated by the decision to plant more tea in 1970 and 1971 (Wood *et al*., 1994). As the result, the assets of Nerada Tea Estates Pty. Ltd. were sold to Tea Estates of Australia Limited in 1973 and the latter thus benefited from some of the errors of Nerada Tea Estates Pty. Ltd., however, the industry remained marginal. In 1974, a packaging plant was established at Innisfail, with government assistance, thus enabling Queensland tea to be retailed rather than sold into wholesale markets for blending. The tea was marketed under the brand "Nerada" and gradually made available to wider markets as production increased. Attention was also given to improving the quality of the tea without planting any new tea until 1977.

By the early 1980s, the Queensland tea industry consisted of one major plantation owned by Tea Estates of Australia Limited and five smaller outgrower plantations. In 1982, Nerada tea was selling in all mainland states of Australia (Taylor, 1982) and production reached 450 t (Johns, 1998). In 1991, Tea Estates of Australia Limited replaced the original factory with a highly automated factory at Malanda in North Queensland to reduce the maintenance and labour costs, to increase processing capacity, and to provide economies of scale. In an attempt to capture a market advantage, this

company has minimised the blending with imported teas for the production of Nerada black tea, which is advertised as the only 100 % Australian tea on the market (Wood *et al*., 1994). In October 1998, Tea Estates of Australia Limited was renamed as Nerada Tea Pty. Ltd. consisting of Glen Allyn Tea Estates located at Malanda in North Queensland and a packaging factory in Brisbane, the capital city of Queensland, Australia (Benson, 2001).

2.2.5.2 Expansion, area, production and imports

Tea was planted in other areas of Australia from 1977. Michael Grant Cook commenced a tea plantation near Condong in north New South Wales, called "Madura Tea" . In 1982, "Madura Tea" was produced for the first time in small quantities (Johns, 1998).

Tea plantings in Australia expanded rapidly in the 1980s in response to a rapid short-term increase in world tea prices. The planting area arose from 100 ha in 1982 to 775 ha in 1991, while the annual gross value of black tea production in Australia reached ca 4 million dollars (Wood *et al*., 1994). Since then, the area under tea has fluctuated, reaching 900 ha in 1994 (with 700 ha in North Queensland), and dropping to 750 ha in 1998 (Chudleigh, 1999). Currently, in New South Wales, Madura grows 25 ha of tea, and Northern Rivers also in north New South Wales grows 106 ha. In North Queensland, Glen Allyn Tea Estates of Nerada Tea Pty. Ltd. grows around 230 ha of tea, while the individual growers nearby, which are called outgrowers, grow ca 300-400 ha of tea. The green leaves harvested from the outgrowers are sold to the factory of Glen Allyn Tea Estates for processing. The size of farms ranges 15-40 ha and the annual production of tea by individual growers has not changed significantly over recent years. The Daintree Tea Company at Mossman in far North Queensland is a family-owned business that manages about 40 ha of tea, of which the company owns 20 ha. This tea was sold as green leaf to Glen Allyn Tea Estates before 1994, but has since been processed on a small scale in an on-site factory, where it is packaged and retailed as "Johnstone Valley Tea", which was changed to "Daintree Tea" in 1999 (Nicolas, 2001).

Harvesting of green leaf occurs about every three weeks in North Queensland, except during winter when it occurs about every four weeks (Chudleigh, 1999). It is believed that there is no reduction in the quality of tea produced over winter months in this region. In northern New South Wales, there is a 4-6 month period over the winter months where the tea bushes are not harvested. Here, tea is harvested every three weeks at the beginning of the harvest season, and then reduces to every 7-10 days during the peak season, January to March (Chudleigh, 1999).

Wilson and Steel (1988) classified the land suitability for tea growing in Queensland into: suitable, 63 169 ha; suitable with negligible to minor limitations, 39 620 ha; and suitable with moderate limitations, 23 549 ha. Chudleigh (1999) suggested that the north-eastern seaboard of Australia is perceived as suitable for tea production.

The quantity of black tea consumed in Australia is estimated to be about 16 000 t annually (Johns, 1998; Chudleigh, 1999). The quantity of total consumption has declined 13 % from 1988/1989 to 1993/1994, while consumption has fallen from 1.1 kg/head to 1.0 kg/head of population in that time (Chudleigh, 1999). Tea production in Australia has increased nearly twice, while tea imports have decreased by ca 2 000 t in past 10 years (Table 2.3).

All tea grown in Australia was established from seed, which is recognised as being marginally economic. Consequently, growth of the Australian tea industry has been slow. There is a potential to improve the average yield through, for example, fertiliser application and the use of cuttings or clones (Chudleigh, 1999). Practice in the other tea producing countries has shown that although the establishment costs are much higher for clonal tea, the returns are also higher (Hobman and Nimmo, 1972). Information about fertiliser application is unavailable for Australian tea growers and this is considered to be a significant gap in the knowledge for efficient tea production (Chudleigh, 1999). However, the tea industry in North Queensland appears to be a viable and profitable venture.

Table 2.3 Tea production and imports for consumption in Australia (Adapted from International Tea Committee, 2001).

Year	Production (t)[a]	Import (t)[a]
1990	773	16 459
1991	720	17 242
1992	525	16 075
1993	789	17 291
1994	1 340	17 255
1995	699	16 515
1996	1 142	17 639
1997	1 200	15 551
1998	1 250	16 662
1999	1 300	14 420

[a] Metric tonnes of made tea

2.2.6 Black tea processing

2.2.6.1 Harvesting of the leaves

According to Millin and Rustige (1967), the composition of tea shoots (percent of dry mass) is 30 % polyphenols, 4 % amino acids, 4 % caffeine, 4 % sugars, 0.5 % organic acids, 15 % protein, 13 % polysaccharides, 7 % cellulose, 6 % lignin, 3 % lipids, 0.5 % pigments, 5 % ash, and 0.01–0.02 % volatiles, as well as about 5 % phenolic acids and 3-4 % flavandiols and flavonol glycosides. Tea shoots are harvested manually or mechanically. Manual plucking of the terminal bud and two adjoining leaves yields the finest quality of tea, but the high cost of labour in some countries makes mechanical harvesting an economic necessity. The differences in chemical composition of young shoots result in corresponding quality differences in the processed tea (Nakagawa and Torri, 1964; Hara *et al*., 1995). Intervals of tea plucking affect the final quality of tea (Baruah *et al*., 1986). Coarse plucking or mechanical harvesting usually takes more mature leaves known to be of poorer quality. This is because as the leaf ages, the content of total catechins and the gallates decreases, with the amount of EGCG and ECG falling sharply, and that of EGC and EC rising. In addition, the activity of polyphenol oxidase, an enzyme of prime importance in black tea processing, may decrease by about 70 % during maturation of tea leaves (Takeo and Baker, 1973). Thus, good quality black tea is produced from tea leaves from high polyphenols and caffeine contents, a relatively low protein content, and an adequate amount of polyphenol oxidase (Bhatia, 1964).

Table 2.4 shows the variation in distribution of the flavanols and caffeine in different parts of the tea shoot. Among individual bushes (clones), the distribution pattern of polyphenols varies a great deal, whereas in a clone the patterns appear to be more or less fixed (Bhatia, 1963). Despite marked changes in the quantities of polyphenols, the relative proportions of certain important polyphenols, such as EGCG, ECG and EGC do not appear to deviate a great deal from their mean values (Bhatia, 1963). Thus, maintaining the plucking criteria for a particular type of tea would provide a consistent basis for making good quality tea.

Studies in central Africa showed that the level of flavanols in fresh apical shoots of tea is the highest during the cold season (Hilton *et al*., 1973). Shoots plucked during slow growth conditions contained a higher proportion of simple catechins to catechin gallates, with EGC being most significantly affected. There is a direct relationship between the level of EGC in tea shoots grown in central Africa and the total level of theaflavins that can be produced from those shoots during processing (Hilton, 1972). Thus, the variation of EGC with season may be regarded as a chemical basis for seasonal variations in tea quality in central Africa. In contrast, in the Northern Hemisphere, the total flavanols

Table 2.4 Distribution of flavanols and caffeine in tea shoots (Adapted from Bhatia, 1963).

Leaf component	Content (%, dry basis)	
	Flavanols	**Caffeine**
Bud	26.5	4.7
First leaf	25.9	4.2
Second leaf	20.7	3.5
Third leaf	17.1	2.9
Upper stem	11.5	2.5
Lower stem	5.3	1.4

Is greatest during the height of the growing season and least at the end of the season (winter). The level of EGCG is about three times of the level of EGC in the green leaves (Chu, 1997). Further, EGCG has been found to be significantly related to the formation of theaflavins (Obanda *et al*., 1997). Thus, EGCG has also been regarded as a critical compound for producing good quality black tea.

After harvesting, the leaves must be transported with great care to the factory as soon as possible to avoid any crushing or other damage. Rough handling may nullify the efforts of careful plucking. This is because any leaf damage will initiate unexpected chemical and biochemical reactions at this early stage (Hara *et al*., 1995). In addition, damage or injury to the tissues of the tea leaf may inactivate the coenzymes involved in carbohydrate oxidation (Deb and Roberts, 1940).

2.2.6.2 Withering and preconditioning

The primary role of withering is to reduce the moisture content of the fresh leaves, making them amenable to subsequent processing steps. In addition, diverse and important biochemical changes occur during withering (Hara *et al*., 1995). Traditionally, the flushes (young shoots) were spread on beds of bamboo or nylon netting with a ceiling fan to accelerate removal of moisture from the leaves (Hara *et al*., 1995). More recent methods involve loading the leaves into troughs fitted with a powerful exhaust fan underneath to draw the moisture from the tea leaves and to carry the humid air out of the workshop. Alternatively, hot air can be blown from underneath the trough, through a layer (10-20 cm in thickness) of tea leaves, to remove the moisture.

The level of total solids reduces slightly during the withering because of the respiration of green leaves (Wood *et al*., 1964b). Some important compounds are developed during withering and these compounds may ultimately influence the flavour, aroma and/or the character of the tea brew (Opie, 1992; Hara *et al*., 1995). Theaflavins are known to be important to black tea quality and are usually formed during the processing steps following withering, but withering conditions may affect their formation, with humid withering being favoured (Obanda *et al*., 1997b). Chemical withering refers to the breakdown of proteins into amino acids, and other chemical changes that take place during this withering period. These changes are not influenced by the leaf losing moisture and the progress of withering (Bhatia, 1964). Short chemical withering periods favour the formation of theaflavins, thus increasing the brightness of black tea liquors (Obanda and Owuor, 1992). Combined physical and chemical withering produces the highest quality black tea because it results in higher theaflavin content (Owuor *et al*., 1986b). In a recent study, when the theaflavins, thearubigins, total colour and brightness were examined, it was found that the quality of Indian orthodox black tea increased with the extent of withering, which in turn was increased significantly dependent upon the prevailing dry and low humidity weather conditions (Sud and Baru, 2000).

The biochemical changes taking place during withering of tea leaves involve changes to proteins, caffeine, sugars, organic acids, polyphenol oxidase activity, chlorophyll, minerals, volatile components, and permeability of cell membranes (Dev Choudhury and Bajaj, 1980). The level of

polyphenol oxidase activity in the plucked tea flush (young shoot) has been shown to fluctuate during the first 24 h of storage from more than double the initial level (at harvest) to less than the initial level (Sanderson, 1964). If unwithered fresh leaf is used for the processing of black tea, the product will have a higher proportion of theaflavins and lower thearubigins, resulting in bright, brisk, but thin liquors (Ullah *et al*., 1984). In addition, the withering process is important for the formation of volatile compounds during black tea fermentation (Takeo, 1984). Thus, appropriate withering may result in good quality teas, while insufficient or overwithered leaf may result in poor liquoring teas.

Preconditioning involves gentle rolling action is to breakdown some of the cell walls separating the individual cells in the leaf epidermis . Rotorvanes are usually used for this practice. The rationale for this process is to mix the polyphenol oxidase with its substrate, the polyphenols, and to reduce the size of the tea leaves for further processing. The polyphenols are located in the palisade layer of the tea leaves, whereas the enzyme is located in the epidermis (Hara *et al*., 1995). After preconditoning, the leaves are ready for the following rolling step.

2.2.6.3 Rolling

During rolling of the tea leaves, the leaves are macerated and the cell structures are disrupted, which brings various enzymes into intimate contact with their substrates, the polyphenols. Rolling of tea leaves may be accomplished by orthodox rollers (e. g. rotorvanes), Lawrie Tea Processor (LTP), or crush-tear-curl (CTC) machines. Orthodox rolling is widely used in Sri Lanka, the world's major producer of orthodox teas (Hara *et al*., 1995). The orthodox process is the method is of choice for maximum realisation of the innate potential quality of tea leaves, but it is not amenable to batch processing. Thus, it has been gradually superseded by continuous leaf processing methods using CTC or LTP.

The major producers of CTC teas are India and Kenya. In the processing, after preconditioning, tea leaves are fed between a pair of stainless steel cutters with etched surfaces, one rotating clockwise, the other anti-clockwise at different speeds. LTP processing is popular in Central Africa and many other tea producing countries (Opie, 1992). The LTP is a pulverising machine containing a shaft that has many sets of beaters and knives to beat and cut the tea leaves. The chemical and biochemical reactions initiated in the leaves during preconditioning proceed at an accelerated rate during and after the rolling, before the leaves progress to the next stage of black tea processing, referred to as fermentation (Hara *et al*., 1995).

The main oxidation products of the catechins and their gallates are the theaflavins and thearubigins. The contents of the theaflavins and thearubigins formed in black tea depend not only on the flavanol and protein contents, and the oxidase activity of the green leaf, but also on the resistance of the leaf to mechanical damage (Wood and Roberts, 1964) and the processing method (Owuor *et al*., 1986c). This resistance has less influence in CTC processing than in orthodox processing (Wood and Roberts, 1964). A high flavour index and high theaflavin contents are associated with general good quality of teas. There are large differences between theaflavin and thearubigin contents of orthodox and CTC teas. In general, orthodox teas have low theaflavin contents while CTC teas have high contents of theaflavins and thearubigins (Owuor *et al*., 1986c). The differences in the theaflavin and thearubigin contents due to these two processing conditions are less for the thearubigins than for the theaflavins. The same differences are produced by the geographical location of tea production (Owuor *et al*., 1986c). Variable climatic factors on the tea farm and the growth rates of tea bushes have also been found to affect the amounts of theaflavins in the resulting black tea (Owuor *et al*., 1986c).

Related to the effect on flavonoids is the difference in the content of volatile compounds produced by each individual processing method (Takeo and Mahanta, 1983a). Thus, the observation that CTC tea was less fragrant than other teas could be explained by the lower amount of volatile constituents with floral notes, such as linalool and its oxides, compared with the orthodox black teas (Takeo and Mahanta, 1983b).

2.2.6.4 *Fermentation*

The principal reaction in fermentation is the oxidation of catechins and catechin gallates by the enzyme polyphenol oxidase, together with other enzymatic (e.g. peroxidase) and nonenzymatic reactions to form the unique character of black tea (Hara *et al*., 1995). There are two important reactions during this stage. First, there is the development of colour, strength and quality of tea brews from the production of non-volatile compounds through the enzymatic oxidation of catechins and their gallates. Second, there is the production of volatile compounds responsible for the characteristic aroma of black tea. Thus, fermentation is the critical step in black tea processing, and the chemical and biochemical reactions are the most complicated ones in tea processing.

According to Roberts (1957ab), Roberts and Russell (1957) and Roberts *et al*. (1957), the mechanism of fermentation is the oxidation of polyphenols catalysed by polyphenol oxidase. This enzyme of tea leaf catalyses the oxidation of catechins and chlorogenic acid. The order of oxidation of individual polyphenols is dependent on their redox potentials. In a tea leaf, the order of redox values is chlorogenic acid > catechins > gallocatechins. For the oxidation of a mixture of substances, those compounds with the lowest redox values are likely to be oxidised the first. Gallic acid, theogallin, and flavone glycosides are rarely oxidised unless a suitable carrier is present (Roberts and Russell, 1957). In a black tea extract, if the EGC and its galloyl esters have not been oxidised completely during the fermentation, the catechins and chlorogenic acid will not undergo permanent change (Roberts *et al*., 1957. Further, apart from slight oxidation of gallic acid, it is unlikely that any coupled oxidations will occur while any of the gallocatechins remain unoxidised. Therefore, the main products of oxidation in black tea are probably derived from two substances, EGC and its gallate (Roberts, 1957ab).

There are large differences in the rates and extent of enzymatic oxidations of EGCG and ECG during fermentation (Bhatia and Ullah, 1962). EGC and its gallate (EGCG) are consumed at faster rates compared to ECG during the oxidative phase of tea processing (Bhatia and Ullah, 1961; Bhatia, 1964), whereas EGC content was found to be the limiting factor in theaflavin production (Hilton, 1972; Hilton and Palmer-Jones, 1973). Degallation and epimerisation of EGCG and ECG were observed when incubated with polyphenol oxidase (Coggon *et al*., 1973). Both of these flavonoids were epimerised at the C_2 position. Thus, the polyphenol oxidase mediates oxidation of the *ortho*-diphenolic group on the B ring in tea flavanols during fermentation, and induces electron displacements over the flavanol molecules, which produce some oxidative changes remote from the B ring (Coggon *et al*., 1973). Flavandiols are a monomeric complex of flavanols at a labile state between flavanols and their oxidation products. It has been proved that flavandiols are oxidised very rapidly and disappear completely, while the flavonols were relatively unaffected (Millin, 1987). During fermentation, thearubigins are formed very slowly at first, but increase after the theaflavins reach their peak levels (Millin, 1987).

The volatile flavour compounds (VFC) are thought to be important to the quality of black tea. Owuor *et al*. (1986bc, 1992ab) classified the volatile flavour compounds into two groups, referred to as VFC I and VFC II. The former has a "grassy-type" flavour, while the latter has a "flowery-type" flavour during the quality formation of black tea. Thus, a flavour index, the ratio of VFC II to VFC I, was suggested to describe the flavour of black tea, although the levels of these two groups of VFC also contribute (Owuor *et al*., 1986bc, 1992ab; Owuor and Orchard, 1991). The larger the flavour index is, the more superior the tea flavour will be. During black tea fermentation, the flavour index value and the VFC II level decrease progressively, while the VFC I level increases (Owuor *et al*., 1994).

The levels of theaflavins in black tea increase quadratically with fermentation time, while the rate of production of individual theaflavins vary between clones as fermentation progresses (Owuor *et al*., 1994). Studies on clonal tea revealed that long fermentation under warm conditions enhanced the formation of these flavonoids leading to increased brightness and total colour of black tea liquors (Obanda and Owuor, 1993). However, fermentation under warm conditions for too long usually results in more intensely coloured black teas with high contents of thearubigins and low levels of theaflavins (Owuor and Obanda, 2001). The estimation of theaflavins gives useful information

regarding the approximate optimum fermentation time for a particular leaf processed in a particular way (Bhatia, 1960). In Central Africa, it is agreed that the theaflavin content is the best objective indicator of the quality and value of plain teas, with the greater the theaflavin content, the higher the market price (Cloughley, 1979). Thus, the accurate and objective determination of optimum fermentation time in the factory assumes great economic importance (Cloughley, 1980a).

Four methods have been used to determine the optimum fermentation time. The first is to measure the reduction of EGCG, as used in India (Bhatia, 1960). The second is to detect the formation of theaflavins, called the in-line theaflavin method, used in Malawi (Cloughley, 1980a). The third is a sensory test by tea tasters that is used in Kenya (Owuor, 1984). The fourth is to measure the theaflavin content in made tea, a method also used in Kenya (Owuor, 1984). Comparing the methods used in Malawi and Kenya, the optimum fermentation time assessed by the in-line theaflavin method peaked ahead of the optimum fermentation time assessed by the tea taster or the made tea theaflavin method (Owuor, 1984). Thus, the made tea theaflavin method is preferred in Kenyan tea processing in the absence of tasters (Owuor and Reeves, 1986). Moreover, in Sri Lanka, the optimum fermentation time was measured by monitoring the absorption of diluted tea liquor at 380 nm, following the development of theaflavins and thearubigins (Roberts and Chandradasa, 1982). However, Owuor (1987a) suggested that photometric evaluation did not provide precise information on the optimum fermentation time, and that sensory assessment might still be the first choice for the control of fermentation in Kenyan tea processing.

The rate of fermentation is profoundly influenced by genetic constitution, seasonal and climatic factors, agronomic and management practices, and systems of processing (Cloughley, 1980a). High temperatures usually increase the rate of fermentation. Temperature varies throughout the day and this variation is more obvious in the summer than in the winter. Thus, some attempt is usually made to alter the fermentation time according to the temperature change during processing (Cloughley, 1980a). In addition, activities of both polyphenol oxidase and peroxidase declined as a function of fermentation time and temperature (Cloughley, 1980b). Adjusting the pH of the fermenting tea from 5.5 to 4.5-4.8 resulted in an increase in theaflavin levels and a reduction in thearubigin levels, which may be due to lower turnover of formed theaflavins to thearubigins (Subramanian *et al*., 1999). Industrial-scale acidification is practicable and the market price for such treated teas has been reported to be higher than the control consignments on the London auction floors (Hilton and Ellis, 1972; Hilton and Palmer-Jones, 1975; Cloughley and Ellis, 1980).

Theaflavins are stable in the presence of air and tea polyphenol oxidase. However, these flavonoids are decomposed rapidly to yield polymeric materials under the action of peroxidase derived from tea (Dix *et al*., 1981). Thus, it has been postulated that the nature and distribution of the pigments formed during the fermentation of tea are governed in part by the relative actions of polyphenol oxidase and peroxidase. These actions were, in turn, influenced by the availability of oxygen and the action of catalase (Dix *et al*., 1981). A significant decrease in the levels of all flavonol glycosides occurs in the presence of peroxidase, whilst a decrease in the levels of myricetin glycoside only occurs in the presence of polyphenol oxidase (Finger, 1994).

In an *in vitro* fermentation system, Robertson (1983a) found that low oxygen concentration inhibited the formation of theaflavins and promoted thearubigin production. Increases in the total flavanol (catechins and catechin gallates) concentrations have been found to inhibit the polyphenol oxidase activity, which depress the formation of theaflavins (Robertson 1983b). Among the individual catechins and catechin gallates, the effects on the oxidation enzyme are different. ECG and EGCG are responsible for enzyme inhibition, whereas EC and EGC are not (Robertson 1983b). These results indicate that a high ratio of simple catechins to catechin gallates in the leaf will facilitate a high ratio of theaflavins to thearubigins in the resulting black tea.

2.2.6.5 Drying, grading, blending and storage

Drying

Drying or firing of fermented tea leaves (called dhool) is primarily intended to cause cessation of enzyme activity and reduce the moisture content to about 3 % of the dry mass so that the tea can be stored (Hara *et al*., 1995). Endless chain pressure driers have been used by the industry for many years. Fluidised bed drying has been recently applied to the firing of tea, using a temperature of 127 °C for 20 min, with discharge temperatures between 88-93 °C (Hara *et al*., 1995). Changes other than removal of moisture that occur during drying include a significant loss of volatile compounds, an increase in the levels of amino acids, the binding of polyphenols to other tea components, and an increase in carboxylic acids, and Maillard reactions. Firing at an elevated temperature is necessary for the development of the taste, colour, and aroma of black tea (Hara *et al*., 1995).

Grading

This is an important stage for the marketing of tea, ensuring the correct particle size, shape, and cleanliness. The major tea grades usually fall into four groups: leaves, broken, fannings and dust. In the tea trade, commonly found grades include broken orange pekoe (BOP), flowery broken orange pekoe (FBOP), and broken orange pekoe fannings (BOPF) (Hara *et al*., 1995).

Blending

In most cases, tea is traded in bulk by tea traders or blenders in the consuming countries. The blenders then blend the tea to suit their market demand. Once bought by the blender, tea loses all identity as to its original source (Othieno and Owuor, 1984). The quality of the blend is dictated by two main factors: the market demand that prevails and the profit that the blender can maximise. The five parameters, colour, strength, briskness, flavour and quality are the best and simplest descriptors used to describe the sensory characters (Roberts, 1962).

The non-volatiles most highly correlated with tea quality are the complex pigmented polyphenols, theaflavins and thearubigins (Roberts, 1958ab; Roberts and Smith 1961, 1963; Wood and Roberts, 1964). Blending is used to bring some grades of teas with some quality variations into relatively consistent products, both in the sensory and physical properties of the teas (Othieno and Owuor, 1984; Hara *et al*., 1995).

Storage

Thearubigins have been classified into two groups, group I thearubigins referred to as SI TR and the more polymerised group II thearubigins referred to as SII TR (Roberts, 1962). The SI TR has been regarded as a contributor to good quality, while the SII TR may influence the brightness of tea liquor (Roberts, 1958ab, 1962; Roberts and Smith 1961, 1963; Wood and Roberts, 1964). During storage, levels of the SII TR fraction and caffeine increase in tea, while flavanols and other soluble solids decrease (Cloughley, 1981a). The loss of theaflavins during storage is the major factor responsible for the deterioration in the quality of black tea. Oxidative enzymatic activity is retained in the processed tea: peroxidase activity is regenerated continuously throughout storage, but residual polyphenol oxidase activity decreases as storage progresses (Cloughley, 1981a).

The rate of theaflavin deterioration is reduced when tea is stored under conditions of low temperature, low moisture and low oxygen availability (Cloughley, 1981b). Teas processed and stored during the hot and humid main production season have been found to deteriorate more rapidly with respect to theaflavin content (Cloughley, 1981b). Further, introduction of variations into each phase of processing alters the storage behaviour of teas. Teas produced from different clones vary significantly in the rate and extent of theaflavin loss during storage (Cloughley, 1981c). In addition, studies in India have shown that the principal changes undergone by tea polyphenols during storage are due to the formation of compounds related to the thearubigins (Roberts *et al*., 1981). All the post-processing

chemical changes in stored black teas have been shown to be independent of tea grade or type of packaging (Obanda and Owuor, 1995).

2.2.7 Polyphenols in tea

2.2.7.1 Polyphenols in green leaf and green tea

The production of polyphenolic constituents in the tea plant is assumed to be a means of chemical defense against insects, birds, and animals, which would consume the plant as food (Beart *et al*., 1985). Green tea is made without enzymatic oxidation of polyphenols, as polyphenol oxidase is inactivated by heat during the early stages of green tea processing (Hara *et al*., 1995). Thus, the polyphenols present in green tea should be the same as those found in fresh tea leaves. In a broad sense, green tea polyphenols consist of simple and complex compounds, the large majority of which are the flavonoid monomers catechins, catechin gallates and flavonols. Catechins and their gallates are members of a more general class of flavonoid, the flavan-3-ols or flavanols. The *epi*-isomers of the catechins and catechin gallates are the principal components found in tea (Figure 2.3).

The tea catechins, a term commonly used to refer to both catechins and catechin gallates, make up as much as 30 % (w/w) of the dry mass of tea (Baruah *et al*., 1986; Harbowy and Balentine, 1997). In a more specific sense, catechins include EC, C, EGC, and GC, while catechin gallates include ECG, CG, EGCG, and GCG (Forrest, and Bendall, 1969; Hilton *et al*., 1973; Hara *et al*., 1995). Two minor catechin digallates, epicatechin digallate (ECDG) and epigallocatechin digallate (EGCDG) (Coxon *et al*., 1972; Nonaka *et al*., 1983; Hashimoto *et al*., 1987) have also been considered as catechin gallates (Opie, 1992). The four most common catechins and catechin gallates are EGCG, EGC, ECG, and EC. Other catechins such as C and GC are present in smaller quantities in tea, whereas the gallates GCG and CG found in tea may be products of racemisation and not "native" to the tea plant (Roberts, 1962). Figure 2.3 shows the structures of the catechins and catechin gallates observed in tea. The methyl esters of ECG and EGCG were also recently identified in tea by Zeeb *et al*. (2000).

In an early study, the chemical constituents of polyphenols in tea shoot were shown to be (% of total polyphenols): C 0.4; EC 1.3; GC 2.0; EGC 12.0; ECG 18.1; EGCG 58.1; while the other polyphenols were 6.67 % (Bokuchava and Skobeleva, 1969). EGCG is the major constituent in all parts of the tea shoot. Graham (1992) showed that the principal catechins and catechin gallates in fresh leaf were in agreement with Hilton (1973) (% of dry tea): C 1-2, EC 1-3, ECG 3-6, EGC 3-6 and EGCG 7-13. In another study, the six catechins and catechin gallates, C, GC, EC, ECG, EGC, and EGCG, were found to represent about 80 % of the total polyphenols in green tea and in fresh tea leaves (Opie *et al*., 1988).

Seasonal variations in the content of polyphenols in some jats (cultivars) and clones grown in north eastern India have been observed (Bhatia and Ullah, 1968, Singh *et al*., 1999). Towards the end of a plucking season, the total content of polyphenols and the oxidase activity of Assam tea leaf tends to fall (Wood *et al*., 1964). Changes in the qualitative and quantitative composition of tea leaf catechins and catechin gallates resulting from the changes in the plucking seasons may result from the biochemical activity during the growth of tea plant. An increase in the content of catechins and catechin gallates in raw tea leaf in summer months is due mostly to an active synthesis of EGCG and ECG (Bokuchava and Skobeleva, 1969). This finding reveals that the accumulation of catechin gallates during summer months may result from the irradiation effects of sunlight.

The precursors of catechins and the gallates, along with theogallin, are presumably present in tea seeds (Bhatia and Ullah, 1962). However, Forrest and Bendall (1969) showed that the only catechins and their gallates that are detectable in the embryo are C and EC. Bhatia and Ullah (1962) suggested that the ratio of EGCG/ECG could be used to monitor the development of new organs of the seedling, increasing from 1.7 for epicotyl, to 2.3 for cataphylls and to 6.3-11.7 for the foliage leaf. The relative constancy of this ratio under diverse cultural conditions, and its sole dependence on the nature and origin of the leaf suggested that this ratio could be genetically controlled (Table 2.5).

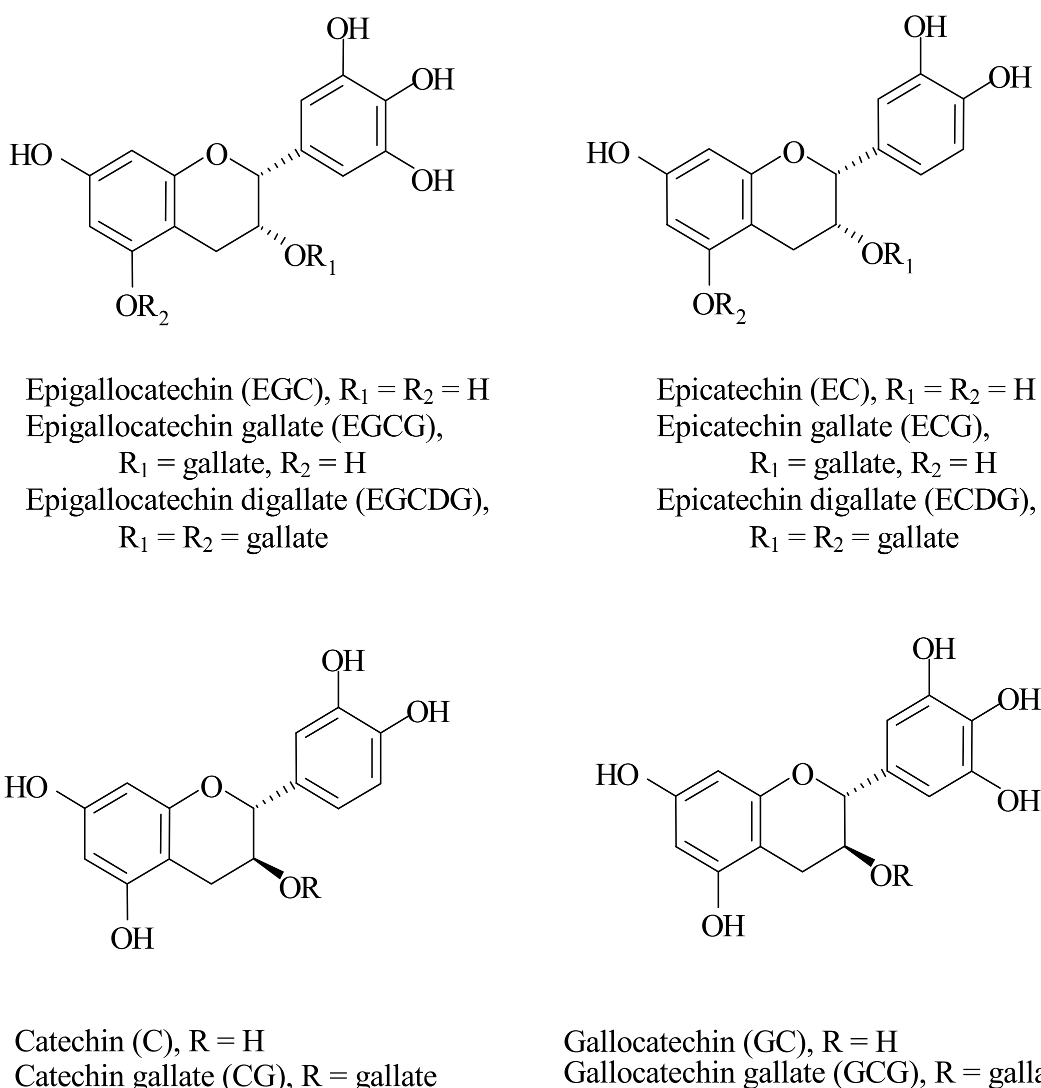


Figure 2.3 Structures of tea catechins (Adapted from Harbowy and Balentine, 1997).

Table 2.5 Contents of characteristic constituents in leaves of various *Camellia* species (Adapted from Chu, 1997) (g/100g dry basis).

Species	Catechins					Theanine	Caffeine
	C	EC	EGC	ECG	EGCG		
Var. *sinensis*[1]	0.07	1.13	2.38	1.35	8.59	1.21	2.78
Var. *assamica*[1]	0.02	1.44	0.35	3.35	12.10	1.43	2.44
C. taliensis	trace	0.58	0.80	1.90	6.84	0.27	2.54
C. irrawadiensis	0.03	0.72	0.12	0.67	0.21	0.21	0.00

[1] Variety belongs to *Camellia sinensis*

Studies on the properties of the catechins and their gallates of green tea revealed that these compounds show a strong UV light absorption (Bradfield *et al*. 1947; Bradfield and Penny, 1948). The range of the absorption is 266-280 nm (ethanol). The absorption maxima for catechins, catechin gallates and simple phenolic acids in alcohol are: catechol 278 nm; pyrogallol 266 nm; gallic acid 272 nm; EC 280

nm; GC 271 nm; ECG 280 nm; GCG 275 nm and 279.5 nm. Coxon *et al*. (1972) showed a similar results with EC 280 nm, ECG 279 nm, ECDG, 282, EGC 271 nm, EGCG 275 nm and EGCDG 283 nm.

The flavonols (e.g. kaempferol, quercetin, and myricetin) and their glycosides (Figure 2.4) have been found in tea as trace but significant constituents (Engelhardt *et al*., 1992, 1993). The total number of flavonoid glycosides isolated from tea is 23 (Nakabayashi, 1953; Oshima and Nakatani, 1953), whereas 19 glycosides have been found in Japanese green tea (Sakamoto, 1967, 1969, 1970). Flavonoid glycosides in tea contain sugars such as glucose, rhamnose, galactose, arabinose, are rutinose (Bokuchava and Skobeleva, 1969). The di- or tri-glycosides of flavonols usually contain a di-saccharide or tri-saccharide moiety (Finger *et al*., 1991ab, 1992; Engelhardt *et al*., 1992). A recent study showed that tea flavonols made up 2-3 % of the water-soluble solids from tea leaves (Wang and Helliwell, 2001). The particle size of ground tea leaves significantly influenced the yield of flavonols. HPLC analysis of flavonoid glycosides in tea are available (McDowell *et al*., 1990; Finger *et al*., 1991ab, 1992; Engelhardt *et al*., 1992, 1993). Thus, the levels and types of flavonoid glycosides are avriable in tea, dependent on the types of tea and the analytical method.

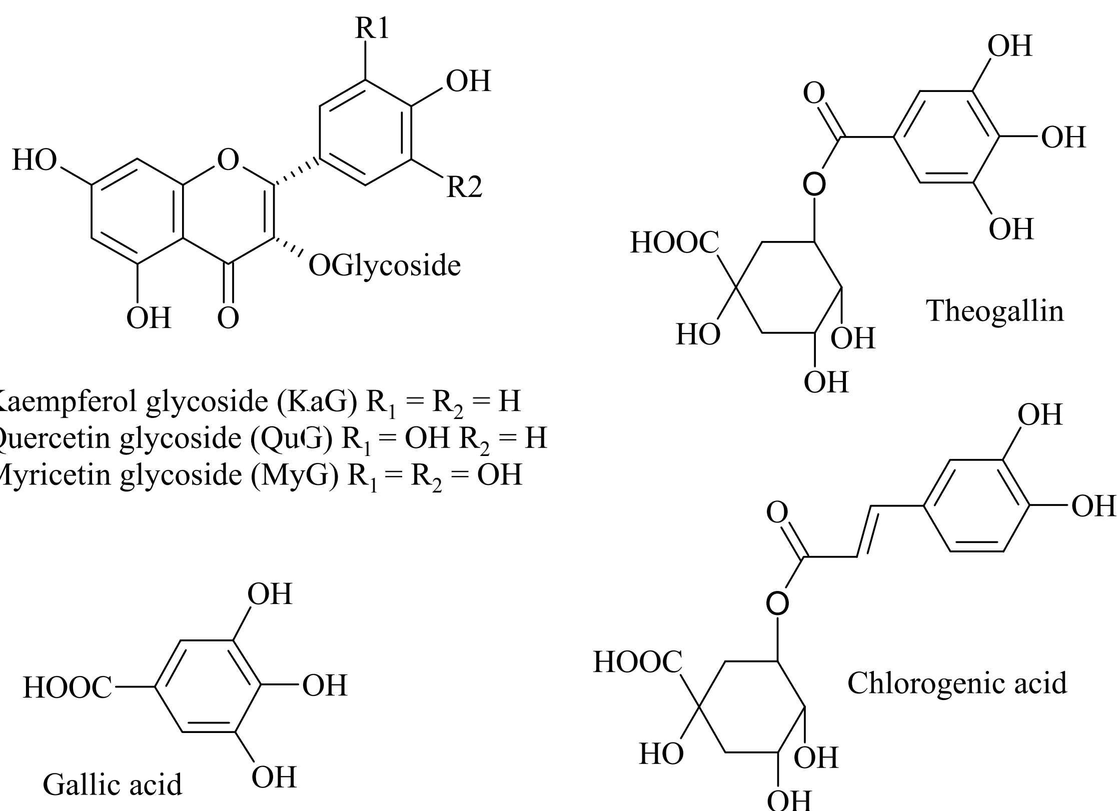


Figure 2.4 Structures of representative phenolic acids and flavonol glycosides in tea (Adapted from Harbowy and Balentine, 1997).

Apart from flavone aglycones and glycosides, which represent a very small fraction of tea polyphenols (Engelhardt *et al*., 1993; Hertog *et al*., 1993ab), there are a number of proanthocyanidins ($C_{30}H_{24}O_{14}$) in fresh leaf and green tea that have been isolated. These include prodelphinidin gallates ($C_{42}H_{34}O_{20}$), assamicains ($C_{42}H_{36}O_{20}$) and theasinensis ($C_{42}H_{34}O_{20}$) (Nonaka *et al*., 1983, 1984; Hashimoto *et al*., 1989). Further, some simple polyphenols have also been isolated from tea, such as gallic acid and its quinic acid ester theogallin (Figure 2.4) (Cartwright and Roberts, 1954a, 1955; Roberts and Myers, 1958; Collier and Mallows, 1971b), and cinnamic acid derivatives, chlorogenic and isochlorogenic acids (Cartwright *et al*., 1955).

2.2.7.2 Polyphenols in black tea

Polyphenols occurring in black tea usually consist of residual green tea polyphenols such as catechins (Bailey *et al*. 1990; Ding *et al*., 1992), flavonols (Bailey *et al*. 1990; McDowell *et al*., 1990), and oxidation products of green tea polyphenols such as theaflavins and thearubigins. Some catechins and catechin gallates may be epimerised or degallated during the processing of black tea; thus, it is possible to increase the levels of gallic acid and isomers of the catechins (Coggon *et al*., 1973). Most of the catechins and their gallates undergo known enzymatic oxidation to form more polymeric polyphenols that are characteristic of black tea, namely theaflavins and thearubigins. Apart from the simple phenolic acids, some other polyphenols have been detected in minor quantities in black tea, including epiafzelechin gallate ($C_{21}H_{16}O_8$) (Hashimoto *et al*., 1987), bisflavanols ($C_{30}H_{22}O_{14}$), proanthocyanidins, theogallinin ($C_{14}H_{16}O_{10}$) (Hashimoto *et al*., 1988), and theaflavonin ($C_{41}H_{35}O_{22}$) (Hashimoto *et al*., 1992). Polyphenols in black tea produced in India vary with the variety of tea, its geographical origin, environmental conditions, agronomic situations and the processing methods (Stephen-Thanaraj and Ramaswamy, 1981). For black tea produced in Kenya, although the contents of theaflavins and thearubigins vary with localities, and the patterns of variation change from clone to clone, there is no significant effect of location or altitude on the contents of theaflavins and thearubigins (Owuor *et al*., 1987).

Theaflavins are known as fermentation products and provide a bright, yellowish appearance to the beverage and have long been positively correlated with the quality and market value of black tea (Roberts, 1958ab, 1962). Theaflavin with a formula $C_{29}H_{24}O_{12}$ (Brown *et al*., 1966) may contain 3.5 moles of water in the crystallisation (Takino *et al*., 1965). All the theaflavins and related compounds, including TF, TF3G, TF3′G, TFDG, isotheaflavin (isoTF, $C_{29}H_{24}O_{12}$), neotheoflavin (neoTF, $C_{29}H_{24}O_{12}$), and theaflavic acids ($C_{21}H_{16}O_{10}$), possess a similar unique benzotropolone ring (Figure 2.5) (Roberts, 1958ab, 1962). This characteristic structure produces yellowish and bright reddish colours, and makes this group of flavonoids easily distinguishable from others. The synthesis of theaflavins from pairs of flavanols or with gallic acid, along with their UV maximum, is shown in Table 2.6, and the four principal pigments in black tea, usually referred to as theaflavins are TF, TF3G, TF3′G and TFDG (Collier *et al*., 1973) (Figure 2.5).

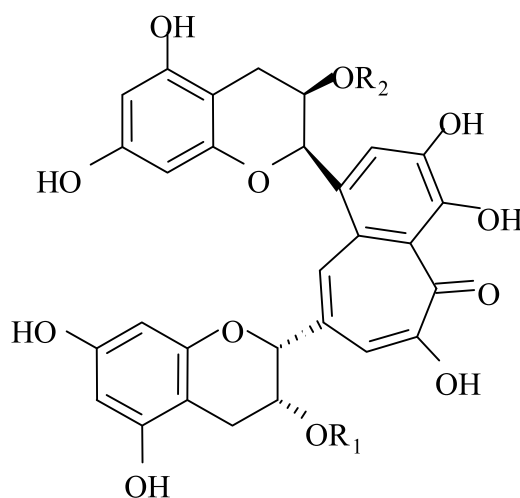
The content of total theaflavins in black tea does not usually exceed 2 % and can be as low as 0.3 % (Balentine *et al*., 1997), whereas Graham (1992) reported that theaflavins ranged 1.5-2.5 % in the dry leaf. Analysis of commercial tea samples from Sri Lanka, Kenya, India and other countries in the German market has found that the total theaflavins range 0.45-1.45 %, with an average of 0.92 % (Steinhaus and Engelhardt, 1989). A recent study on the commercial black tea from the Kenyan market showed a range of total theaflavins from 1.89-2.27 %, with an average of 2.14 % (Owuor and Obanda, 1995a). On comparing the tea processed from different methods, the levels of total theaflavins found in orthodox black tea is 2.12 % and in CTC tea is 4.01 % (Unilever, 1996). These results indicate that the content of theaflavins varies due to differences in clones or cultivars of the fresh leaves, in processing method, in storage conditions and times (teas from market should have a period of storage), and in analytical method (McDowell *et al*., 1985).

Furthermore, the level of theaflavins has been found to fluctuate following climatic variations, decreasing after rainy periods, and the total colour of black tea shows a good correlation to the levels of theaflavins (Malec and Vigo, 1988). The fraction of black tea theaflavins has been shown to contain about 8 % TF, 30 % TF3G, 20 % TF3′G, 40 % TFDG, and 4 % isoTF, as well as minor levels of theaflavic and epitheaflavic acids (Coxon *et al*., 1970a). Another study of black tea reported that the contents of TF, TFG (TF3G and TF3′G) and TFDG in total content of theaflavins are about 35 %, 46 % and 19 %, respectively (Takeo and Oosawa, 1976). Recently, a study on individual and total theaflavins using HPLC found that theaflavins are clonal dependent (Obanda *et al*., 1997a). Thus, the proportion of individual theaflavins in the total theaflavins may vary accordingly to the variety, processing, and storage (samples from market).

Table 2.6 Synthesis of theaflavins and related compounds and their absorption spectra λmax (Adapted from Coxon *et al*., 1970abc; Opie, 1992).

TFs	Parent PPs		M.W.[1]	λmax in ethanol (nm)			
TF	EC	EGC	564	268	294sh	379	467
IsoTF	EC	GC	564	270	297sh	378	462
NeoTF	C	GC	564	270	295	378	465
TF3G	EC	EGCG	716	275	-	378	462
TF3′G	ECG	EGC	716	275	-	378	462
TFDG	ECG	EGCG	868	278	-	378	460
ETFA	EC	GA	416	280	-	400	-
ETFAG	ECG	GA	586	-	-	-	-
TFA	C	GA	416	280	-	404	-
TFAG	CG	GA	586	-	-	-	-

[1] M.W. = molecular weight



Theaflavin (TF), $R_1 = R_2 = H$
Theaflavin 3-gallate (TF3G), R_1 = gallate, R_2 = H
Theaflavin 3'-gallate (TF3'G), R_1 = H, R_2 = gallate
Theaflavin 3,3'-gallate (TFDG), $R_1 = R_2$ = gallate

Figure 2.5 Structure of four major theaflavins in black tea (Harbowy and Balentine, 1997).

The combined oxidation of EC and EGC leads to the formation of TF, whereas the oxidation of EGCG with EC yields TFG (Coxon *et al*., 1970b). Chemical oxidation of ECG alone produces theaflavate A (Wan *et al*., 1997), while the chemical oxidation of EGCG alone forms a similar phenolic pattern to that of Malawi black tea (Yao and Nursten, 1997). Some EGCG was detected during the oxidation of ECG alone in the same chemical model system (Yao and Nursten, 1998), suggesting that the B ring be activated during the oxidation of ECG. The oxidation and decarboxylation of the A ring occur in the reaction of EGCG and EGC with H_2O_2, suggesting that the A ring of EGCG and EGC may also be an antioxidant site (Zhu *et al*., 2000). In conclusions, these results suggest that there may be an additional reaction pathway for the oxidation of tea catechins and their gallates.

Coxon *et al*. (1970b) suggested that it is most unlikely that isoTF, a minor theaflavin component in black tea, could have arisen as an artefact derived from TF during acidic isolation procedures, because TF does not isomerise when kept in acidic solvents for several months. It seems probable that isoTF is formed from EC and GC as precursors.

Further oxidation of theaflavins produces a group of brown pigments called thearubigins (Roberts, 1958a, 1962). In a model system, it was found that the primary oxidation products formed from the oxidation of EC or ECG with gallic acid in short reaction periods were bright red condensation products named epitheaflavic acid (ETFA) and 3-galloyl epitheaflavic acid (ETFAG) (Berkowitz *et al*., 1971). ETFA was not reactive itself but was rapidly transformed to thearubigins when EC was present in the system. The oxidation rate of theaflavins in the processing of black tea varies among the individual theaflavin compounds (Bajaj *et al*., 1987). Here, oxidation of TF3G and TF3′G has been found more rapid than that of TFDG in the presence of EC (Bajaj *et al*., 1987). These results suggest that because the redox of EC is the highest among the tea catechins and catechin gallates, the quinone of EC acts as an electron donor for the other compounds, such as theaflavins, and the quinone reverts to EC. Thus, considerable amounts of theaflavins synthesised during black tea fermentation may be oxidised by EC (Bajaj *et al*., 1987).

The brown acidic pigments in black tea liquor were first named by Roberts (1958a) as thearubigins (TRs), which was mistakenly written as thearubigens in some literature. In the opinion of Roberts (1958ab), *o*-quinones of EGC and EGCG are condensed spontaneously to diphenoquinones. As a result of oxido-reductive reactions, two molecules of the diphenoquinones form dicatechin and TF (or TFG). Further transformations of dicatechins and theaflavins yield the thearubigins which, together with theaflavins, represent 30 % of the dry mass in black tea (Opie *et al*., 1988).

Early studies by Brown *et al*. (1969) suggested that the thearubigins were polymeric proanthocyanidins, which could be degraded to catechins and their gallates, anthocyanidins, and gallic acid. Cattell and Nursten (1976) using column chromatography of the ethyl acetate-soluble fractions of thearubigins found that they were pentameric flavanols or their gallates with both hydrolysable and non-hydrolysable interflavanoid links and benzotropolone units. The ethyl acetate-soluble fractions of thearubigins might not be polymeric proanthocyanidins (Cattell and Nursten, 1976). Furthermore, there were two extra peaks of thearubigin fractions observed when monitored at 380 nm, compared to monitoring at 460 nm (Cattell and Nursten, 1977). These two peaks were suggested to be two flavonol glycosides (Cattell and Nursten, 1977) that did not undergo enzymatic oxidation during the black tea processing (Biedrich *et al*., 1989). HPLC analysis also showed that the thearubigins measured by early spectrophotometric methods included some flavonol glycosides that showed similar absorption maxima to the thearubigins (McDowell *et al*., 1990; Whitehead and Temple, 1992). Thus, the value of thearubigins determined simply by the spectrophotometric method may not be accurate as the true value of thearubigins as defined by Roberts (1958ab). Similarly, using the spectrophotometric method at the same wavelength of 380 nm for the measurement of theaflavins may also affect the accuracy of theaflavin estimation (Collier and Mallows, 1971a).

Thearubigins consist of the three subclasses SI, SIIa and SIIb based on the analysis by paper chromatography (Roberts *et al*., 1957). These compounds were classified into the three groups, I, II and III by HPLC analysis (Bailey *et al*., 1991, 1994a). The paper chromatographic analysis of thearubigins by Roberts *et al*. (1957) dealt with the unresolved fractions as thearubigins, whereas Bailey *et al*. (1991, 1994a) separated these compounds into “resolved” and “unresolved” thearubigins. However, no one unique chemical structure seems to be indicative of a thearubigin to date. The presence of brown-coloured products that partition into all of the phases used in solvent extraction, including the aqueous phase, butanol and ethyl acetate phases, suggests the need for a number of different chemical moieties to be elucidated, rather than a single structured group of compounds as for the theaflavins.

Methods for analysing tea composition are important in assessing the quality of a tea (Crispin *et al*., 1968). Paper chromatography (Roberts, 1962) and column chromatography (Vuataz *et al*., 1959) were

once the best choice for the separation of polyphenols from tea leaf. As soon as HPLC was introduced into the analysis, these techniques were superseded by this much more powerful analytical technique. Normal-phase HPLC (Wedzicha and Donovan, 1989; Wedzicha *et al*., 1990) was used for the separation of thearubigins but did not lead to their identification. However, the reversed-phase separation technique made the identification of thearubigins possible (Bailey and Nursten, 1994). Based on HPLC analysis using the reversed-phase techniques, Bailey *et al*. (1991) classified the thearubigins into three groups: Group I as anthocyanidins; Group II as theaflavins and types I and II resolved thearubigins, and Group III as unresolved thearubigins. The resolved thearubigins were analysed based on their absorption at 460 nm, where most of the pigments show strong absorption. Following on this study, a mixture of phenolic polymers was isolated from thearubigins (Bailey *et al*., 1992, 1994bc). This brown polymeric mixture is free of protein, caffeine and flavonol glycosides, and is a flavanol polymer with various intermonomer linkages to proanthocyanidin polymers. This brown fraction of thearubigins was designated as the theafulvin fraction (Bailey *et al*., 1992, 1994bc). Chemical oxidations of catechins and catechin gallates formed the same resolved and unresolved pigments found in the black tea (Bailey *et al*., 1993).

Model fermentation systems (Opie *et al*., 1990; Opie, 1992; Opie *et al*., 1993, 1995) have been used in combination with HPLC techniques for the classification of thearubigins. The oxidation products were monitored at 450 nm (Powell *et al*., 1993). The proanthocyanidins from the theafulvin fractions of thearubigins were found to contain prodelphinidins, procyanidins and propelargonidins (Powell *et al*., 1995). Temple and Clifford (1997) found that the peaks of the theaflavins and thearubigins of the decaffeinated aqueous extract of black tea remained stable for up to 850 min, thus permitting an automated HPLC analysis over a certain time (e. g. overnight). These researchers (Temple and Clifford, 1997) also found that the use of citric acid to acidify the chromatographic solvents did not produce superior separation to the use of acetic acid in the HPLC analysis, which is not in agreement with the result of Bailey *et al*. (1991) who recommended using citric acid solvents for the HPLC analysis of black tea liquor without being decaffeinated.

In conclusion, thearubigins is a collective name for the brown acidic pigments or the coloured phenolic oxidation products that remain after removing of the yellow neutral pigments from a black tea liquor (Roberts, 1958a; Millin, 1987). The molecular weight and spectral characteristics of thearubigins are known to be very heterogeneous; thus, the quantitative measurements of this group of pigments based on their absorbance at particular wavelengths has been recognised to be rather meaningless (Millin, 1987). Moreover, although thearubigins have not been fully characterised, it has become clear that this complex consists of a group of polymers with various properties. Thus, it is likely that the complex will be fully elucidated and characterised in the near future, with the further development of modern analytical techniques.

2.2.7.3 Polyphenols and tea quality

The quality of a tea is formed during the growth and development of the tea plant, when the compounds responsible for quality are synthesised (Bokuchava and Skobeleva, 1969). The chemical constituents synthesised in the tea shoots may exert positive and/or negative effects on the quality of the made tea. The quality index [(EGCG+ECG)/EGC] has been found to be directly related to the sensory properties of green tea (Yuan, 1962). Thus, this index has been used as an objective parameter for assisting the evaluation of green tea quality in China (Liang, 1990). A comparison of the polyphenolic profiles of different types of teas showes that different dominant catechins and catechin gallates occur in green teas, and different profiles of theaflavins occur in fermented teas (Shao *et al*., 1995). The content of theaflavins is far less than that of thearubigins in black tea, but theaflavins are of primary importance to tea quality, since they impart the specific bright and vivid colour to the liquor; and further, the ratio of theaflavins to thearubigins has been found to be responsible for the strength of the tea liquor (Bokuchava and Skobeleva, 1969).

Good quality teas contain less high molecular weight compounds than those of inferior quality (Bradfield, 1946). For black tea, a high proportion of extractable polyphenols may indicate a good

quality liquor, with astringency and a bright reddish colour. As an aid to judging quality, a finely divided precipitate formed during the cooling of black tea liquor is referred to by tea tasters as the "cream down" of tea (Bradfield, 1946). This cream consists largely of extractable polyphenols such as theaflavins and thearubigins, and other flavonoids in combination with caffeine. Interactions between caffeine and the polyphenols are primarily responsible for this cream (Collier *et al*., 1972). The composition of a cream in an Assam tea infusion brewed with a tea/water ratio of 1/40 is: ca 15 % theaflavins, 65 % thearubigins, 14 % caffeine, 3 % ash and other compounds (Smith, 1968).

Roberts and Fernando (1981) showed that theaflavin content in black tea is not quantitatively related to the polyphenol content or polyphenol oxidase activity in fresh leaves. These researchers showed some correlation between the quantity of theaflavins and the quality of black tea, but no statistical correlation was found between total or individual polyphenols and quality ranking of orthodox Sri Lanka black tea. Further, Sivapalan *et al*. (1985) indicated that theaflavins as a criterion of quality evaluation might not be accurate for orthodox Sri Lanka tea, but the theaflavins were found to make important contributions to sensory properties of black tea infusion. However, these are the only reports that have not found statistical correlation between theaflavins and orthodox black tea. In India, a non-linear relationship has been found between polyphenol oxidase activity of clonal fresh tea shoots and the content of theaflavins in the resulting black teas (Stephen-Thanaraj and Seshadri, 1990). Black tea produced from buds has the highest amount of theaflavin gallates, whereas black tea produced from the internodes has the lowest, suggesting tea made from buds possesses higher quality (Stephen-Thanaraj and Seshadri, 1990) . In Japan, levels of theaflavins and thearubigins, and especially their total contents showed high positive correlation with the evaluation of black tea quality (Takeo, 1974).

Regardless of tasters and the methods of processing, the quality of north eastern Indian plains black tea depends mainly on briskness, with theaflavins being the main factor for briskness (Biswas and Biswas, 1971; Biswas *et al*., 1971, 1973). After years of research, Eden (1976) concluded that the dynamics of the production of theaflavins is the most potent single factor in promoting good quality in tea. In addition, Davies (1983) suggested that the content of theaflavins and the percent of extractable solids in a black tea could be used as objective measures of the tea quality. Patterns or levels of phenolic compounds in black tea liquor have been used as means of predicting price and country of origin (McDowell *et al*., 1991, 1995ab). A good quality tea possessing brightness, briskness, and good colour and body may possess a ratio of theaflavins:thearubigins of 1:10 (Deb and Ullah, 1968). Thus, phenolic compounds, particularly the theaflavins, play an important role in determining the quality of black tea, and the use of polyphenols as quality indicators for black tea is discussed below.

2.2.7.4 Quality indicators

Factors that influence tea quality can broadly be divided into controllable factors and non-controllable factors (Odhiambo *et al*., 1988). The controllable factors are field cultural factors and factory practices, and to some extent genetic factors, whereas the non-controllable factors are environmental and genetic factors. Sometimes, tea price is determined by factors outside the quality of the tea, for example, the supply and demand of the tea, and consumer's preferences (Owuor and Obanda, 1995a). Thus, quality related factors should be used as indicators for producing good black tea for good prices when the quality factor overrides the other factors. These quality factors should be used as part of an objective method of ensuring consistently good processing (Owuor and Obanda, 1995a).

The content of total polyphenols has shown least variation with time of a year, and correlated significantly with plain tea quality parameters (Obanda *et al*., 1992). The level of total polyphenols of fresh tea shoots is important to black tea quality, and may be a reliable parameter for identifying and propagating potential high quality clonal tea plants (Obanda *et al*., 1997a). Furthermore, flavanols were found to be clonal dependent . In green leaf, either ECG or EGCG was the dominant flavanol present. Tea tasters' preferences for black teas showed positive and significant correlation with the contents of ECG and EGCG in the green leaf (Obanda *et al*., 1997a). Therefore, ECG and EGCG can

be used as potential quality indicators for the clonal selection and propagation of tea plants that are more suitable for black tea processing.

Theaflavins have been found to possess the most important influence on the taste and colour of black tea liquor (Ellis and Cloughley, 1981). These compounds produce intense, bright orange and /or yellow colour, and a strong astringency in their pure state. Thearubigins are a very variable mixture of compounds and less well defined chemically than the theaflavins, as discussed earlier. In their original work, Roberts (1958ab, 1962), Roberts and Smith (1961, 1963), Wood and Roberts (1964) showed that theaflavins are the characteristic constituents of good quality teas, and that the content of theaflavins is an extremely important factor in determining the quality of black tea. Numerous studies have since displayed a close and statistically significant linear relationship between the content of theaflavins and sensory evaluations of tea tasters and market prices (Hilton and Ellis, 1972) of teas from different countries of origin (Ellis and Cloughley, 1981).

Hazarika *et al*. (1984b) showed that theaflavins have distinct organoleptic properties and their variation is reflected in the overall quality of black tea (Table 2.7). During the sensory evaluation of black tea, the tasters' judgement is usually based on the colour, strength, briskness, flavour and overall quality of the tea (Hilton and Ellis, 1972). Theaflavins contribute to all of these characteristics but some other components such as thearubigins, caffeine and volatile flavour compounds also have some effects (Owuor, 1982). Thus, the total evaluation of black tea appears to be determined by a complex combination of chemical parameters. In addition to the main quality components mentioned above, the levels of certain pigments are also suggested as a means of predicting black tea quality (Taylor *et al*., 1992). These pigments (e.g. carotenoid and chlorophyll) in green tea leaf could be also used to differentiate teas from various clones (Taylor *et al*., 1992). In fact, although tea tasters make every effort to evaluate the quality of tea, using their sense of taste and sight and integrating them into a value judgement, it is the theaflavins that tea tasters are most sensitive during their evaluation (Ellis and Cloughley, 1981).

In the quality assessment of black tea, theaflavins analysis shows two important merits: "Objective" and "quantitative." These properties of theaflavins analysis enables the comparison and quality monitoring on the black teas produced in different places (e.g. countries) and at different times (e.g. years). Therefore, theaflavins are used by the major tea research institutes in central Africa and other countries as a routine quality indicator in biochemistry, agronomy and plant improvement for tea (Ellis and Cloughley, 1981).

Table 2.7 Content of theaflavins in leaf components and sensory comments on the resulting black tea (Adapted from Hazarika *et al*., 1984b).

Leaf component	TF content (% dry basis)	Tasters' comments
Bud	1.55	Very good
First leaf	1.49	Good
Second leaf	1.23	Fair
Third leaf	1.10	Poor
Stem	1.15	Fair

In Kenya, the correlation between the content of theaflavins and the evaluation of tea tasters has been shown a generally positive but statistically non-significant correlation (Owuor *et al*., 1986a). Further, the level of theaflavins is loosely related to the price of the teas (Owuor *et al*., 1986d). However, the formation rate of theaflavins during fermentation varies between clones and within a clone (Owuor and McDowell, 1994). In addition, individual theaflavins formed at different rates during the fermentation result in the levels and ratios of the theaflavins being different in the final black tea (Obanda *et al*., 1997a). Astringency of black tea varies with composition and total amounts of theaflavins, since individual theaflavins have different astringencies. Thus, total theaflavins in the tea liquor may not correctly represent the total astringency and hence the quality of the tea. Owuor and

Obanda (1995) showed that TFDG possesses the strongest astringency among the main theaflavins, and proposed that using TFDG or the TFDG equivalent of the total theaflavins could better describe the quality of Kenyan black teas than the use of total theaflavins. Furthermore, Owuor (1996) found that astringency is a better estimator of tea quality than aroma, with the four main theaflavins, TF, TF3G, TF3′G and TFDG being contributors to the astringency. Therefore, theaflavins may be the best choice as a quality indicator for black tea, both in the processing line and the end product.

2.2.8 Alkaloids and other chemical components in tea

2.2.8.1 Alkaloids

The study of tea chemistry may be said to have begun with the isolation of the alkaloid caffeine (Figure 2.6) from tea in 1827 (Bradfield, 1946). Tea has been valued historically for its caffeine content, which is between 2 and 5 % (w/w) depending on variety. High caffeine content in fresh leaf may be one of the important factors ensuring good quality of the resulting black tea (Bhatia, 1964). Caffeine is regarded as an important constituent of tea, bestowing mood and cognitive-enhancing properties (Bokuchava and Skobeleva, 1969; Chow and Kramer, 1990). In Kenya, caffeine is used as an important quality parameter for the evaluation of plain black tea quality (Owuor *et al*., 1986c). The quantity of caffeine infused into a tea brew is determined by the infusion time and by the leaf style (Harbowy and Balentine, 1997). In green tea, the infusion is slightly affected by tea clones, but significantly affected by the temperature and infusion time (Yao *et al*., 1992). The temperature and time also affects caffeine infusion in black tea, with water volume being significant (Yao *et al*., 1993). Thus, the actual content of caffeine in a brew depends on many factors, mainly the method of brewing. No significant difference in caffeine levels have been found when brewing green and black teas under similar conditions (Hicks *et al*., 1996), discrediting the theory that withering and fermentation have a significant impact on caffeine content (Sanderson, 1972).

Owuor (1987b) suggested that seasonal, genetic, agronomic and cultural factors, as well as processing practices might influence the caffeine content of made teas to some extent. In Argentina, caffeine content in tea leaf decreased gradually during most of the season after an early rapid increase (Malec and Vigo, 1988). In central Africa, the highest level of caffeine was found during the peak harvesting season when shoot growth rate was most rapid (Cloughley, 1982). In addition, studies on Assam tea by Wood *et al*. (1964a) showed that caffeine content decreased progressively through the season. Shoot maturity, variety, season, fertilisers, pruning, processing, grading and location have effects on the caffeine content of tea (Dev Choudhury *et al*., 1991). Caffeine levels vary from 5.30 % for 1 bud 1 leaf; to 4.20 % for 1 bud 2 leaves; to 3.80 % for 1 bud 3 leaves; and to 3.20 % for 1 bud 4 leaves (Dev Choudhury *et al*., 1991). Thus, the caffeine content decreases as the leaf ages or matures. Accordingly, teas made from pruned shoots have higher caffeine contents because of more young tender shoots. During withering and the entire processing of black tea, caffeine content increases marginally. Caffeine of an infused brew is responsible for the briskness of the tea liquor; this is due to its association with theaflavins.

Caffeine is one of the most comprehensively studied ingredients in food. Caffeine acts as a diuretic, cardiac muscle stimulant, central nervous system stimulant, smooth muscle relaxant, gastric acid secretion stimulant, elevates plasma free fatty acids and glucose (Harbowy and Balentine, 1997). The caffeine content of a typical tea beverage ranges 20-70 mg/170 mL of infusion, with the infusion being prepared from 2-2.5 g of tea leaves, while a coffee brews typically contain 40-155 mg caffeine/170 mL beverage (Harbowy and Balentine, 1997). Caffeine is absorbed rapidly and reaches peak levels in the body about 1 h after ingestion (Graham, 1978).

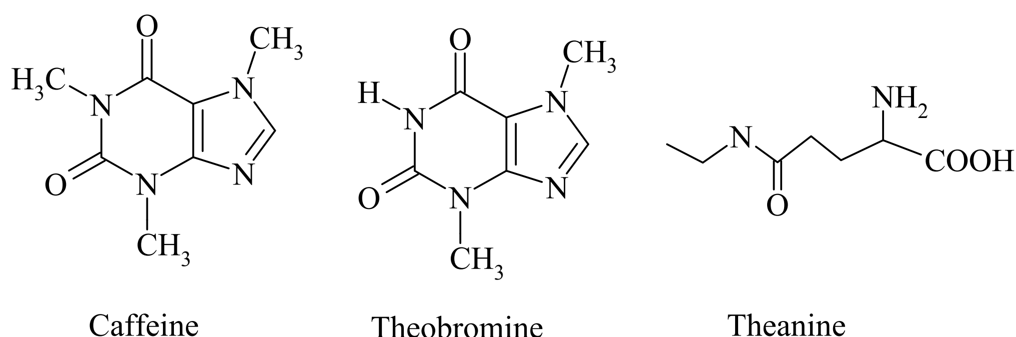


Figure 2.6 Structures of some nitrogenous tea phytochemicals (Adapted from Harbowy and Balentine, 1997).

Theobromine (Figure 2.6) and theophylline are another two alkaloids that occur in tea, but are present in much lower quantities than caffeine . In Assam black tea, alkaloids were estimated on a dry basis (w/w) as 1-5 % caffeine, 0.05 % theobromine, 0.0002-0.0004 % theophylline (Stagg and Millin, 1975). This xanthine content of teas is an area that may require further and more careful research.

2.2.8.2 Other components

Eighteen amino acids have been identified in two kinds of green teas (Liang *et al.*, 1990), while more recently the identified numbers of amino acids present in green tea leaves have reached 26 (Wang, 1994). Among those compounds is theanine (Figure 2.6), a unique amino acid only present in tea (Cartwright *et al.*, 1954b). Theanine is the major amino acid in tea, comprising about 3 % (w/w) of the tea extract (Harbowy and Balentine, 1997). Carbohydrates contribute approximately 11 % (w/w) of extract solids of tea (Sanderson *et al.*, 1976). Approximately, 11 free sugars have been detected in tea by paper chromatography (Cartwright and Roberts, 1954b), while 1 free sugars was found in green tea or fresh leaves using HPLC (Anan *et al.*, 1981, 1985). Tea also contains various organic acids (Sanderson and Selvendran, 1965; Jayman and Sivasubramanian, 1975). Shikimic and quinic acids occurring in tea are considered very important for the biosynthesis of polyphenols (Bokuchava and Skobeleva, 1969).

Tea contains several vitamins such as vitamin B, but vitamin C may be in a significant amount (Sanderson, 1972; Bokuchava and Skobeleva, 1980). Mineral content measured as ash has long been recognised in the international standards (ISO 1575, 1987; ISO 1576, 1988; ISO 1577, 1975). Excellent reviews and research works on minerals of tea are available (Wood *et al.*, 1964a; Bokuchava and Skobeleva, 1969; Lu, 1987; Natesan and Ranganathan, 1990). The main pigments in the fresh tea leaves are chlorophylls and carotenoids. In black tea, there have been twenty-eight pigments detected by HPLC (Taylor and McDowell, 1991). The composition of these components in the green leaf has been shown to exert a strong impact on the quality of black tea (Hazarika and Mahanta, 1984; Taylor *et al.*, 1992) and green tea (Wang, 1994).

Tea flavour is unique among various beverages, and is the most important factor in determining tea quality and hence the market prices of tea. In the 1960s, only 83 aroma compounds of black tea were identified (Bondarovich *et al.*, 1967), which reached 638 in the 1990s, although the quantity of these compounds in tea is only 0.02 % (w/w) (Hara *et al.*, 1995). Excellent reviews and studies on volatile flavour compounds are available (Yamanishi *et al.*, 1968ab, 1989; Bokuchava and Skobeleva, 1969; Sanderson *et al.*, 1971, 1976; Wickremasinghe, 1973, 1974; Howard, 1978; Fernando and Roberts, 1984; Hazarika *et al.*, 1984a; Mahanta *et al.*, 1985; Owuor, 1996; Harbowy and Balentine, 1997).

2.2.9 Chemical composition of Australian tea

Phenolic compounds have long been recognised as the important quality indicators for tea, both fresh leaves and the final black tea. However, there is no published data available on either total or individual chemical compounds, including phenolic compounds, in Australian grown and made tea prior to this study. Thus, investigation of the types and contents of phenolic compounds in Australian grown and made teas, at various growing seasons and different processing steps would provide very useful information to assist with the improvement of the quality of tea, and to judge its quality relative to tea produced in other countries.

2.3 Summary

This review has generally examined the important information about phenolic compounds in tea beginning with flavonoids and their health benefits, and detailing the tea flavonoids, their changes during processing, particularly their commercial importance as quality parameters for tea. It was suggested from the review of the literature that some of the individual phenolic compounds occurring in tea shoots could be used as indicators for the quality of the produced black tea. In addition, those phenolic components, mainly catechins and/or catechin gallates, could be used for monitoring the progress of black tea processing. In particular, theaflavins were recognised as the main chemical component for monitoring the fermentation, a critical stage during black tea processing.

From the literature, no published information about the composition of Australian grown and made tea has been found, especially information for phenolic compounds occuring in tea shoots and the resulting black tea, and their changes during processing. Thus, reliable objective analysis of the phenolic compounds in Australian grown and made tea should help to identify the optimum harvest date for tea leaves, and the best processing conditions, that together maximise the quality of the resulting black tea.

3 Agronomic aspects of the tea farm and the on-site processing of black tea

3.1 Introduction

Agronomic aspects and environmental factors have been known to play important roles in the determination of tea quality (Sanderson, 1964; Sanderson and Kanapathipillai, 1964; Millin, 1987; Hara *et al*., 1995). However, there has been little published information detailing the agronomic aspects, environmental factors and processing of Australian tea. Therefore, presentation of general information on the tea farm and the factory at Malanda, North Queensland is necessary. The aim of this Chapter is to overview the operations of the main tea producer in Australia. This overview covers an introduction to the tea farm; details the climate data around the farm areas; includes records of fertiliser applications; and details the black tea manufacturing process.

3.2 Tea farm

3.2.1 Glen Allyn Tea Estates

The main tea producer in Australia is Glen Allyn Tea Estates (GATE, owned by Calata Pty. Ltd.), which comprises a tea farm and a processing factory at Malanda, North Queensland. The tea farms of Glen Allyn Tea Estates and nearby outgrowers are located at the triangle areas between Malanda, Millaa Millaa and Innisfail in North Queensland, as shown in Figure 3.1. These tea growing areas are on the Atherton Tablelands, southwest Cairns, and belong to a warm rainforest climate zone. All farms are approximately 35-40 km from the nearest coast. The elevation of the tea farms ranges 245-823 m, with an average of 762 m. The tea factory is located on the tea farm of Glen Allyn Tea Estates.

3.2.2 Areas under tea

The total area under tea is 463 ha. Glen Allyn Tea Estates owns 216 ha of tea plants whereas the outgrowers, who provide or sell their fresh tea leaves (green leaves) to Glen Allyn Tea Estates, have 247 ha. The locations and areas of outgrowers are as follows:

- Barclay 20 ha, located at Topaz.
- Melhuish 30 ha, located at Topaz.
- Muir 64 ha, one farm is located near Glen Allyn Tea Estates, the other farm is in the north of Butcher's Creek.
- Ramjat Verkerk 80 ha, located at Gadgarra.
- Nucifora 41 ha, plus a 12 ha new planting in 1999 which has not been harvested. It is located on the eastern Palmerston Highway between Millaa Millaa and Innisfail.

Figure 3.1 Tea Farms in North Queensland, Australia. T1-Glen Allyn Tea Estates; T2-Other outgrowers (Topaz and Gadgarra); B-Butcher's Creek; N-Nucifora.

3.2.3 Origin of tea clones on the farm

There are three origins of the tea clones grown on the farm. Firstly, some of the seeds were from Bingil Bay, Innisfail, North Queensland, Australia. The trees were planted in 1884 and the seeds were originally from Kew Botanic Garden, London, UK. Secondly, some of the seeds were from Malaysia, with part of the poly-clonal seeds from the Sri Lanka TRI 2000 series plus TV9. Tea trees on the Glen Allyn Tea Estates farmland are mostly of the second origin with some of the first origin. Thirdly, some outgrowers planted tea trees with seeds from Malawi. For example, Verkerk used 100 % Ramjat seeds from Malawi, while Nucifora used 20 % of this origin. The other outgrowers used seed originating from Bingil Bay.

In general, the size of tea leaves of the Glen Allyn Tea Estates are smaller than those of the outgrowers who used Ramjat seed (Benson, 2001). All of the tea species are the *Camellia sinensis v. assamica*, which are suitable for the processing of black tea. The tea bushes that provide green leaves for the processing of black tea were planted on the farm of Glen Allyn Tea Estates between 1985 and 1992. About 80 % of Australian grown and made tea is produced by Glen Allyn Tea Estates (Benson, 2001).

3.2.4 Management of the tea farm

3.2.4.1 Skiffing and pruning

The tea bushes on the farm are usually harvested up to a height of 1.50 m. When they reach this height, they are pruned back to 0.56 m. Then it takes 1 year for them to get back to normal production. After three years of normal harvesting they are skiffed, a technique of lightly cutting off the top of the small and fine tea shoots to maintain an even tree crown for efficient mechanical harvesting. The tea bushes are then pruned in the following year.

3.2.4.2 Fertiliser application

The main constituents of the fertilisers applied to tea plants on the farm of Glen Allyn Tea Estates are nitrogen (N), phosphorous (P) and potassium (K), and these fertilisers are usually applied in a mix with the ratio of 5:1:2, respectively. The quantity of fertilisers used is based on the production of tea. The production of each 10 kg made tea requires one unit of N. Occasionally, fertilisers containing

zinc are also applied. Application details of fertilisers on the paddocks that were used for sampling during the experimental periods (Chapter 4) are given in Table 3.1.

3.2.4.3 Harvest

Green tea leaves on the farm are normally harvested at 21 day intervals, except during the cooler months of winter when they are harvested at up to 30-35 day intervals. Each harvest is called a round. At harvest, a 4.88 m long harvester is operated 24 h a day. A Universal machine is used for spraying weedicide and fertilisers.

Other tea growing countries use fully mechanized harvesters to harvest green tea leaves while others use small hand-operated mechanical harvesters (controlled by one or two people) in a small scale. This harvest in Australia is referred to as "low labour, high mechanisation, high throughput." Otherwise, it would be uneconomical to grow and harvest tea in Australia (Benson, 2001).

Table 3.1 Fertiliser application on the three paddocks used for this study.

Month	Paddock A (kg/ha)			Paddock B (kg/ha)			Paddock C (kg/ha)		
	N	P	K	N	P	K	N	P	K
03/00	81.8	16.4	32.5				81.6	16.4	32.5
04/00				82.2	16.5	32.7			
06/00	59.5			59.8			59.8		
11/00	140.0	28.0	55.5	143.0	28.7	56.9	142.0	28.5	56.6
01/01	82.2	16.5	32.7	82.2	16.5	32.7	82.2	16.5	32.7
05/01	123.0	24.8	49.1	123.0	24.8	49.1	123.0	24.8	49.1

3.3 Weather data

The yearly rainfall on Glen Allyn Tea Estates farm area is around 3 000 mm (Benson, 2001). The rainfall and temperature data recorded on a monthly basis on the farm and the nearby weather stations during the period of sampling for the experimentation detailed in Chapter 4 are shown in Table 3.2.

The data in Table 3.2 show that during the experimentation detailed in Chapter 4 the main periods of rainfall at Glen Allyn Tea Estates and Malanda were February-April, and the secondary periods were during November-January and May-June. The available data on the temperatures were recorded at the weather sites near the farm areas (including the farms of Glen Allyn Tea Estates and outgrowers) (Table 3.2). The lowest means for monthly temperatures during the experimentation period were from June to July, while the warmest months were from November-March. Since the weather data indicate that the rainfall only occurs in a short period and not evenly over the year, and there are more warm days than cool days during the year, it is understandable why tea production continues all year around. This is unlike the Northern Hemisphere where tea bushes cease growing during the winter.

Table 3.2 Rainfall and temperature recorded on Glen Allyn Tea Estates and the weather sites nearby.

Month	Rainfall (mm)[a]		Mean air temperature (°C)[b]							
	GATE	Malanda	Innisfail		Kairi		Atherton		Mareeba	
			Min	Max	Min	Max	Min	Max	Min	Max
01/00	129.0	150.2	22.8	30.9	19.1	28.1	18.4	28.2	21.0	31.7
02/00	1274.0	911.0	23.9	29.9	20.5	26.7	20.0	26.7	21.8	29.6
03/00	584.0	293.4	22.5	29.6	18.4	25.7	18.0	25.7	19.9	29.1
04/00	651.0	287.4	22.2	28.1	18.7	24.6	18.1	24.5	20.0	28.1
05/00	325.0	92.6	19.7	26.2	15.6	22.9	14.8	23.1	17.5	26.8
06/00	254.0	119.6	16.3	22.3	12.3	19.2	12.0	19.4	14.7	23.2
07/00	30.0	14.0	14.5	24.4	8.9	20.9	8.4	21.3	11.8	24.7
08/00	81.0	28.8	16.1	25.7	11.4	23.6	12.6	23.2	13.5	26.8
09/00	23.0	5.4	17.9	27.8	13.0	25.7	12.5	26.4	15.4	28.6
10/00	65.0	40.4	21.3	28.6	16.9	26.8	15.6	27.1	18.5	29.6
11/00	263.0	224.0	22.3	30.1	18.7	27.9	18.0	28.3	20.4	31.2
12/00	409.0	206.1	22.6	29.2	19.0	25.7	17.9	25.8	20.4	28.6
01/01	403.0	326.6	22.8	30.5	18.7	27.1	17.8	27.5	20.3	30.5
02/01	920.0	607.2	23.3	29.4	19.8	25.7	19.2	26.0	20.9	28.4
03/01	274.5	151.8	22.9	31.0	18.6	27.4	18.7	27.1	19.9	30.5
04/01	404.0	159.4	21.6	28.5	17.9	24.4	17.1	24.4	19.2	28.0
05/01	17.0	6.6	16.6	27.5	11.3	24.0	9.8	24.2	14.7	27.4
06/01	353.5	137.6	19.3	24.8	15.2	21.1	14.5	20.7	16.7	24.8
07/01	30.0	11.4	15.1	25.0	10.2	21.8	9.0	22.1	12.5	25.4
08/01	41.0	21.8	15.9	25.6	11.0	22.9	9.9	23.5	13.3	26.3

[a] GATE rainfall was recorded on the farm by Glen Allyn Tea Estates; Malanda rainfall was recorded by Malanda Post Office and supplied by the Brisbane Regional Office of the Bureau of Meteorology (BOM).
[b] Data were supplied by BOM.

3.4 Tea factory at Glen Allyn Tea Estate

There was once a tea factory operated in Innisfail from the 1970s and all the tea farms nearby sold their green tea leaves to this factory for processing, until it was shut down because it was too small to cope with new plantings around the tableland area. The tea factory of Glen Allyn Tea Estates was established in 1992, and has since operated with a “low labour, high mechanisation and high throughput” regime.

3.4.1 Operation of the tea factory

The outturn of dry tea is about 20 % from the green leaves. The yearly production of black tea in the factory of Glen Allyn Tea Estates is about 1.2 million kg (1 200 t) (Benson, 2001).

3.4.2 Processing of black tea

Initially, freshly harvested green tea leaves with a moisture content of 76-80 % are held in large bins. During rainy season, the harvested tea leaves usually contain water. In this case, the water can flow out of the bin through the holes during the harvest.

After storage, these leaves are transferred from the bin to the Classifier, where there is hot air blown upwards through the tea leaves to remove some non-tea matter. Then the leaves are transferred into the Shredder (chopper) to be chopped into smaller sizes (Figure 3.2). Next, the tea leaves are passed through a 2 m long channel with hot air blowing through. This channel, which is called an Incline

Witherer (Figure 3.2), is actually an exhauster used to evaporate off some moisture as the leaves travel through the channel. Only a slight withering occurs during this short period.

The green leaves are then transferred to a pair of parallel Rotorvanes (Figure 3.2), where the leaves are ruptured and crushed into further smaller sizes. These rotorvanes consist of a rotating screw auger fixed with resistors protruding around a cylindrical barrel closely fitted with internal vanes. At the end of the barrel, there is a resistor plate that can be adjusted to restrict the quantity of green leaf from being forced out. Tea leaves that usually fill about three-quarters of the barrel are delivered to the end of the screw auger against the protruding resistor in the barrel. It is this resistance, or back pressure, that crushes the leaf, or causes rupturing and maceration of the leaf.

Following the rotorvanes there are four Crushing, Tearing, and Curling (CTC) machines (Figure 3.2) in succession, which consist of a pair of stainless steel rollers with etched surfaces. When the CTC is working, one of the rollers rotates clockwise, and another anticlockwise at different speed. The principles of CTC are crushing the cells of the tea leaves, tearing apart the tissues, and then curling (twisting) the cells into fine particles. The curling effect of the machine will eventually affect the density, shapes and grades of the final tea. After passing through rotorvanes, the size of the tea leaves is still big enough to resist the fierce action of normal CTC machines, particularly for mechanically harvested green leaves where more mature leaves are present, which produce more resistance for the processing machines. Thus, in this Australian factory, the four sets of CTC machines are designed with large teeth, followed by smaller teeth, and finally fine teeth, which allow the machine to macerate the tea leaves progressively to form the required black tea particles. The tea leaves pass through these four CTC machines in a very short time (about 1 min in total), and the enzymes and substrates are considered fully mixed up for further the fermentation stages. Thus, the CTC stage is one of the critical steps in processing CTC tea. During this stage, the moisture content of the on-line tea leaves is almost the same as that of the green leaves in the incoming bin.

Following the four CTC machines is a fermentation process, which brings the temperature of the dhool from 32 °C (the dhool temperature after CTC) to the room temperature within 2 h. Dhool is the term usually used to describe tea samples from the fermenting leaf to the final tea before sorting (some researchers refer to it only as the fermenting leaf, Hara *et al*., 1995). Fermentation is another critical stage for black tea processing, and involves the enzymatic oxidation of tea polyphenols and the formation of theaflavins and thearubigins.

The Australian factory contains a five-step fermentation (Figure 3.2) but the fifth fermenter is usually only used for the dhool which is thought to be under-fermented. In this factory, all the fermenters are called Jumbo Lindsey Fermenters, consisting of a pair of chambers and using a series of paddles running on a shaft to push (slightly screwing) the dhool forward and allowing air to mix with the dhool. When temperature is too high, the cold air blown through the dhool can bring the temperature down to the desired region. Since the fermentation is actually an enzymatic oxidation of polyphenols and an oxygen consuming process, an appropriate supply of air for the fermentation ensures development of the aroma (flavour) and the overall quality of the final tea

After passing through the fermentation process, the dhool enters two parallel dryers called Hull Dryers.. The Hull Dryers are the dryers incorporated with fluidised bed drying (FBD) technology, extensively applied to tea industry since 1980s (Hara *et al*., 1995). When the dhool is introduced into the dryer, hot air is blown through the dhool, which will take off the moisture and inactivate the enzymes. These dryers are used for the preliminary drying of tea to reduce the moisture content from 80 % to 46 %.

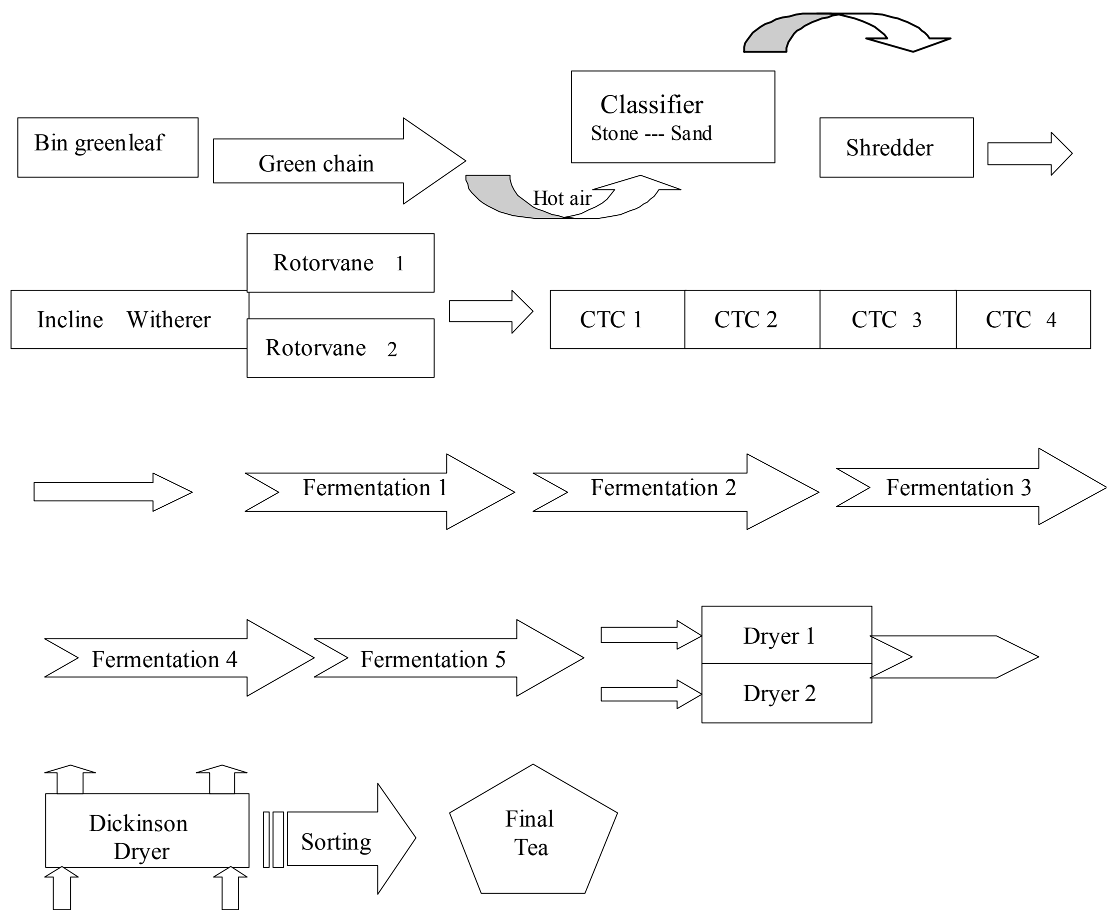


Figure 3.2 Processing flow of the black tea manufacture at the Glen Allyn Tea Estates.

Finally, the tea is dried in a Dickinson dryer which is specially designed for this factory with a larger size than the Hull Dryers. The fluidised bed drying technique has also been incorporated into this dryer; thus, the tea is dried by flowing through the hot air blowing from underneath of the "bed". After this drying, the moisture content of the black tea is about 2.5-4.5 %. Traditionally, only tea passing through this drying stage can be called black tea.

This tea then undergoes a sorting process where it is prepared for further sieving or separating into different kinds of products for sale to packers. In this factory, sorting of the dried tea is undertaken on 2-3 sets of parallel sorting beds, and operation of these sets of beds is dependent on the season or throughput requirement. That is, during the high season, three of the sorting beds are in use. On each of the sorting beds, five pairs of static electricity stalk pickers are installed in consecution. When the tea passes underneath the rotating static electricity stalk pickers, the stalks and fibres are attached or absorbed onto one side of the surface of the hub and scraped off on the another side, when they pickers rotate to a transport conveyor. This process enables the black tea processed from mechanically harvested tea leaves in Australia to attain a similar purity (free from stalks) to those teas produced in other countries. Thus, more stalkers than usual are combined and used in this factory. In addition, it takes approximately 175-200 min for a tea sample to pass via the conveyor belt through the whole process (Figure 3.2).

3.4.3 Comparison of CTC teas produced in Australia and other countries

The main difference between the processing of CTC black tea at the factory of Glen Allyn Tea Estates and in the other countries is that this factory does not use the traditional withering method due to a lack of a large space and the intensive labour required. Instead, this factory keeps the freshly harvested green leaves in a bin and blows cold air through the leaves. In this way, the processing of black tea can be operated mechanically, which saves on labour costs and factory space.

3.4.4 Marketing of Australian grown and made tea

The tea processed in the Glen Allyn Tea Estates is sold to the Brisbane Packaging Factory of Nerada Tea Pty. Ltd. The Brisbane Packaging Factory normally requires a slightly over-fermented tea in line with the preference of Australian consumers. Therefore, the tea quality at the factory of Glen Allyn Tea Estates is graded as an average quality (due to being slightly over-fermented) compared with tea produced in other countries. The assessment of the quality of the black tea in the factory is done by sensory evaluation based on comparisons with the standard black tea samples provided by tea buyers (Benson, 2001). Only when the sensory characteristics (e.g. colour and taste) of a tea matches with the standard will the tea buyer be satisfied.

Due to climatic variation during the year, the tea produced during wet seasons is recognised as being of lower quality, but during cool seasons the quality is higher (Benson, 2001). Sometimes the Brisbane Packaging Factory will reject the tea if the quality of the black tea does not meet the minimum quality requirements. Thus, Glen Allyn Tea Estates must maintain the quality that the Brisbane Packaging Factory requires. The tea is then packed according to the market demands and sold as 100 % pure Australian tea bag and/or leaf tea, or sometimes as blended leaf tea when Australian grown tea is less available (Benson, 2001). The black tea in Australia is manufactured mostly for local markets.

3.5 Conclusions

Glen Allyn Tea Estates has developed a unique tea growing and processing system that is characterised by low labour, high mechanisation and high throughput. Thus, the industry is viable and profitable. However, information on the aspects of the black tea process that affects final black tea quality needs researching.

4 Methodology for the analysis of flavonoids and other polyphenols in Australian tea

4.1 Measuring phenolic compounds in teas from Australian markets

4.1.1 Introduction

Traditional methods for the preparation and determination of polyphenols from tea fresh shoots or manufactured tea have been described by several researchers (Roberts *et al*., 1956, 1957; Roberts & Myer, 1958; Roberts & Smith, 1961; Roberts, 1962) and widely used. The most commonly used methods are paper chromatography (Roberts & Wood, 1951, 1953; Oshima & Nakabayashi, 1953a; Roberts *et al*., 1956, 1957; Roberts & Myer, 1958), column chromatography (Oshima & Nakabayashi, 1953b; Whitehead & Temple, 1992) and colorimetric measurement (Oshima & Nakabayashi, 1953c) or spectrophotometric analysis (Roberts & Smith, 1961; Takino *et al*., 1967; Muralidharan, 1997). All those methods are based on the oxidation and reduction properties of tea polyphenols to form a system of colored mixtures. More recently developed analytical techniques have been able to isolate, identify and determine individual polyphenol compounds (Harbowy, 1997) such as high performance liquid chromatography (Roberts *et al*., 1981; Wellum & Kirby, 1981; Robertson & Bendall, 1983; Steinhaus & Engelhardt, 1989; Bailey *et al*., 1991; Temple & Clifford, 1997). However, colorimetric methods are still the most practical in the determination of total phenolic compounds, theaflavins and thearubigins (Bhatia, 1960; Bhatia & Ullah, 1968; Harbowy, 1997; Lakenbrink, 2000). This is because that no other methods can be used to determine those compounds at the same time, particularly the thearubigins, a group of complex compounds, which so far have not been completely isolated and identified.

4.1.2 Materials and methods

4.1.2.1 Tea samples

Leaf tea and teabags commercially available from supermarkets in Queensland, Australia were used, except for one of the leaf tea that was made in Food Science and Technology laboratory (The University of Queensland, Gatton, Australia) from the fresh leaves provided by Glen Allyn Tea Estates (GATE), Malanda, Queensland, Australia. One crude black tea sample also provided by GATE was used for comparison. Teabags were either heat-sealed as in UK type or double chamber US-type. The sampling method used was according to Standard ISO 1839 of the International Standard Organisation (ISO 1980).

The 56 samples included 9 Australian teas. There were 3 green leaf teas, 5 green teabags, 16 black leaf teas and 32 black teabags.

4.1.2.2 Moisture analysis

Tea moisture was measured by vacuum oven according to Standard ISO 1573 of the International Standard Organisation (ISO, 1980).

4.1.2.3 Measurement of total phenolic compounds

The method was based on that of Roberts (1962) and the Handbook of the Chinese National Centre of Tea Quality Control and Inspection (CNC, 1991), with modification from the recent work of some researchers (Yao et al., 1992; Harbowy & Balentine, 1997; Muralidharan, 1997). Details of the method are as follows:

Preparation of the tea solution. 200 mL boiling water was added to 2 g of leaf tea or 1 teabag in a 250 mL conical flask and stirred by a magnetic bar on a heated (~90 °C) hot plate for 10 minutes. After filtration, the tea solution was allowed to cool down to room temperature and then made up to 250 mL with distilled water.

Tartrate solution. 1 g $FeSO_4$ and 5 g $KNaC_4H_4O_6$ were dissolved in distilled water and made up to 1000 mL.

Buffer solution. 23.377 g Na_2HPO_4 was dissolved in distilled water to 1000 mL. 9.078 g KH_2PO_4 was dissolved in distilled water to 1000 mL. 85 % (v/v) Na_2HPO_4 solution and 15 % (v/v) KH_2PO_4 solution were mixed as the buffer solution.

Measurement. 1 mL tea solution, 4 mL water and 5 mL tartrate solution were added in a volumetric flask. The buffer was added to make up the mixture to 25 mL. The mixture was measured using a Pharmacia Ultrospec III uv/visible spectrophotometer at 540 nm.

Calculation. Total polyphenols were calculated as follows:

$$\text{Polyphenols (\%)} = 3.914\ E / 1000 * V_0 / V_1 / W * 100$$

Where E is the reading of the spectrophotometer; V_0 is the total volume of the tea solution (250 mL); V_1 is the volume used for the measurement (1 mL), and W is the dry weight of the tea sample.

4.1.2.4 Measurement of theaflavin (TF), thearubigen (TR) and theabrowin (TB)

The method was based on Roberts's (Roberts & Smith, 1961; Roberts, 1962) description, Physical and Chemical Analysis on Tea Quality (CMC, 1989) and Sensory Evaluation and Inspection of Tea (Zhang, 1987), with modifications from the work of some researchers (Bhatia, 1960; Bhatia & Ullah, 1968; Takeo & Oosawa, 1976; Spiro & Siddique, 1981a, 1981b; Yao *et al*., 1993; Harbowy & Balentine, 1997; Muralidharan, 1997). Details of the method used are as follows:

Extraction. 3 g of leaf tea or 2 tea bags were added to 125 mL boiling water in a 200 mL conical flask and stirred by a magnetic bar on a heated (~90°C) magnetic stirrer for 10 minutes. After filtration, the tea solution was allowed to cool down to room temperature.

Ea. 30 mL tea solution was mixed with 30 mL EtOAc in a separating funnel and shaken for 5 minutes. 2 mL of the EtOAc layer was added with 95 % ethanol to 25 mL.

Ec. 15 mL of the EtOAc layer was mixed with 15 mL 2.5% $NaHCO_3$ and shaken for 30 seconds. The aqueous layer was discarded and 4 mL of the EtOAc layer was added with 95 % ethanol to 25 mL.

Ed. 2 mL of the aqueous layer of Ea was added 2 mL saturated oxalic acid, 6 mL distilled water, and 95 % ethanol to 25 mL.

Eb. 15 mL tea was mixed with 15 mL η-butanol and shaken for 3 min. 2 mL of the aqueous layer was added with 2 mL saturated oxalic acid, 6 mL distilled water, and 95 % ethanol to 25 mL.

Measurement. The absorbency of the above solutions was measured with the Pharmacia Ultrospec III spectrophotometer at 380 nm with 95 % of ethanol as a blank.

Calculation.

TF % = 2.25 x Ec / (1-M)
TR % = 7.06 x (2Ea + 2Ed – 2Eb – Ec) / (1-M)
TB % = 7.06 x 2Eb / (1-M)

Ea, Eb, Ec and Ed are the corresponding readings from the spectrophotometer of the above solutions, while M is the moisture content of the tea sample. If the sample is not 3 g, the calculations should be multiplied by 3 and then divided by the practical weight of sample analysed because this empirical formula was originated from a 3-g basis. The percentage of each compound was calculated on a dry weight basis.

4.2 Development of methods for the analysis of flavonoids and other polyphenols in Australian tea

4.2.1 Introduction

4.2.1.1 The HPLC system for tea analysis

Hoefler and Coggon (1976) first introduced the HPLC system for the analysis of both black and green tea components. A μBondapak C_{18} reversed phase packing (10 μm) column was used with an isocratic elution system. This system was improved by Robertson and Bendall (1983) who replaced the HPLC column with a 5 μm Hypersil ODS column. This replacement of column packing materials from a larger pore size (10 μm) to a much smaller size (5 μm) ensured the introduction of the sample close to the column surface, and hence, maximum separation efficiency could be achieved (Robertson and Bendall, 1983). These researchers also used the isocratic elution system. Although this system provided good separation of the theaflavins, the quantitative reproducibility was still very poor.

The HPLC system provided a very powerful tool for studying black tea chemistry with the addition of photodiode array (PDA) detection in the late 1980s. Since then, the system has been used to analyse the products from model *in vitro* fermentation systems used to study thearubigin chemistry (Opie *et al*., 1988, 1990, 1993, 1995; Powell *et al*., 1993, 1995). Further, Bailey *et al.* (1990, 1991, 1992, 1993, 1994abcd) successfully applied this HPLC system to the analysis of the chemical oxidation of green tea polyphenols in a chemical fermentation model, including a comparison with the enzymatic oxidation products in black tea liquor. Both of the above models employed a similar HPLC system with PDA detection and a gradient elution system. This elution system usually combined an acidified mobile phase with an organic solvent. As a result, the HPLC system was gradually improved and analysis became more productive, diverse and compatible. Therefore, the isolation of closely eluting compounds and separation of polymerised thearubigins have since become possible in the progress of thearubigin chemistry studies.

Due to having an improved HPLC analysis system available, a new type of tea pigment from the chemical oxidation of ECG alone has been separated and identified (Wan *et al*., 1997). Theaflavate A, a benzotropolone derivative formed by the coupling of one galloyl ester group of one ECG molecule to the B ring of another molecule, was isolated from black tea. This finding illustrated the complexity of theaflavin-type compounds in black tea and provided an additional reaction pathway for the formation of thearubigins. In addition, this HPLC analysis system was successfully used to monitor the chemical oxidation of the green tea polyphenols, EGCG and ECG (Yao and Nursten, 1997, 1998), through identification of the polyphenols and their oxidation compounds (Bailey *et al*., 1990, 1991, 1993). These studies suggest that the HPLC system is a useful tool for the analysis of flavonoids and other polyphenols in Australian grown and made tea.

4.2.1.2 *Preparation of tea liquor*

The preparation of the tea extract is a critical step in the analysis. Firstly, solvent extraction of the tea components from the tea leaves may initiate the action of polyphenol oxidase (PPO) that causes the oxidation of tea polyphenols (Hara *et al*., 1995). Secondly, solvent extraction influences the extraction rate of tea components and hence the composition of the mixture subjected to HPLC analysis (Harbowy and Balentine, 1997).

Oxidation of tea polyphenols occurs when the cells that separately contain the substrates (polyphenols) and the enzymes (polyphenol oxidase and other oxidase) are crushed; i.e. the polyphenols come in contact with the enzymes. Furthermore, the tea polyphenols easily form complexations with other tea components when these latter compounds are also extracted (Roberts, 1962), e.g. the formation of "cream down" in the black tea (Roberts, 1963). Both of these are recognised as potential problems that affect analytical results such as the yield and composition of the extracted phenolic components. Thus, the type of solvent, the extraction time, and the type of extraction are considered important in the development of the extraction protocol.

The method of extraction is based on an international standard (ISO 3103, 1980) and previous work (Robertson and Bendall, 1983; Opie *et al*., 1988, 1990, 1994, 1995; Bailey *et al*., 1990, 1991). Approximately 15-20 g of hand plucked green tea leaves (equivalent to about 4-5 g tea on a dry basis) is blended with a solvent. The ratio of solvent to tea leaves on a dry weight basis is about 40:1 (v/w) for all the extractions. Boiling water is usually used for extraction of polyphenols from dried black tea and dried green tea (Roberts, 1962; Bailey *et al*., 1990, 1991, 1992). However, Waterman and Mole (1994) recommended methanol as the choice for the extraction of phenolic compounds from fresh plant tissues. Thus, both boiling water and methanol were used for the method development and its optimisation in the current study.

4.2.2 Materials and methods

4.2.2.1 *Preparation of tea samples*

Fresh green tea leaves consisting of one apical bud and the two adjoining leaves were hand plucked from the fields of the tea farm at Malanda in North Queensland. As soon as possible after collection, the samples were wrapped in washed calico and packed in dry ice in polystyrene foam boxes. Other samples collected from the processing line were placed in plastic bags and packed in a similar way to the fresh green tea leaves. All the samples were then delivered with dry ice to the laboratory at Gatton by overnight cargo flight from Cairns. On arrival, all the samples were stored at −80 °C before analysis. This protocol was developed in an endeavour to prevent oxidation of the tea polyphenols by the polyphenol oxidase present in the fresh green tea leaves.

4.2.2.2 *Chemical solvents*

The solvents used for the extraction of tea samples were analytical grade methanol, Milli-Q (Millipore Australia Pty. Ltd., North Ryde, New South Wales, Australia) distilled water. The solvents used for the HPLC analysis were HPLC grade acetonitrile and acetic acid, Milli-Q distilled water.

4.2.2.3 *Extraction of polyphenols from green tea leaves*

4.2.2.3.1 Extraction of tea components from fresh green tea leaves using boiling water

Green leaves (ca 15 g) were blended with boiling water (180 mL) for 4 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The mixture was filtered through cotton wool and the residue was washed with water (3 × 10 mL). The combined extract was then concentrated to dryness under vaccum using a rotary evaporator at 40 °C. The dry extract was stored at −24 °C prior to HPLC analysis. The storage of tea extracts at −24 °C in a dry form was considered to produce less decomposition of the extracted polyphenols than storing in solution. Storage of

extracts was necessary due to the large number of samples that needed to be analysed later in the main trials.

For the HPLC analysis, re-extraction of the dry extract was required. Selection of the solvents for re-extraction was based on the following considerations. Flavonoid aglycones such as polyphenols are slightly acidic, and are generally moderately soluble in polar solvents such as ethanol, methanol, butanol, acetone, and water (Geissman, 1962; Harborne, 1967, 1975). Combinations of the above organic solvents with water are better solvents for glycosides. The less polar aglycones, such as flavonols, flavanones and the highly methoxylated flavones and flavonols, which tend to be more soluble in solvents such as ether and chloroform, are usually extracted with these solvents including ethyl acetate. The more polar aglycones such as hydroxylated flavones are generally isolated by extraction with acetone, alcohol, water or a combination of water and methanol (Geissman, 1962; Harborne, 1975). A diverse range of flavonoids makes up the polyphenols in tea.

The selected solvents for this trial involving tea flavonoid re-extraction were chloroform, ethyl acetate, methanol and water. Solvent was added to completely dissolve the tea components in the dried solids (obtained from water extract), and each extract was diluted to 25 mL. Each solution was then filtered through a 0.45 μm membrane filter and immediately analysed by HPLC. The whole of the experiment was repeated a total of two times, from the water extraction of fresh green tea leaves, evaporation to dryness and dissolution of the solid with solvent.

4.2.2.3.2 Methanol extraction of tea components from fresh leaves with a drying step

Green leaves (ca 15 g) were blended at room temperature with methanol (180 mL) for 4 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The resultant mixture was filtered through cotton wool, and the residue was washed with methanol (3 × 10 mL). The extracts were combined and then evaporated to dryness under vaccum using a rotary evaporator at 40 °C. The dry extract was stored at −24 °C prior to HPLC analysis. Next, methanol was added to completely dissolve the tea components in the dry extract, and the extract was diluted to 25 mL with methanol. The whole experiment of extraction of the green tea leaves with methanol followed by concentration to dryness and then re-dissolution in methanol was repeated a total of two times. In each trial, the solution was then filtered through a 0.45 μm membrane filter and immediately analysed by HPLC.

4.2.2.3.3 Methanol extraction of tea components from fresh leaves without a drying step

Green leaves (ca 15 g) were blended with methanol (180 mL) for 4 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The volume of the methanol extract was reduced due to evaporation occuring during the blending. The resultant mixture was filtered through cotton wool and the residue was washed thoroughly with methanol (3 × 10 mL). The combined extract was then diluted to 200 mL with methanol. The whole experiment was repeated a total of two times. Each solution was then directly filtered through a 0.45 μm membrane filter and immediately analysed by HPLC.

4.2.2.3.4 Extraction of tea components from fresh leaves with different blending times

Green leaves (ca 15 g) were blended with methanol (180 mL) for 3, 4, 5, 6 or 7 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The resultant mixture was filtered through cotton wool and the residue was washed thoroughly with methanol (3 × 10 mL). The combined extract was diluted to 200 mL with methanol. Each solution was then directly filtered through a 0.45 μm membrane filter and immediately analysed by HPLC. Each blending time was repeated four times on successive days in a completely randomised design (i.e. 5 extraction times × 4 replications).

4.2.2.3.5 Test of enzymatic activity

Polyphenol oxidase is the main enzyme in green tea leaves, whereas peroxidase is the most heat stable enzyme of the tea enzyme systems (Roberts, 1952, 1962; Cloughley, 1980b, 1981a). The enzymatic activity was examined by using guaiacol (*o*-methoxyphenol) as an indicator as well as a reductive reagent. The method used was described by Miller (1998) and Gullet *et al*. (1984) and is detailed as follows.

Green tea leaves (ca 15 g) were blended with methanol (180 mL) for 0.5, 1, 2, 3, 4, 5, 6, and 7 min, respectively. Each solution was then filtered through cotton wool and the residue was washed with methanol (3 × 10 mL).

The extract (2 mL) was added to distilled water (20 mL) for the control. The extract (2 mL) was added to distilled water (20 mL) for the test. Guaiacol solution (0.5 % in 50 % ethanol) (1 mL) and hydrogen peroxide solution (0.8 %; 1 mL) were added to the test solution. Peroxidase activity was indicated by the development of a reddish colour. If no colour developed within 3.5 min, there was no peroxidase activity, thus no enzymatic activity remaining in the solution, and thus in the green tea leaf extract. The whole experiment was repeated two times for each of the 8 blending times (i.e. 8 blending times × 2 replications).

4.2.2.3.6 Repeatability of the methanol extraction method

The extraction described in 4.2.2.3.3, but with a 5 min blending time was repeated seven times using seven sub-samples of green leaves from the same gross sample. Each extract was filtered through a 0.45 μm membrane filter and immediately analysed by HPLC. Standard deviation, means and coefficient of variance [CV % = (standard deviation/mean) × 100] for the levels (mg/g) of the compounds EC, ECG, EGC, EGCG and caffeine, and for the total levels of these compounds in the extract were compared.

4.2.2.3.7 Recovery trials for the main components in the methanol extraction method

Green leaves (ca 15 g) and five added standards were blended with methanol (180 mL) for 5 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The resultant mixture was filtered through cotton wool and the residue was washed thoroughly with methanol (3 × 10 mL). The combined extract was diluted to 200 mL with methanol. Each solution was then directly filtered through a 0.45 μm membrane filter and immediately analysed by HPLC. The standards added were the four principal tea compounds, ECG, EGCG, gallic acid and caffeine, and one minor tea component coumarin. The amounts of each that standard were added were: ECG 50 mg; EGCG 80 mg; gallic acid 80 mg; caffeine 80 mg; coumarin 50 mg. The whole experiment was repeated three times on three successive days in a completely randomised design.

4.2.2.3.8 Final optimised method for the extraction of tea flavonoids and other polyphenols from green leaves from the field

The green tea leaves from the field were defrosted sufficiently to allow separation of the mass before extraction. Green leaves (ca 15 g) were blended with methanol (180 mL) for 5 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The resultant mixture was filtered through cotton wool and the residue was washed thoroughly with methanol (3 × 10 mL). The extract was combined and diluted to 200 mL with methanol. The solution was then directly filtered through a 0.45 μm membrane filter and immediately analysed by HPLC. (It is noteworthy that after the blending of green leaves with methanol, the total volume of 180 mL methanol is not recovered because of evaporation that occurs during the blending and the absorption of methanol by the mass. Thus, thorough washing of the residue with methanol is needed). This procedure can be used for extracting fresh green tea leaves and for extracting in-line samples taken off the processing line from the Bin (green leaves) to Rotorvane (Figure 3.2).

4.2.2.4 Extraction of tea flavonoids and other polyphenols from samples off the black tea processing line

Tea samples collected from the processing line (only from the CTC stage onwards) were defrosted sufficiently to allow separation of the mass before extraction. The extraction method was a modification of a method based on previous work (Bailey *et al*., 1990, 1991; Finger *et al*., 1991ab, 1992, 1994; Kuhr and Engelhardt, 1991; Ding *et al*., 1992; Engelhardt *et al*., 1992, 1993; Degenhardt, 2000ab, 2001). The modified method is as follows. The in-line sample (ca 10 g) was extracted with 100 % (v/v) methanol (100 mL) for 10 min at 40 °C in a rotary evaporator without vacuum. The extract was then filtered through cotton wool, and the residue was continuously extracted with 70 % (v/v) aqueous methanol (2 × 50 mL) at 40 °C in the rotary evaporator without vacuum. The extract was then filtered through cotton wool. The final residue was washed with 70 % (v/v) aqueous methanol (3 × 10 mL). All the extracts were combined, cooled and diluted to 200 mL with 70 % (v/v) aqueous methanol. The solution was passed through a 0.45 μm membrane filter and immediately analysed by HPLC.

4.2.2.5 Moisture analysis and freeze-drying

Moisture content for tea samples from the field and factory was determined based on international standards (ISO 1573 and ISO 9768) and AOAC Official Methods No. 925.19 and No. 934.01 (Cunniff, 1995), with the exception that the temperature of the vaccum oven was 75 °C and the oven was operated at a negative pressure of 65 kilopascals (kPa). After extraction and moisture measurement, the unused frozen samples from the field and from the factory were dried to a moisture content below 0.5 % (w/w) using a freeze dryer (Lindner & May Pty. Ltd., Brisbane, Australia) and then stored in the cold room at 4°C.

4.2.2.6 HPLC analysis of tea flavonoids and other polyphenols

4.2.2.6.1 Chemical standards for flavonoids and other polyphenols

The standard tea components used in this study, with their source are listed in Table 4.1.

Table 4.1 Chemical standard compounds for tea.

Common name	Abbreviation	Source
Caffeine	-	1, 2
Caffeic acid	-	1, 2
Catechin	C	3
Chlorogenic acid	CA	2
Catechin gallate	CG	3
Coumarin	-	1
p-Coumaric acid	-	1
Epicatechin	EC	2, 3
Epicatechin gallate	ECG	1, 3
Epigallocatechin	EGC	3, 4
Epigallocatechin gallate	EGCG	1, 3
Gallic acid	GA	1
Gallocatechin	GC	3
Gallocatechin gallate	GCG	3
3-(*p*-hydroxyphenyl)-propionic acid	3PA	1
Kaempferol	-	1
Theaflavin	TF	3
Theaflavin 3-gallate	TF3G	3
Theaflavin 3′-gallate	TF3′G	3
Theaflavin 3,3′-digallate	TFDG	3
Theobromine	THB	1

Source: 1. College of Food Science, Southwest Agricultural University, Chongqing, China. 2. Sigma Chemical Co., St Louis, MO, USA. 3. Food Research Laboratories, Tokyo Food Techno. Co. Ltd. (Mitsui Norin Co. Ltd.), Miyabara, Fujieda, Shizuoka, Japan. 4. Tea Research Institute, Chinese Agricultural Academy, Zhejiang, China.

4.2.2.6.2 HPLC analysis system for flavonoids and other polyphenols

A Shimadzu Class-VP HPLC control system was used for this study consisting of a computer-controlled system with the Class-VP 5.03 software and an SCL-10A VP System controller. The HPLC system consists of a Shimadzu GT-104 Degasser, an FCV-10AL Mixer, two LC-10AD Shimadzu Liquid Chromatography Pumps, an SIL-10a XL Auto Injector, a CTO-10A Column Oven, and an SPD-M10A VP Diode Array Detector.

The Shimadzu Class-VP 5.03 software used for the HPLC system with a photodiode array (PDA) detector in this study can collect information from one chromatographic run as follows: (1) a UV or UV/VIS spectrum at any retention time, which provides a complete UV/VIS spectrum for each data point, including each selected peak; (2) a three-dimensional plot (absorbance versus time versus wavelength), which permits monitoring of the absorption profile of a selected peak at any wavelength as the elution progresses or after the elution; (3) a chromatogram at any wavelength, which permits comparison of the chromatograms of all the peaks eluted from a complex of compounds at any wavelength of interest to justify the chromatogram for analytical purposes; (4) mixed view of the above 3 types of information, which allows loading of a particular wavelength at a particular elution time to obtain the above information for a particular peak; (5) peak purity testing, which provides information about the resolution of a peak within a particular elution system and how much the peak spectrum matches a standard spectrum of a particular compound so that the user of this HPLC can integrate the peak in the chromatogram to ensure the identification to be accurate.

In addition, it permits the following manipulations to be done: (1) superimposition of chromatograms at different wavelengths, which allows comparison of the chromatographic behaviour of all the compounds at different wavelengths during or after the elution; (2) comparison of spectra for components within a chromatogram with those of standards, which permits matches of the spectra for the unknown compounds to those for standards for identification on-line and/or off-line; (3) storage of spectra in a library, which enables spectra extracted from standards and/or newly identified compounds to be stored in a spectrum library as a database for future use, particularly, for the analysis carried out with a similar elution system and under similar conditions (e.g. column, oven temperature, solvents, gradient, flow rate and PDA detection parameters); (4) integration and re-integration of chromatograms, which allows the splitting and integrating of selected peaks close to one another, based on the purity of a peak, its elution time, absorption threshold, width, separation (e.g. valley to valley) status and its resolution status in the chromatogram. It can be done by a combination of manual and automatic analysis (by software when the parameter imported). This integration program is more or less an experience dependent operation of HPLC in some critical analysis. Particularly, for the less resolved and small asymmetrical peaks, manual integration and peak purity control are required. Peak purity control or testing includes the following parameters to compute the purity of a defined peak: wavelength range and interval, sensitivity (to modify the impact of the noise spectrum), purity index mode (e.g. graph mode with similarity plus threshold), background compensation. Once the peaks are defined, manual splitting of conjugated peaks by enlarging them in the chromatogram, plotting their start and stop times, inputting events of threshold, shoulder sensitivity, and peak maxima are followed. Then, the software computing program will calculate the requested analytical data for these peaks and export them separately; (5) calculation of spectral derivatives, which can provide information on peaks with similar spectrum and how much the derivation of these peaks (compounds) reflecting in their absorption shift (derivatisation) in the spectra; (6) opening of the current data at the same time as the data in stored files so that specific chromatographic comparison can be done.

The column used for the analysis was a Hypersil ODS S5 250 × 4.6 mm reversed phase HPLC column (TheroQuest Hypersil, Runcorn, Cheshire, UK). A 10 × 4 mm guard column packed with Exsil ODS 5 µm packing material and contained in a 10GCH-guard cartridge holder (SGE Exsil, Ringwood, Victoria, Australia) was used.

The spectroscopic data were detected from 220 nm to 600 nm, and chromatograms were monitored at 280, 310, 340, 380, 450 and 510 nm. The temperature of the column oven was 35 °C. Mobile phase A = 2 % aqueous acetic acid and mobile phase B = 100 % acetonitrile. Gradient elution was programmed from 8 % to 31 % B over 50 min, then to 100 % B over 2 min, maintained at 100 % B for 3 min, and then returned to 8 % B for 10 min. The flow rate was 1.2 mL/min. An auto injector was used to inject 20 µL of the test solution into the HPLC system.

4.2.2.6.3 Identification of the tea flavonoids and other polyphenols

By utilising linear PDA technology, the multiwavelength detector can monitor continuously between 190 and 800 nm, which enables the UV and visible (VIS) regions to be monitored simultaneously. Identification of tea polyphenols was done using multi-wavelength detection and the UV/VIS spectra reported in the literature (Opie *et al*., 1988, 1990, 1993, 1995; Bailey *et al*., 1990, 1991, 1992, 1993, 1994abcd; Powell *et al*., 1993, 1995). The PDA detector was used in the role of a coupled chromatographic-spectroscopic technique to obtain information about the complex tea liquor analysed during this study. The retention time and the spectrum of each peak were proved to be very strong evidence for the identity of an unknown compound (Bailey *et al*., 1990, 1991, 1992, 1993; Opie *et al*., 1990, 1993, 1995; Powell *et al*., 1993, 1995). Thus, criteria for the identification of tea compounds were established based on comparisons of the retention time and spectrum of an unknown compound with the previously generated HPLC data library of standards. The purity of each peak was determined to ensure correct identification. For closely eluting peaks in the chromatogram, an integration program in the HPLC software was used to split the peaks and produce data for the calculation. For example:

(1) for well resolved or symmetric peaks, such as peaks 1, 6, 10, 13, 18 in Figure 5.2, the spectrum match of an unknown compound was at a level of 99 % or above against the spectrum of a standard compound;
(2) for less resolved or asymmetric peaks, such as the pairs of peaks 7 and 8, 14 and 15, enlargement of these peaks and then manual splitting them with the peak purity control program were used. Following this process, re-integration of each peak was applied by using the chromatographic program. All of these peaks were identified by their spectral matches at a level of 90 % or above with the spectra of their standards. For those of spectral matches less than 90 %, the peaks were not included in the identification for further analysis.

4.2.2.6.4 Quantification of the tea flavonoids and other polyphenols

The quantification of the tea components was done using the external standard method. It was based on the use of response factors (= concentration of standard / peak area of standard) determined for solutions of various standards. A solution of each standard compound was injected into the HPLC system and the peak area and thus the response was recorded under the same operating system and conditions as for the samples. Concentration of a compound = peak area of the compound × response factor (Table 4.2).

Eight catechins and catechin gallates (C, CG, EC, ECG, EGC, EGCG, GC and GCG) and four black tea theaflavins (TF, TF3G, TF3′G and TFDG) were quantified using the response factors of authentic standards (Table 4.2). In addition, gallic acid, caffeine and theobromine were quantified against authentic compounds using response factors (Table 4.2). For compounds where no authentic standards were available, selection of the reference standard was based on its chromatographic and spectroscopic behavior under the same conditions (Table 4.2).

Table 4.2 Response factor and their use for quantification of chemical compounds in tea.

Standard compound	Response factor	Compound quantified
Catechin (C)	1.10×10^{-7}	C
CG Catechin gallate (CG)	2.72×10^{-8}	CG
Epicatechin (EC)	9.15×10^{-8}	EC
Epicatechin gallate (ECG)	4.09×10^{-8}	ECG, EGDG
Epigallocatechin (EGC)	3.38×10^{-7}	EGC
Epigallocatechin gallate (EGCG)	5.27×10^{-8}	EGCG, EGCDG
Gallocatechin (GC)	1.09×10^{-7}	GC
Gallocatechin gallate (GCG)	4.55×10^{-8}	GCG, theogallin
Theaflavin (TF)	5.44×10^{-8}	TF
Theaflavin 3-gallate (TF3G)	4.38×10^{-8}	TF3G
Theaflavin 3′-gallate (TF3′G)	4.79×10^{-8}	TF3′G
Theaflavin 3,3′-digallate (TFDG)	3.66×10^{-8}	TFDG
Gallic acid	3.05×10^{-8}	Gallic acid
Caffeine	3.11×10^{-8}	Caffeine
Theobromine	4.79×10^{-8}	Theobromine
Caffeic acid	1.71×10^{-8}	*p*-Coumaryl quinic acid
p-Coumaric acid	1.14×10^{-8}	*p*-Coumaric acid
Chlorogenic acid	3.73×10^{-8}	Chlorogenic and isochlorogenic acids
Kaempferol	3.27×10^{-8}	QG, Q3G, Q3RG, KG, K3RG
3-(*p*-Hydroxyphenyl)-propionic acid	1.02×10^{-7}	3-(*p*-Hydroxyphenyl)-propionic acid

4.3 Analysis of flavonoids and other phenolic compounds in fresh Australian grown tea leaves

4.3.1 Introduction

Fresh tea leaves are rich in the flavanol group of phenolic compounds known generally as catechins, which usually refers to both catechins and catechin gallates. Tea of the highest quality has been found to be made from young shoots consisting of the tender bud and first two leaves (Baruah *et al*., 1986). This portion of tea shoots contains the highest level of catechins and the gallates. The content of total catechins and catechin gallates decreases as the leaf ages (Bokuchava and Skobeleva, 1969). In a tea, catechins and catechin gallates may constitute up to 30 % of the dry leaf weight (Millin and Rustige, 1967; Graham, 1992; Wang, 1994). The differences in the chemical composition, including phenolic compounds, of young shoots have been considered to be the reason for the differences in the quality of the tea processed from these green leaves (Nakagawa and Torri, 1964ab; Hara *et al*., 1995). The content of total polyphenols in tea shoots grown in Kenya has been found to correlate significantly with Kenyan plain tea quality parameters (Obanda *et al*., 1992). Clones with low total polyphenol content may produce low quality black teas, and *vice versa* (Obanda *et al*., 1997ab). The levels of total polyphenols are also important to black tea quality and are affected by the levels occurring in the fresh tea shoots grown in other countries in the world (Hara *et al*., 1995; Harbowy and Balentine, 1997). Thus, the total polyphenol content of fresh tea shoots has been proposed as a reliable parameter for determining the quality of black teas (Obanda *et al*., 1997ab). No such total polyphenol data on fresh tea shoots for Australian grown tea exist to make such a determination.

Of all the polyphenols, both epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) in fresh tea shoots have been proposed as quality indicators for black tea production in Kenya (Obanda *et al*., 1997ab). Firstly, this is because they are the main flavanols present in green tea leaves. Secondly, the level of preferences of tea tasters for black teas shows a significant, positive correlation with the level of these two compounds in the original green leaves (Obanda *et al*., 1997ab). ECG, EGCG and theogallin of black tea were found to be the main guiding constituents of a desirable tea from the northeastern Indian plains (Biswas *et al*., 1971). The levels of the main flavanols, EGCG and ECG, fall sharply during the aging process of tea shoots, whereas the levels of another two catechins epigallocatechin (EGC) and epicatechin (EC) rise slightly (Bokuchava and Skobeleva, 1969). The biochemical changes occuring in the tea shoots may thus affect the quality of produced black tea (Baruah *et al*., 1986). The levels of these particular polyphenols, which are so important to black tea quality (Bokuchava and Skobeleva, 1969; Biswas *et al*., 1973; Graham, 1992; Obanda *et al*., 1997ab) are not known for tea grown in Australia. Such data could be useful for monitoring the quality of tea over a growing season, and enable Australian tea producers to know at what time of the year the highest quality black tea is likely to be produced from their green leaves in the field.

Clonal studies show that the pattern of distribution of polyphenols appears to be more or less constant in a clone, whereas the phenolic patterns vary a great deal among individual tea bushes of various clones (Bhatia, 1962, 1963). Despite marked changes in the quantities of polyphenols, Bhatia (1962, 1963) noted that the relative proportions of certain important polyphenols did not vary a great deal from their mean values. Thus, studies on the phenolic composition of tea shoots could assist in the identification of the tea clones, which would enable the Australian tea industry to select the appropriate tea clones for producing teas with constant and high quality (Chudleigh, 1999).

Studies in central Africa have shown that the concentration of flavanols in fresh apical shoots of tea was highest during the cold season (Hilton *et al*., 1973). Tea shoots plucked during slow growth conditions such as in the winter contained a higher proportion of simple catechins relative to catechin gallates, with EGC being the most significantly affected (Hilton *et al*., 1973). In contrast, in the Northern Hemisphere, total flavanol content is the greatest during the height of the growing season (i.e. summer) and the least at the end of the season (late autumn) (Hilton *et al*., 1973). Hilton (1972) reported a direct relationship between the level of EGC in a shoot and the total level of theaflavins in the black tea produced. Thus, the seasonal variations of EGC in green leaves may be regarded as a chemical basis for seasonal variations in made tea quality in central Africa. Nakagawa and Torri

(1964a) reported that EGC, along with ECG and EGCG, were the main flavanols in green tea shoots for both *Camellia sinensis* var. *sinensis* and *Camellia sinensis* var. *assamica* grown in Japan, with EGCG predominating. EGC showed a higher level in spring than in summer, while ECG and EGCG showed higher levels in summer than in spring (Nakagawa and Torri, 1964b). Further, it has been found that the levels of ECG and EGCG are higher in young and tender shoots, while EGC is higher in the fully developed shoots (Nakagawa and Torri, 1964b). This variation of leaf flavanol constituents has been suggested as the main factor affecting the quality of resulting tea (Nakagawa and Torri, 1964ab; Bokuchava and Skobeleva, 1969; Biswas *et al*., 1973; Graham, 1992; Obanda *et al*., 1997ab). Thus, data on the EGCG, ECG and EGC levels in green shoots of tea grown in Australia may be able to be used to monitor seasonal variations in the quality of the resultant black tea. However, no such data presently exists.

The tea polyphenols, theaflavins, impart to black tea the distinct organoleptic properties of colour and taste. Their variations affect the overall quality of the made tea (Hazarika *et al*., 1984b). The level of total theaflavins of the resultant black teas is highly correlated to the content of EGC in the shoots (Hilton, 1972). However, this result does not represent the contribution of the other flavanols to the formation of theaflavins, since there are only two out of ten individual theaflavins that involve EGC during their formation (Hilton, 1972; Hilton and Ellis, 1972; Hilton and Palmer-Jones, 1973). Under field conditions, the phenolic composition of tea shoots varies considerably with the effect of seasonal, genetic and agronomic factors (Hilton and Palmer-Jones, 1973). In his early work, Roberts (1958ab) found that EGC and EGCG were responsible for the formation of theaflavins. Bryce *et al*. (1970), Coxon *et al*. (1970abc) and Sanderson *et al*. (1972) found that more than seven flavanols (C, EC, CG, ECG, GC, EGC and EGCG) and one phenolic (gallic acid) in the fresh tea shoots were responsible for the formation of various of theaflavins. Therefore, the quality of black tea is dependent in the first instance on the chemical composition of the harvested shoots and subsequently on the way in which they are handled, processed, and stored (Millin, 1987). There is no published information on the chemical composition of Australian grown tea, thus, it is important to collect this data and compare the findings with those previously reported in other countries.

The aims of this study were to determine the principal individual flavonoids, total catechins, total catechin gallates, and total phenolic compounds in fresh leaves of Australian grown tea and to determine the variations of those compounds among the locations of the plucking area and over a full year of production. The variations in the compounds between hand plucked and mechanically harvested fresh tea leaves were also compared. The results of this study provide information to assist in the processing of high quality black tea in Australia.

4.3.2 Materials and methods

4.3.2.1 Sampling of green tea leaves from the field

4.3.2.1.1 Harvest date

Green tea leaves were collected from the farm of Glen Allyn Tea Estates, Malanda, North Queensland, at three-weekly intervals, except during the winter seasons, when the harvest was at four to five weeks apart, from April 2000 to May 2001, in accordance with the commercial harvest by the factory (Table 4.3). Collection of the samples by hand plucking was conducted just before each commercial mechanical harvest.

Table 4.3 Experimental harvest dates.

Month and year	Location and harvest date		
	Paddock A	Paddock B	Paddock C
April 2000	12	11	–
May 2000	9	9	–
June 2000	*	*	–
July 2000	19	19	–
August 2000	23	23	–
September 2000	28	27	27
October 2000	24	23	17
November 2000	14	13	6 & 28
December 2000	6 & 28	6 & 27	20
January 2001	16	15	9 & 31
February 2001	2 & 27	1 & 22	20
March 2001	20	19	14
April 2001	10	9	3 & 30
May 2001	8	2	–

* The harvested tea green leaves were dumped due to being burnt by frost.

4.3.2.1.2 Sampling location and sampling points

The sampling locations (Table 4.3) were selected on the basis of the age, cultural management, clones of tea trees, and the geological location of the paddocks. For instance, the paddocks selected as sampling locations should not have been skiffed in the past 6 months or pruned in the past 12 months, and would not be skiffed or pruned during the sampling period under Glen Allyn Tea Estates normal production schedules. This is because it takes time for the new shoots to come back to normal harvest after skiffing or pruning, although the quality of resulting black tea from the new round of tea shoot would be markedly improved (Owuor and Langat, 1988; Dev Choudhury, 1991).

The criteria for the sampling points within each location were as follows. Firstly, each sampling point covered about 10 m^2 of tea bushes to ensure enough quantity of shoots for selection as representatives at each harvest. Secondly, the sampling points were at least 80 m away from the edge or corner of the paddock to avoid being affected by any other agricultural activities that occurred during the sampling period. Thirdly, the sampling points were marked with red plastic ribbons on the tea bushes and nearby fences or signs to ensure that the samples were collected from the same bushes at each harvest. Fourthly, the sampling points were selected with reference to the area of the paddock, and the distance between points was 500 m within each location.

4.3.2.1.3 Sampling plan

Initially, two paddocks called Paddock A and Paddock B were selected as the two locations for sampling from April to August 2000. Each location consisted of three sampling points (A1-A3 and B1-B3). However, based on the results from the preliminary chemical analysis, from September 2000 onwards a third location in a paddock called Paddock C was included in the sampling plan and then only two sampling points were included in each of the three sampling locations. From September 2000, the sampling points were: A1 and A3; B1 and B3; and C1 and C2.

- **Hand plucked samples**: within each sampling point (ca 10 m^2) within each location at each harvest time, one gross sample of ca 200 g of green shoots consisting of one apical bud and two expanded leaves were randomly hand plucked from designated bushes.

- **Mechanically harvested samples**: at each harvest time, green tea leaves mechanically harvested at each location were randomly sampled from the harvester bin at five different points to obtain the gross sample (ca 2 kg). This gross sample was then well mixed and a sample of about 500 g green tea leaves was randomly taken from the 2 kg gross samples for the April to August 2000 harvests to ensure enough leaves for the analysis. As the analysis progressed, the quantity of each sample taken was reduced from 500 g to 250 g for the months, September 2000 to May 2001, as this was later found to be sufficient for analysis.

More locations for the hand plucking would have been desirable. However, hand plucking is extremely labour intensive and time consuming, and done in difficult circumstances. For example, often hand plucking was done in water up to ones knees and containing leeches during the rainy season. In addition, the rows of tea bushes on the farm of Glen Allyn Tea Estates are very close together, making movement on foot difficult. Thus, it was impractical to sample more than the number of selected locations and sampling points.

4.3.2.1.4 Statistical design

The statistical design for the experiment was a nested design comprising 3 or 2 locations and 2 or 3 sampling points respectively in each location over a period of fifteen harvest times.

- From April to August 2000: 2 locations × 3 sampling points × 1 sample, over 4 harvest times;
- From September 2000 to May 2001: 3 locations × 2 sampling points × 1 sample, over 11 harvest times.

4.3.2.2 Sample preparation

At each harvest, the samples of both hand plucked fresh leaves and mechanically harvested green leaves were separately wrapped in washed calico, then labeled and packed in dry ice in a polystyrene foam box. The samples were then delivered with sufficient dry ice by overnight cargo flight from Cairns to Brisbane and then road transport from Brisbane to the laboratory in Gatton. Sufficient dry ice was still present to keep the samples frozen. After arrival, the samples were stored in a freezer at −80 °C before analysis.

4.3.2.3 Determination of moisture content

The moisture content of tea samples was determined according to the method detailed in Section 4.2.2.5. These moisture contents are attached in the Appendix 5.1.

4.3.2.4 Extraction of green tea leaves

The green tea leaves from the field were defrosted sufficiently to allow separation of the mass before extraction. Green leaves (ca 15 g) were blended with methanol (180 mL) for 5 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan, 2 L). The resultant mixture was filtered through cotton wool and the residue was washed thoroughly with methanol (3 × 10 mL). The extract was combined and diluted to 200 mL with methanol. The solution was then directly filtered through a 0.45 μm membrane filter and immediately analysed by HPLC (Section 4.2.2.3.8).

4.3.2.5 HPLC analysis

For each treatment, the HPLC analyses were carried out on two identical subsamples. Samples of tea leaves were extracted according to the method detailed in Section 4.3.2.4. The extract was analysed using the HPLC system according to the method outlined in Section 4.2.2.6.2. A number of polyphenols were analysed as detailed below.

4.3.2.5.1 Epigallocatechin gallate (EGCG)

Epigallocatechin gallate (EGCG) in the sample solutions was identified by comparing the UV spectrum and retention time to those of a solution of an authentic compound, as described in Section 4.2.6.3. Quantification of EGCG was according to UV absorption against a standard solution using the method detailed in Section 4.2.2.6.4.

4.3.2.5.2 Epicatechin gallate (ECG)

Identification and quantification of epicatechin gallate (ECG) in the sample solutions were done conducted by comparing the UV absorption and retention time to those of a solution of an authentic compound as described in Sections 4.2.2.6.3 and 4.2.2.6.4.

4.3.2.5.3 Epigallocatechin (EGC)

Identification and quantification of epigallocatechin (EGC) in the sample solutions were done by comparing the UV spectrum and retention time to those of a solution of an authentic compound as described in Sections 4.2.2.6.3 and 4.2.2.6.4.

4.3.2.5.4 Total catechins

The analysis of total catechins included the four compounds, catechin (C), epicatechin (EC), gallocatechin (GC) and epigallocatechin (EGC). Identification and quantification of these compounds in the sample solutions were done by comparing the UV absorption and retention times to those of solutions of authentic compounds as described in Sections 4.2.2.6.3 and 4.2.2.6.4.

4.3.2.5.5 Total catechin gallates

The analysis of total catechin gallates included the six galloyl catechin compounds, catechin gallate (CG), epicatechin gallate (ECG), epicatechin digallate (ECDG), gallocatechin gallate (GCG), epigallocatechin gallate (EGCG) and epigallocatechin digallate (EGCDG). Identification and quantification of these compounds in the sample solutions were done by comparing the UV absorption and retention times to those of solutions of authentic compounds as described in Sections 4.2.2.6.3 and 4.2.2.6.4.

4.3.2.5.6 Combined catechins/catechin gallates

The content of combined catechins/catechin gallates was determined by summing the contents of the four catechins and the six catechin gallates detailed above in Sections 4.3.2.4.4 and 4.3.2.4.5.

4.3.2.5.7 Total phenolic compounds

The content of total phenolic compounds was determined by summing the content of the above mentioned four catechins and six catechin gallates, and five flavonol glycosides and seven simple phenolic compounds. The five flavonol glycosides determined were kaempferol glycoside (KG), kaempferol 3-rhamnosylglucoside (K3RG), quercetin glycoside (QG), quercetin 3-glucoside (Q3G)

and quercetin 3-rhamnosylglucoside (Q3RG). The seven simple phenolic compounds determined were gallic acid (GA), theogallin (THG), iso-chlorogenic acid (5-*o*-caffeoylquinic acid) (IsoCA), *p*-coumaryl quinic acid, chlorogenic acid (3-*o*-caffeoylquinic acid) (CA), 3-(*p*-hydroxyphenyl)-propionic acid and *p*-coumaric acid. Identification and quantification of these compounds in the sample solutions were done by comparing the UV/VIS absorption and retention times to those of solutions of authentic compounds using the methods described in Sections 4.2.2.6.3 and 4.2.2.6.4.

4.3.2.6 Statistical analysis

Prior to analysis, data from duplicate samples were averaged. The data for each individual compound or grouped compounds were then subjected to an analysis of variance (ANOVA). Initially a nested model was used allowing for variation between locations and between sampling points within each location. However, this showed that there was as much variation between sample points as there was between locations. Accordingly, a simpler two-way ANOVA model was adopted with 6 unique sample locations and 15 sample times. The statistical adequacy of the model was checked and for all variables where significant F-values ($P < 0.05$) were found, comparisons of least square means were carried out using Fisher's least significant difference (LSD) procedure.

The standard error of each mean was obtained by dividing the pooled standard deviation from the ANOVA by the square root of the number of replicates.

4.4 In-line analysis of flavonoids and other polyphenols during the processing of Australian black tea

4.4.1 Introduction

Black tea in the classical sense refers to the tea that is processed from "the leaves, buds or tender shoots of the tea bush" (Schultert and Gunther, 1998). The flavour (taste and aroma) of black tea is primarily determined by factors mainly consisting of the following four aspects. Firstly, genetic factors such as clones or varieties of the tea bushes determines the types and amounts of useful constituents in the green leaves (Millin, 1987). Secondly, there are environmental factors such as altitude, climate and soil in the growing region (Millin, 1987; Schultert and Gunther, 1998). Thirdly, a number of agronomic factors such as farm management, harvesting and fertiliser applications are important (Millin, 1987). Fourthly, factory practice determines black tea quality (Cloughley and Ellis, 1980; Roberts and Chandradasa, 1982; Millin, 1987; Schultert and Gunther, 1998). Of these four factors, the first and the second are generally considered as uncontrollable factors for tea production, whereas the third and the fourth are controllable factors. Therefore, once the other quality factors (genetic, environmental and agronomic) have been more or less fixed, the processing or factory practice of tea production is considered a critical factor for producing high quality black tea.

After harvesting from tea bushes, the green leaves usually undergo a process called "processing" to produce black tea. The processing of black tea includes withering, preconditioning, rolling, fermentation and drying (Hara *et al*., 1995). During the black tea processing, the quality attributes of black tea are formed gradually by various chemical and biochemical reactions in the leaf. The most complex reactions occur during the fermentation period, with many chemical constituents important to black tea quality formed, including the complicated theaflavins and a group of much more complicated and poorly elucidated polymers with the collective name of thearubigins. As a summary of the studies conducted from 1930s to the early 1960s, Roberts and Smith (1963) considered the theaflavin content as an extremely important factor for black tea quality. High levels of theaflavins and thearubigins in black teas result from the relatively high concentrations of polyphenols in the tender tea shoots (Forrest and Bendall, 1969). Theaflavins and thearubigins influence the colour, taste and other black tea liquor characteristics (Roberts and Smith, 1961; Roberts, 1962). The composition of these compounds in black tea is not affected by the market fluctuations and/or tea taster's preferences. Therefore, chemical analysis of theaflavins and thearubigins is a more preferable and desirable means of maintaining records of the properties of processed teas (Roberts and Smith, 1961). Bailey *et al*. (1990, 1991) and Opie *et al*. (1990, 1992) used HPLC with photodiode array detection for the

measurement of theaflavins and thearubigins. In conclusion, theaflavins have become regarded as the best objective indicators for the quality and value of black tea, with analysis of theaflavins being an objective way of monitoring the quality of black tea, and a way of aiding the control of its processing.

The factors affecting the quality of fresh green tea leaves may also affect the processing of the tea. It has been found that the rate of fermentation is profoundly influenced by the genetic constitution of the tea bush, seasonal and climatic factors, agronomic and management practices, and systems of processing (Cloughley, 1979). In the summer, high temperatures usually accompany fast fermentation. Thus, it is necessary to adjust the temperature and/or the fermentation time in the factory to control the progress of black tea processing to ensure good quality. Cloughley (1980a) indicated that fermentation at 15 °C for a certain time (e.g. 100 min) might achieve desirable amounts of theaflavins in the resulting black tea. However, warm fermentation conditions (e.g. 35 ˚C) within a selected time (e.g. 45 min) may enhance the formation of theaflavins and thearubigins, thus increasing the total quality of black tea (Obanda and Owuor, 1993). In current factory practices, it has been noted that longer fermentation at higher temperature generates more intensely coloured black teas, with higher thearubigin and lower theaflavin concentration. Thus, fermentation at a low temperature (20-25 ˚C) during tea processing is recommended and widely used in many of the tea producing countries (Owuor and Obanda, 2001). In the Australian black tea processing, the temperature is 25-30 ˚C, which is high compared to high quality black tea processing throughout the world. In conclusion, the formation of theaflavins is recognised as a quality parameter for the control of fermentation, regardless of the temperature, time and processing method.

The formation and further oxidation of theaflavins mainly occurs during the fermentation period of black tea processing, so the accurate and objective determination of the optimum fermentation time in the factory will eventually produce tea of great economic value (Cloughley, 1979). In Malawi, central Africa, the monitoring of theaflavins using in-line analysis is used to optimise fermentation during tea processing, whereas in Kenya, theaflavin analysis of the final black tea is used to determine the optimum fermentation time (Owuor, 1984; Owuor and Reeves, 1986). In India, a reduction in the level of principal flavanol, EGCG in green leaves has been used to determine the optimum fermentation time and conditions of the processing (Owuor, 1984). This is because consumption of EGCG is associated with the formation of theaflavins. The youngest plants usually accumulate the greatest levels of catechins and catechin gallates, and these plants have been used as the basis for producing black tea with higher theaflavins levels, and thus of higher quality (Escarpa and Gonzalez, 2001).

Roberts and Smith (1963) reported that the levels of theaflavins, thearubigins and soluble solids in CTC teas fell as the harvest time proceeded. In Kenya, it has also been found that the levels of theaflavins in black tea varied with harvest times of the green leaves, whereas no clear trend was evident due to the application of fertiliser (Owuor *et al*., 1991). Thus, seasonal variations in black tea quality are determined from the corresponding variations of theaflavins presented in the tea. Eden (1976) showed that the production of theaflavins was the most potent single factor in promoting good quality in tea. The levels of catechins and their gallates in tea leaves produced during wet weather are usually at their maximum, whereas the activity of polyphenolic oxidase is only moderate, which thus leads to moderate production of theaflavins in black tea and consequently affect the quality of the black tea (Eden, 1976). However, high levels of catechins and catechin gallates coincide with high enzymatic activity during dry weather in Kenya, leading to high theaflavin levels in the final black tea. Therefore, such seasonal variation of leaf chemical constituents may be used as an indicator of the potential quality of teas (Eden, 1976).

For sensory characteristics of black tea theaflavins, Sanderson *et al*. (1976) demonstrated that the astringencies of individual theaflavins were different: theaflavin digallate (TFDG) is the most astringent (index to 6.4); theaflavin monogallates (TFMG, TF3G and TF3′G) are the next most astringent (index to 2.2); and theaflavin (TF) has the lowest astringency (index to 1.0). Whether the total theaflavins or the TFDG equivalent (converting all individual theaflavins into a general index as mentioned above) is used in the description of black tea quality, different conclusions regarding tea

quality are made (Owuor and Obanda, 1995a). However, TFDG or the TFDG equivalent (of the total theaflavins levels) have been suggested as better quality indicators for Kenyan black teas than total theaflavin levels (Owuor and Obanda, 1995a).

Good quality tea always attracts a good price. However, factors other than tea quality such as the demand and preferences of customers, and the production and supply of the producers, may sometimes determine the price or market value of a black tea (Ellis and Cloughley, 1981). Thus, the chemical indicators of good black tea should be used to ensure the processing of black tea with consistent quality characteristics.

The first aim of this study was to quantify the chemical transformation of the main catechins and catechin gallates, and the formation of the four principal theaflavins during the commercial processing of Australian black tea. The second aim was to determine how the levels of theaflavins in Australian produced black tea compare with those in black tea produced throughout the world, so that some judgement on the relative quality of Australian black tea can be made for the benefit of the Australian tea industry. The third aim was to obtain data on the seasonal variations of leaf catechins and catechin gallates and their effect on the processing of black tea, particularly on the theaflavin levels, so that a recommendation can be made as to which months of the year are likely to produce the highest quality black tea. These data were obtained by analysing tea samples taken from various stages of a processing line in a tea factory, across a number of harvests throughout a year of production. In conclusion, the results of this study provide information on the chemical aspects of the processing of black tea under the current “low labour, high mechanisation, high throughput” model used Glen Allyn Tea Estates.

4.4.2 Materials and methods

4.4.2.1 Experimental design

4.4.2.1.1 Sampling times

Collection of the in-line samples was conducted in the tea factory of Glen Allyn Tea Estates of Nerada Tea Pty. Ltd., Malanda, North Queensland, at three monthly intervals in April, July and October 2000, and January 2001, respectively, in accordance with the commercial processing in the factory. The sampling was originally designed to collect the samples in three consecutive days. Thus, the collection of samples was conducted on 11, 12 and 13 April 2000, and 16, 17 and 18 January 2001. However, since there were only enough green leaves for one day of processing in July 2000 after the June frost, sampling conducted in the July round was modified as three separate processing intervals within one day (21 July 2000). In addition, there occurred an uncontrolled time difference in the harvest of green leaves between paddocks in October 2000. This difference resulted in the processing of black tea with an interval between harvests to ensure enough green leaves for one or two days processing within a week. Thus, the three separate samples collected in the October round were conducted on two days, one sampling conducted on 24 and two on 25 October 2000.

4.4.2.1.2 In-line sampling

The collection of the in-line samples at a number of processing points was conducted from the binned (B) green tea leaves to the end black tea product (T). The actual sampling points in the factory (Figure 4.1) are summarised as follows.

- Bin leaves coded as **B**.
- Leaves after a shredder (the size of the leaves reduced about 1/3 to 1/2) coded as **S**.
- Leaves after Rotorvanes as preconditioning (the size of the leaves reduced to about 1/10 to 1/20) coded as **R**.
- Leaves passed through four consecutive curling-tearing-cutting (CTC) machines and became small particles, which were coded as **C1**.

- Samples after CTC and just before fermentation step 1 were coded as **C2**.
- Samples after each of the four fermentation steps were coded corresponding to the steps as **F1**, **F2**, **F3** and **F4**. (In April 2000, there was another fermentation step 5. This step was removed from the processing line for the other harvests because of concern about over-fermentation. Sampling for this step was incomplete, so no statistical analysis being done and the results not being reported in this Chapter).
- Samples after preliminary drying were coded as **D1** and after full drying as **D2**.
- Samples after the static electricity stalk picking machines were coded as **T**, representing the final black tea product

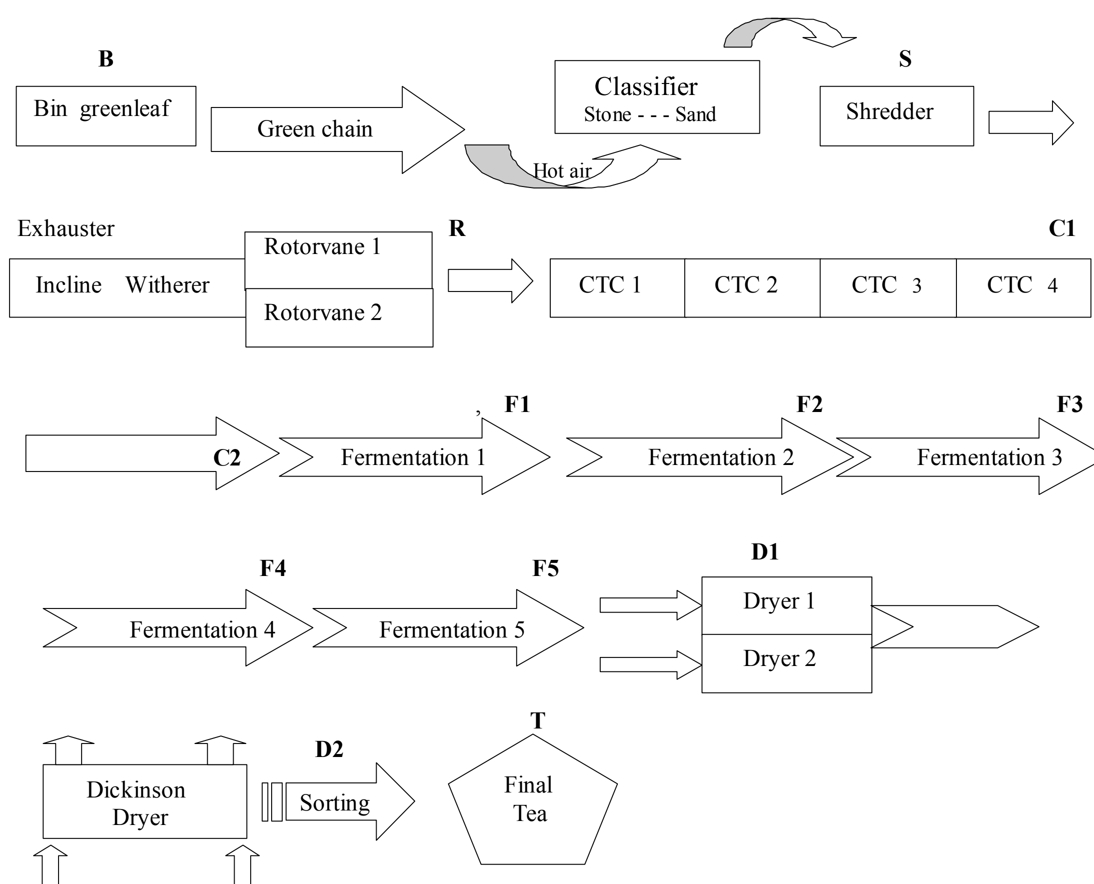


Figure 4.1 Sampling points on the black tea processing line at the factory of Glen Allyn Tea Estates, Malanda, North Queensland.

4.4.2.1.3 Experimental design

The experimental design is statistically called a split unit model. In detail, one sample of ca 200 g in-line tea leaves was collected at each of the twelve sampling points in the tea processing line, at four harvest times (April, July and October 2000, and January 2001) with the following replications:

- April 2000, 3 replications as the 3 days processing on 11, 12, 13 April.
- July 2000, 3 replications on 3 separate processing intervals throughout the one-day processing on 21 July 2000.
- October 2000, 3 replications as the 2 days of processing on 24 (1) and 25 (2) October.
- January 2001, 3 replications as the 3 days processing on 16, 17 and 18 January.

4.4.2.2 Sample preparation

The samples taken off the processing line were separately wrapped in plastic bags and immediately packed in dry ice in a polystyrene foam box. This sampling process requires rapid action to minimise the oxidation occurring in the samples. The samples were then delivered with dry ice using overnight cargo flight from Cairns to Brisbane and then using road transport from Brisbane to the laboratory at Gatton. Sufficient dry ice was still present to keep the samples frozen. All the samples were stored at −80 °C in a freezer prior to analysis.

4.4.2.3 Determination of moisture content

The moisture contents of the in-line tea samples were determined according to the method detailed in Section 4.2.5. These moisture contents are attached in Appendix 5.2.

4.4.2.4 Extraction of flavonoids and other polyphenols from in-line tea samples

4.4.2.4.1 Extraction of in-line samples from Bin to Rotorvane

The optimised methanol extraction method used for fresh green tea leaves and detailed in Section 4.2.2.3.8 was used to extract all samples taken off the processing line at various stages (treatments) from the initial fresh tea leaves in the Bin (B) to those in the Rotorvane (R). Extractions were done in duplicate using identical subsamples of each treatment sample.

4.4.2.4.2 Extraction of in-line samples from the CTC to the final black tea

The methanol extraction method detailed in Section 4.2.2.4 was used to extract all samples taken off the processing line at various stages (treatments) from the initial fresh tea leaves in the CTC (C1) to the final black tea (T). Extractions were done in duplicate using identical subsamples of each treatment sample.

4.4.2.5 HPLC analysis

The methanol extracts were analysed using a HPLC analysis procedure detailed in Section 4.2.2.6.2.

4.4.2.5.1 Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG)

EGCG and ECG were identified according to the UV spectra and retention times of authentic compounds, as described in Section 4.2.2.6.3. Quantification of EGCG and ECG was undertaken using the external standard method detailed in Section 4.2.2.6.4.

4.4.2.5.2 Total catechin gallates

Total catechin gallates included the six galloyl catechin compounds, catechin gallate (CG), epicatechin gallate (ECG), epicatechin digallate (ECDG), gallocatechin gallate (GCG), epigallocatechin gallate (EGCG) and epigallocatechin digallate (EGCDG). Identification of these compounds was done using the UV spectra and retention times of authentic compounds, according to the method detailed in

Section 4.2.2.6.3, and quantification involved the external standard method described in Section 4.2.2.6.4. The content of total catechin gallates was the sum of the content of these six compounds.

4.4.2.5.3 Epigallocatechin (EGC) and total catechins

Total catechins included the four compounds, catechin (C), epicatechin (EC), gallocatechin (GC) and epigallocatechin (EGC). Identification of these compounds was done using the UV spectra and retention times of authentic compounds according to the method described in Section 4.2.2.6.3, and quantification involved the external standard method described in Section 4.2.2.6.4. The content of total catechins was the sum of the content of these four compounds.

4.4.2.5.4 Combined catechins/catechin gallates

The content of this group of compounds involved the sum of the contents of the total catechins (Sections 4.4.2.4.2) and total catechin gallates (4.4.2.4.3).

4.4.2.5.5 Theaflavin (TF), theaflavin 3-gallate (TF3G), theaflavin 3′-gallate (TF3′G), theaflavin 3,3′-digallate (TFDG) and total theaflavins

Identification of the theaflavins, TF, TF3G, TF3′G and TFDG was done using the UV spectra and retention times of authentic compounds according to the method described in Section 4.2.2.6.3, and quantification involved the external standard method described in Section 4.2.2.6.4. The content of total theaflavins was the sum of the content of these four individual theaflavins.

4.4.2.5.6 Total phenolic compounds

The total phenolic compounds included all the above-mentioned 4 catechins, 6 catechin gallates and 4 theaflavins, plus 5 flavonol glycosides and 7 simple phenolic compounds. The flavonol glycosides analysed were kaempferol 3-rhamnosylglucoside, kaempferol glycoside, quercetin glycoside, quercetin 3-glucoside, and quercetin 3-rhamnosylglucoside. The simple phenolic compounds were gallic acid, theogallin, iso-chlorogenic acid (5-cafeoylquinic acid), *p*-coumaryl quinic acid, chlorogenic acid (3-cafeoylquinic acid), *p*-coumaric acid, and 3-(*p*-hydroxyphenyl)-propionic acid. Identification and quantification of the flavonol glycosides and simple phenolic compounds were conducted according to the methods described in Sections 4.2.2.6.3 and 4.2.2.6.4, respectively. The content of total phenolic compounds was the sum of the content of these 26 compounds.

4.4.2.6 Statistical analysis

Prior to analysis, data from duplicate samples were averaged. The data for each individual compound or group of compounds were then subjected to an analysis of variance (ANOVA). A split unit model was used with 12 “main units” (4 seasons × 3 repeats), each split into 12 in line samples. The statistical adequacy of the model was checked and for all variables where significant F-values ($P < 0.05$) were found, comparisons of least square means were carried out using Fisher’s least significant difference (LSD) procedure.

4.5 Development of an on-line method for assessing optimum fermentation of black tea

4.5.1 Introduction

The feasibilty of using the measurement of theaflavins for the on-line determination of optimum fermentation time was investigated. The emphasis was on finding a method which could be adapted for use in a factory where space and technical support are very limited.

Over the years a number of methods have been developed for measuring theaflavins and thearubigins in black tea. These have been mainly spectrophotometric methods. In recent years HPLC methods have been developed as used in Sections 4.2 - 4.4.

Owuor and Obanda (1995) have compared a number of methods for the determination of theaflavin levels in black tea. These methods involve extraction and separation of theaflavins and thearubigens and analysis using spectrophotometric assays. The Flavognost method has been generally accepted as the standard analytical technique for theaflavins analysis. Another method replaces the expensive Flavognost reagent by inexpensive aluminium chloride. Other methods used Sep-Pak cartridges for the separation. These are methods of Whitehead (1991) and Whitehead and Temple (1992). The latter method would appear to be an optimised version of the previously published method by Whitehead (1991).

Ullah (1972) reported on a spectrophotometric method to measure TF and TR in dhool and so determine optimum fermentation time.

A number of indirect methods have been suggested for measuring theaflavins. Davies (1983) stated there was a correlation between TF and soluble solids (TSS).

Total colour (TC) was suggested of use in determining the optimum fermentation time. The spectrophotometric method of Ullah (1972) used to measure TF and TR in dhool was used to measure total colour and also % brightness. Whitehead and Muhime's (1989) paper on the Aluminium Chloride method is entitled "Black Tea Manufacture: A Practical Method to Enable Factory Managers to Control "Quality" during Fermentation". This method measured as well as TF in dhool using a simplified 'in-line' method to assess the progress of fermentation. The Aluminium Chloride TF measurement at 520 nm peaked at the optimum fermentation time while the total colour measurement continued to rise. The ratio of readings at 520 nm and 460 nm dropped at the optimum fermentation time.

The method described by Ullah (1972) for measuring TF and TR can also be used to measure total colour and percentage Brightness. This uses the ratio of the reading at.380 nm and 460 nm. The reading at 380 nm is the spectrophotometric reading used to calculate the TF, so is comparable to the reading at 520 nm from the 'in-line' fermentation monitoring described by Whitehead and Muhime (1989). The drop in the ratio of readings at 520and 460 at the optimum fermentation time can be compared to the drop in % brightness. A comparison of changes in TF, total colour, TR and Brightness (Whitehead and Muhime, 1989) showed that brightness peaked before TF. Thearubigins and total colour rose steadily. They stated that there was a strong positive correlation between the concentration of thearubigins and the level of total colour.

4.5.2 Materials and methods

Tea samples collected from the processing line in the factory at Glen Allyn Tea Estates (see Section 4.4) were analysed for some quality parameters, namely theaflavins, thearubigins, soluble solids, and total colour. The tea samples used were from the fermentation stages (during which theaflavins and thearubigens are formed). There were 3 replicate sets of samples taken during the study at three monthly intervals in April, July and October 2000, and January 2001 (Section 4.4). Samples from January and July were measured here. These represented the highest and lowest levels of theaflavin content and were therefore used as an indicator of the sensitivity of the methods used.

The samples had been previously dried and stored at refrigerator temperature. In some cases there was insufficient sample left to conduct all trials.

4.5.2.1 Direct spectrophotomeric assays

Several methods were used, namely:

1. The method of Whitehead (1991) using Sep-Pak C_{18} Sorbent cartridges. Values for thearubigins were also obtained.
2. The method of Whitehead and Temple (1992) using Sep-Pak C_{18} Sorbent. Values for thearubigins were also obtained.
3. Flavognost Assay (Owuor and Obanda, 1995b)
4. Aluminium Chloride Assay (Owuor and Obanda 1995b; Whitehead and Muhime, 1989)
5. Method of Ullah (Ullah, 1972)
6. Method outlined in Section 4.1, hereafter referred to as Method 6

The method of Ullah (1972) and Method 6 are variations of the method of Roberts and Smith (1963).

Most of the methods were developed for dried tea. Two of the methods were used with dhool.

Some difficulties were encountered with the Aluminium Chloride method as some of the formulas for this method were not cited correctly in the review of Owuor and Obanda (1995). The reference for the method was eventually found which contained the correct formula (Whitehead and Muhime, 1989). This reference gave a formula with a slightly different wavelength as well. As a result, most of the tea samples were measured at 525 nm and some of the later samples were measured at both wavelengths, ie 520 nm and 525 nm.

4.5.2.2 Indirect methods

Total soluble solids (TSS) was measured in samples run towards the end of study.

The procedure described in Whitehead and Muhime (1989) was used for measuring total colour. This method is uncomplicated and uses simple equipment to facilitate it being used on-site. Dhool (0.5 grams) is extracted with iso-methylketonebutanol (IBMK) (10 mls). TF can be measured by taking 1 ml of the resulting IBMK extract and mixing with $AlCl_3$ reagent, and measuring the colour at 520 nm after 4 minutes of colour development. Total colour can be measured by pouring off the IBMK layer from the dhool sample, and adding 10 mls of boiling water. 1 ml of the resulting filtered extract is diluted 1 in 4, and the optical density is measured at 460 nm. The resulting total colour measurement does not include TF because this is extracted by the IBMK layer which is discarded.

The method described by Ullah (1972) was also used to measure total colour as well as percentage brightness.

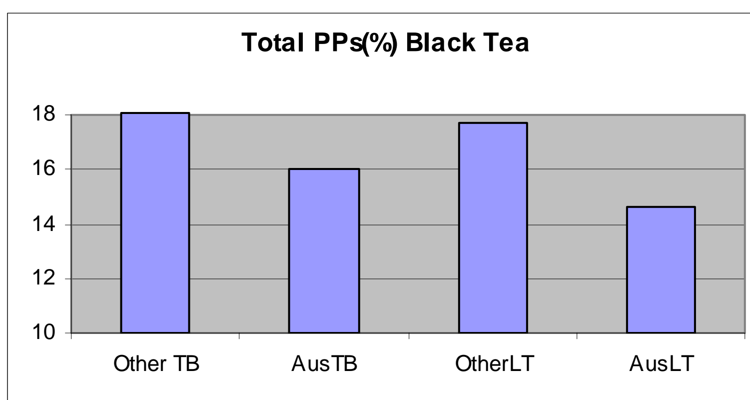
5 Results and discussion

5.1 Measuring phenolic compounds in teas from Australian markets

5.1.1 Polyphenols

Total polyphenols of black tea include some residues of green tea polyphenols that were not oxidised during the processing and the oxidation products of polyphenols such as theaflavins. The content of total polyphenols in black tea leaf was between 14 % and 20 %, with an average of 17 %. This was slightly lower than the total polyphenols detected in black teabags, which ranged from 13 % to 27 % with an average of 18 % (Figure 5.1). Two of the Australian grown and made black leaf teas had the lowest total polyphenol content of 14 %. Another sample, a fresh crude tea provided by GATE also had 14 % of total polyphenol content. The results may suggest that Australian tea providers prefer teas with a slight over-fermentation that allows more polyphenols to oxidise than moderate fermentation during the black tea processing.

The total polyphenol content of green tea was between 15 % and 34 %, with an average of 23 %. The total polyphenol content of green teabags (the majority of the samples) was between 21 % and 33 %, with an average of 25 %. This was much higher than the polyphenols content of black teabags with an average of 18 %. This is because some of the green tea polyphenols are oxidised during the fermentation stage of black tea processing. It is also possible that some of the oxidation products, although still phenolic compounds, could not be detected by the method used, e.g. the more polymerised structures such as the polymerised thearubigins.



AusTB was Australian teabags; AusLT was Australian leaf tea
Othe teabag and leaf tea were the average of the remainder of black tea samples analysed.

Figure 5.1 Comparison of Total Polyphenol Content of Black Tea

The differences in polyphenol content among the black teas and among the green teas were mostly due to differences in their botanical origins. Usually, green teas originating from India or Sri Lanka have higher polyphenol contents (ca 30 %) than those from China (ca 20 %) (Harbowy, 1997). Here, the total polyphenols of green tea samples originating from Sri Lanka were 26 % and 34 %, respectively, whereas the polyphenols of green tea samples originating from China were 15% and 23 % (see Table 5.3). Thus, under such circumstances, the polyphenol content can be used as a quality parameter for tea only if combined with the other parameters such as tea flavor and amino acid and caffeine content. However, the low content of phenolic compounds among teas with the same origins may indicate a long period storage for both black and green teas or over-fermentation for black teas. In this situation, polyphenols could be used as one of the major quality parameters. The green tea sample containing

only 15 % of total polyphenols had contents of TF, TR and TB that showed that the levels of oxidation compounds were similar to those of normal black teas (Table 5.3). Its sensory properties suggested that this tea might have been stored too long to be drinkable.

A comparison of the polyphenols released into hot water from tea with or without a bag showed that on average more polyphenols were extracted from green tea bags in comparison to green leaf tea than from black tea bags in comparison to black leaf tea (Table 5.1). Once again this might be due to the fact that the more polymerised thearubigins of black teas could not be measured. This might be also due to the variations of tea clones used for blending the commercial teas. In general, green teas contain relatively simple polyphenols, mainly catechins and their gallates, so it is also relatively simple to measure the polyphenol content. In addition, the types of additives used in the processing of teabags might also affect the permeability of the filter papers. Nevertheless, the percentage of the polyphenols dissolved from the teabags could be a useful index of the quality of the filter paper used for teabags.

Table 5.1 Solubility (%) of Total Polyphenols in Green and Black Tea

Tea Type	Without bag A	With bag B	Ratio B/A
Green tea	25.25	24.13	95.37
Black tea	17.93	16.34	90.91

5.1.2 Theaflavins, thearubigins and theabrownins

These complex phenolic compounds are usually derived from the oxidation of catechins and their gallates during the fermentation stage of black tea processing. Thus, the black tea trade measures the content of these compounds as part of a regular quality control procedure. TF and their gallates are the first stable oxidation products formed in tea fermentation. They undergo further oxidation to form more polymerised TR and then TB. TR is a group of compounds formerly recognised as insoluble fractions SI and SII of ethyl acetate (Roberts et al., 1956). TR was further separated by butanol into soluble TR and insoluble TB (Yuan, 1983). Of those compounds, TF contributes the brisk and astringent taste and bright golden color to black tea quality, while TR contributes the reddish color and richness in taste. Therefore, both classes of compounds are associated with quality and other desirable liquor characters (Bhatia, 1960; Roberts 1962). On the other hand, TB endows tea liquor and leaf with a dark brown color, which has a negative effect on tea quality (Yuan, 1983).

The average TF content of 30 kinds of teabags was 0.81 % and that of 17 black leaf tea samples was 0.75 %. The contents of TR and TB in teabags were about 1 % higher than in leaf teas (Table 5.2). Further investigation found that TF content of the teabags had a range from 0.29% to 1.25%, which showed higher variation than leaf teas that had a range from 0.32% to 1.10%. This may suggest that some teas were processed to produce special market varieties, such as Earl Grey, English Breakfast, Prince of Wales, etc. During the processing of these teas, the producer might add some flavorings and additives that resulted in low TF contents. In addition, teabags are mainly prepared with a view to quick solubility and convenience for consumers. Thus, components of teabags should be soluble in hot water in a very short time.

During the analysis, the aroma of all the green tea samples was found to be deteriorated, with a kind of unpleasant aroma of long storage rather than that of a fresh tea. Thus, TF, TR and TB were measured in green teas as well as black teas, although those compounds are would not generally be present in green teas. The results are shown in Table 5.3. All the tea analysed were teabags except 2 samples of leaf tea. An average of 0.25 % TF, 6.80 % TR and 5.03 % TB were detected in the teas. This indicates that all the green tea analysed had undergone oxidation. These results imply that the teas were stored too long and were no longer fresh. Even though they were still drinkable under these conditions, they had no green tea taste at all. One of the leaf tea samples smelt stale and had a similar content to the three black tea components suggesting that this tea was undrinkable.

The fresh black tea that was collected from the GATE factory contained 0.76 % of TF, 9.24 % TR and 9.8 % TB. This sample was collected from the GATE factory just after processing and was going to be sorted as leaf teas and/or teabags. Thus, it was considered as a representative of Australian made teas. A comparison of its TF, TR and TB contents with the other samples from Australian markets (Table 5.2) showed that all the teas had similar contents of these compounds. Since black teas with appropriate fermentation should have a TF content of more than 1 % with more than 10 % of TR (Bhatia, 1960; Roberts, 1962), the results of this study imply that the fresh tea sample was over-fermented as a crude black tea because of its lower TF content (0.76 %). It is known that black tea continues fermentation just following the completion of whole tea processing (Cloughley, 1981a, b), which can be called post-fermentation or post-mature progress. Furthermore, oxidation of phenolic compounds occurs in all types of teas during the storage period. Therefore, it is recommended that fresh black teas should have a high TF (> 1 %) with a high TR (>10 %) and an adequate TF/TR ratio (> 0.1) (Bhatia, 1960).

The solubility of TR and TB from teabags was between 82 % to 86 % and between 83 % to 92 %, respectively. This indicates that the permeability of teabags was variable. The variation in TF solubility from teabags was between 75% and 103% suggesting that it is unstable due to the tendency to be oxidised.

Table 5.2 TF, TR and TB Contents (%) in Black Tea

Tea	Check Point	TF	TR	TB
Teabag n=28	Highest	1.25	12.07	11.40
	Lowest	0.29	5.33	7.61
	Average	0.75	8.87	9.77
Leaf n=16	Highest	1.10	10.71	10.52
	Lowest	0.32	3.91	7.32
	Average	0.75	7.61	8.66

Table 5.3 Total Polyphenols, TF, TR and TB Contents (%) in Green Tea

Sample	1	2	3	4	5	6	7
Origin	Sri Lanka	Sri Lanka	Aust/ Overseas Blend	Overseas Blend	China	China	Aust
PP	26.18	33.86	21.35	21.68	23.18	14.76	19.58
TF	0.18	0.18	0.15	0.15	0.18	0.62	0.26
TR	7.44	8.62	6.60	6.19	7.42	5.48	5.85
TB	5.22	3.99	5.04	5.95	3.53	7.94	3.53

5.1.3 Comparison with the work done in other countries

In 1962, Roberts compared the TF and TR contents in teas with the results of sensory evaluation by well-trained tea tasters. Results of three best black teas of his observation were compared with these results and some recent work (Table 5.4). In those analyses, it was found that high TF content in conjunction with high TR content indicated high quality black tea. Generally, teas from Australian markets should have a relatively softer taste than those consumed overseas because of the lower TF contents and lower TF/TR ratios. Some of the imported tea samples analysed in this experiment had similar TF and TR contents and also similar ratio of TF/TR to the overseas counterparts. This implies that teas of high TF contents have a market in Australia. In other words, some Australians select

strong tasting or high TF content teas like overseas consumers. Thus, this result could be used as an incentive for the tea industry to educate Australian tea consumers to consume stronger tasting tea rather than providing softer tasting products.

Table 5.4 Comparison of TF (%), TR (%) and TF/TR in Black Teas from Various Countries*

Area	**Aus	UK	Int A	Int B	US	India	R1	R2	R3
TF	0.79	1.54	1.15	1.47	1.17	2.21	1.10	1.55	1.75
TR	8.64	11.09	11.56	12.18	9.45	16.04	10.30	15.90	15.40
TF/TR	0.09	0.14	0.10	0.12	0.12	0.14	0.11	0.10	0.11

*Int A: Continental Europe; Int B: Middle East. Data of Int A, B, UK and US was based on Lakenbrink & others 2000. Data of India was based on Bhatia 1960, leaf tea. R1-3 was based on Roberts 1962, leaf tea. The data of Bhatia and Roberts have been cited for many years as a comparison because of the similar analytical method.

** This refers to teas available in Australia not Australian grown teas.

The price of the tea samples was also compared with their TF, TR contents as well as TF/TR ratio. There were no direct relationships. This may be because the price of the teas on markets was not only based on tea quantity, but also on packaging, re-processing, and blending of the teas. However, the contents of TF, TR and ratio of TF/TR could be a useful index of quality for tea exporters and importers (Takeo & Oosawa, 1976). They could also be used by the tea producers to monitor their tea processing.

5.1.4 Conclusion

Although this was a relatively small observational study it did point to quality differences in teas available in Australia.

It indicated that total polyphenols of black and green teas could be used as one of the quality indicators for tea processing and marketing. The percentage of the polyphenols dissolved from teabags suggests the quality of the filter papers used for teabags is variable. The price of black teas from markets was not related to the content of TF, TR, or TF/TR though it was assumed to be for the tea producers. Similarly, the price of green teas from the markets showed the same trend. TF, TR and TF/TR are very important quality indicators of black teas. Black teas from Australian markets had lower contents of TF and TR as well as TF/TR ratio than those from overseas markets. This implies that education of Australian consumers is necessary to encourage the consumption of quality black teas with higher TF content and TF/TR ratio.

5.2 Development of methods for the analysis of flavonoids and other polyphenols in Australian tea

5.2.1 Identification of tea components

5.2.1.1 Identification of tea compounds in Australian grown fresh green tea leaves

UV spectra were extracted from the principal peaks of the chromatogram of an extract of Australian grown fresh green tea leaves (Figure 5.2).

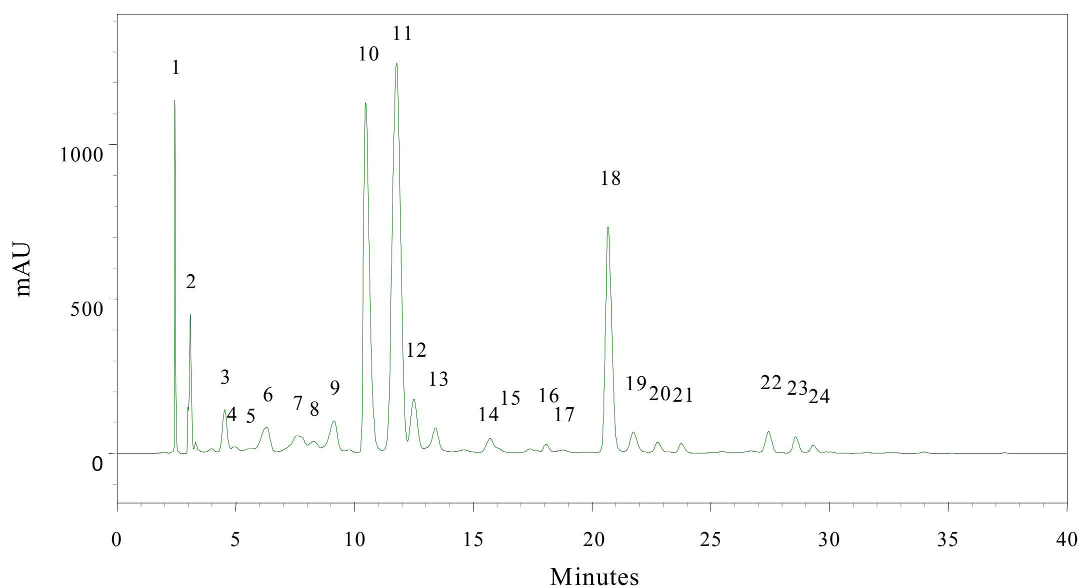


Figure 5.2 HPLC chromatogram of Australian grown fresh green tea leaves monitored at 280 nm (eluted in the first 40 minutes). Mobile phase: A = 2 % acetic acid, B = 100 % acetonitrile. Linear gradient: 8 % to 31 % B over 50 min, then 100 % B over 2 min, maintained at 100 % B for 3 min, returning to 8 % B for 10 min. The tea leaves (*Camellia sinensis var. assamica* were from Glen Allyn Tea Estates, Malanda, North Queensland).

These PDA UV spectra extracted were used in combination with retention time and spectral data of available standards and other reference compounds to identify the compounds responsible for the peaks. It should be noted that there was, in some cases, a small shift between the peak retention time and the corresponding peak spectrum time because of the photodiode array software, which divides the time scale of the chromatogram into very small sections. As a result, the spectrum was sometimes extracted from slightly off the top of the peak. This shift or difference, however, was usually very small. The retention times, spectral data and the identity for the peaks in the chromatogram are shown in Table 5.5. Discussion of the identification of these peaks is detailed below.

Table 5.5 UV absorption and identity of peaks in the chromatogram.

Peak	Time (min)	Peak maxima (nm)*	Identity
1	2.43	236, 271	Theogallin
2	3.09	232, 273	Gallic acid
3	4.53	233, 272	Theobromine
4	4.97	235, 298sh, 323	Iso-chlorogenic acid (5-*o*-caffeoylquinic acid)
5	5.60	234, 275	Gallocatechin (GC)
6	6.30	235.5, 269	Epigallocatechin (EGC)
7	7.58	233, 263	Catechin (C)
8	8.28	234, 283, 325	*p*-Coumaryl quinic acid
9	9.14	235, 301sh, 325	Chlorogenic acid (3-*o*-caffeoylquinic acid)
10	10.48	234, 270	Caffeine
11	11.78	236, 272	Epigallocatechin gallate (EGCG)
12	12.50	234, 277	Epicatechin (EC)
13	13.42	234, 310	*p*-Coumaric acid
14	15.71	232.5, 276	3-(p-Hydroxyphenyl)-propionic acid
15	16.00	235, 273	Gallocatechin gallate (GCG)
16	18.06	257, 306sh, 354	Quercetin 3-rhamnosylglucoside (Q3RG)
17	18.77	237, 275	Epigallocatechin 3,5-digallate (EGCDG)
18	20.67	234, 275.5	Epicatechin gallate (ECG)
19	21.75	233, 277	Catechin gallate (CG)
20	22.75	255, 265sh, 353	Quercetin 3-glucoside (Q3G)
21	23.76	254, 262sh, 352.5	Quercetin glycoside (QG)
22	27.44	266, 346	Kaempferol 3-rhamnosylglucoside (K3RG)
23	28.57	264, 346	Kaempferol glycoside (KG)
24	29.31	233, 278	Epicatechin 3,5-digallate (ECDG)

* sh-shoulder.

The PDA spectrum (Figure 5.3) of peak 1 in Figure 5.2 is very similar to the spectrum of gallic acid. The spectrum of peak 1 was once mistaken as that of a digallic acid (Cartwright and Roberts, 1954a) before being affirmed as that of theogallin (Cartwright and Roberts, 1954ab; Roberts and Myers, 1958). In addition, the HPLC retention time of peak 1 was found to be close to that of theogallin (Bailey *et al.*, 1990) (Table 5.6). Therefore, peak 1 was assigned to the compound, theogallin.

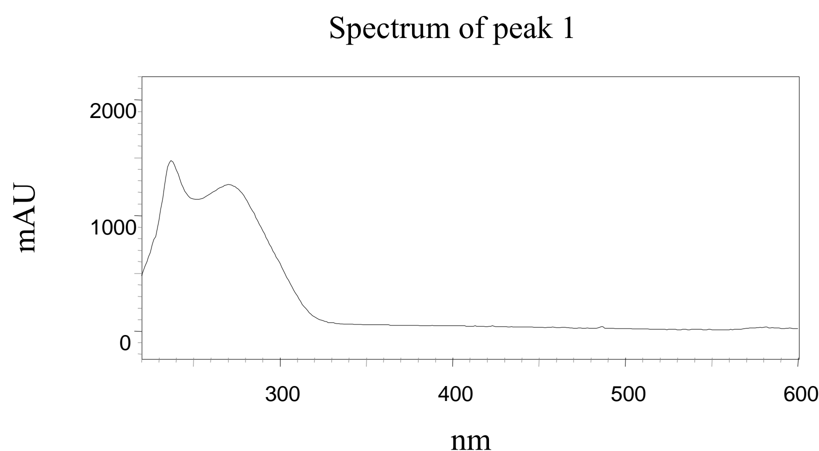


Figure 5.3 PDA UV spectrum of theogallin.

Table 5.6 Published UV absorption peak maxima of compounds found in tea.

Compound	Peak maxima (nm) and reference*
Gallocatechin	271a
Epigallocatechin	271a, 272b
Catechin	280a
Epigallocatechin gallate	275a, 279.5a, 277b, 226.5c, 274.5c
Epicatechin	280a
Epigallocatechin 3,5-digallate	283d
Epicatechin gallate	280a, 226.5c, 276.5c, 279d
Epicatechin 3,5-digallate	282d
Quercetin 3-rhamnosylglucoside	256.5c, 264shc, 352.5c, 259e, 266she, 359e
Quercetin 3-glucoside	256.5c, 264shc, 354.5c, 257e, 269she, 362e
Quercetin glycoside	256.5c, 264shc, 352.5c
Kaempferol 3-rhamnosylglucoside	264.5c, 344.5c
Kaempferol glycoside	264.5c, 344.5c
Gallic acid	272a, 273b, 226.5c, 270.5c
Theogallin	276b, 226.5c, 274.5c, 275f
p-Coumaryl quinic acid	313b, 228.5c, 310.5c, 314c
Theobromine	226.5c, 272.5c
Caffeine	226.5c, 272.5c
5-*o*-Caffeoylquinic acid	296.5c, 324.5c
3-*o*-Caffeoylquinic acid	292.5c, 320.5c

* sh = shoulder. a. Bradfield and Penny, 1948; b. Roberts and Williams, 1958; c. Bailey *et al*., 1990; d. Coxon *et al*., 1972; e. Mabry *et al*., 1970; f. Cartwright and Roberts, 1954ab.

The PDA spectra and retention times of peaks 2 and 3 compare satisfactorily with the spectra and retention times of the authentic samples of gallic acid and theobromine. The spectrum of peak 3 (Figure 5.4) contains details that were also similar to UV spectral details of Bailey *et al*. (1990). Thus, the peaks 2 and 3 were assigned to gallic acid and theobromine, respectively.

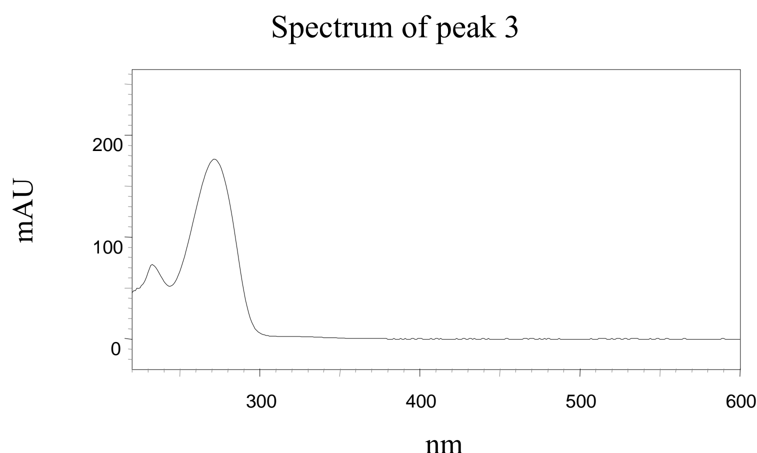


Figure 5.4 Photodiode array UV spectrum of theobromine.

The spectrum and retention time of peak 9 compared satisfactorily with the spectrum and retention time of a standard chlorogenic acid (3-*o*-caffeoylquinic acid); thus, peak 9 was assigned to this compound, whereas peak 4 was assigned to its isomer, iso-chlorogenic acid (5-*o*-caffeoylquinic acid)

(Table 5.5). This assignment is supported by the HPLC analysis of black tea liquor (Bailey *et al.*, 1990), which showed a similar chromatographic and spectroscopic behavior for these two compounds.

The PDA spectrum of peak 8 has a similar spectrum profile, with two partially resolved bands at 234 nm and 325 nm (Figure 5.5), suggesting it is due to a quinic acid derivative. Moreover, it also has a UV spectrum similar to authentic *p*-coumaric acid. Based on the observations of Roberts (1962) and the HPLC analysis of Bailey *et al.* (1990), peak 8 was assigned to *p*-coumaryl quinic acid.

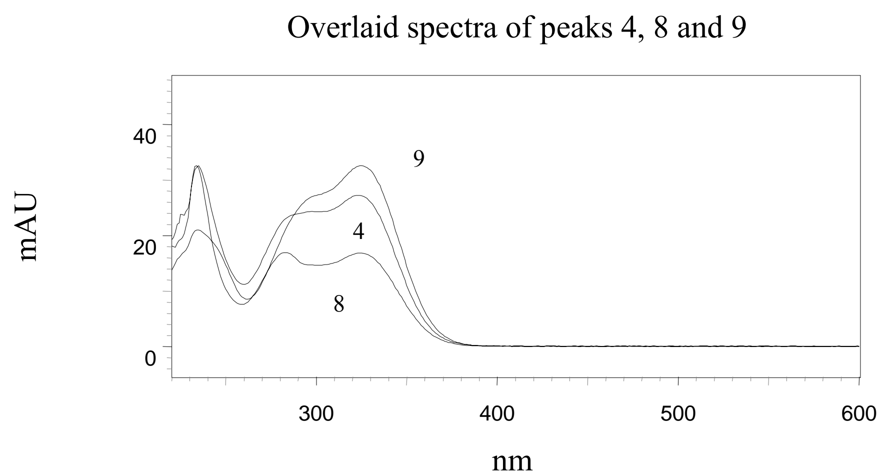


Figure 5.5 Photodiode array UV spectra of quinic acid derivatives: peak 4, iso-chlorogenic acid; peak 8, *p*-coumaryl quinic acid; peak 9, chlorogenic acid.

The PDA UV spectra of peaks 5 to 7, 11, 12, 15, 18, and 19 are characteristic spectra of green tea catechins and their gallates. All of them have two specific resolved bands (Figure 5.6). Band I ranges 232-235 nm and is little affected by the galloyl status of the compounds. Band II ranges 260-285 nm and the shift of absorption depends on the galloyl status of the compounds. The retention times and spectra of a number of authentic catechins and their gallates compare satisfactorily with the spectra and retention times of these compounds permitting their identification (Table 5.6). The four principal compounds present in green tea leaves are EC, ECG, EGC, and EGCG (Figure 5.6), which together usually make up 70 % of the polyphenols in tea green leaves (Roberts and Wood, 1951; Roberts, 1962; Sanderson, 1972; Hilton, 1973; Millin, 1987; McDowell and Taylor, 1993).

It is noteworthy that the literature peak maxima of the catechins and catechin gallates contain only a UV peak maximum between 270-280 nm (Table 5.7). The UV absorption maxima of those compounds at 232-236 nm are not listed in the literature (Table 5.7). This is due to the fact that in the past most of the UV absorption maxima were measured photometrically or under a UV light with a certain wavelength. The UV absorption of these flavanols is usually extracted with their maximum absorption regions, such 270-280 nm for the tea flavanols. In their systematic identification of flavonoids using the UV spectra, Mabry *et al.* (1970) showed that "only the maxima for those peaks at wavelengths longer than 240 nm were tabulated." Thus, the UV maxima for tea flavanols in Table 5.7 were extracted from the available data listed in literature.

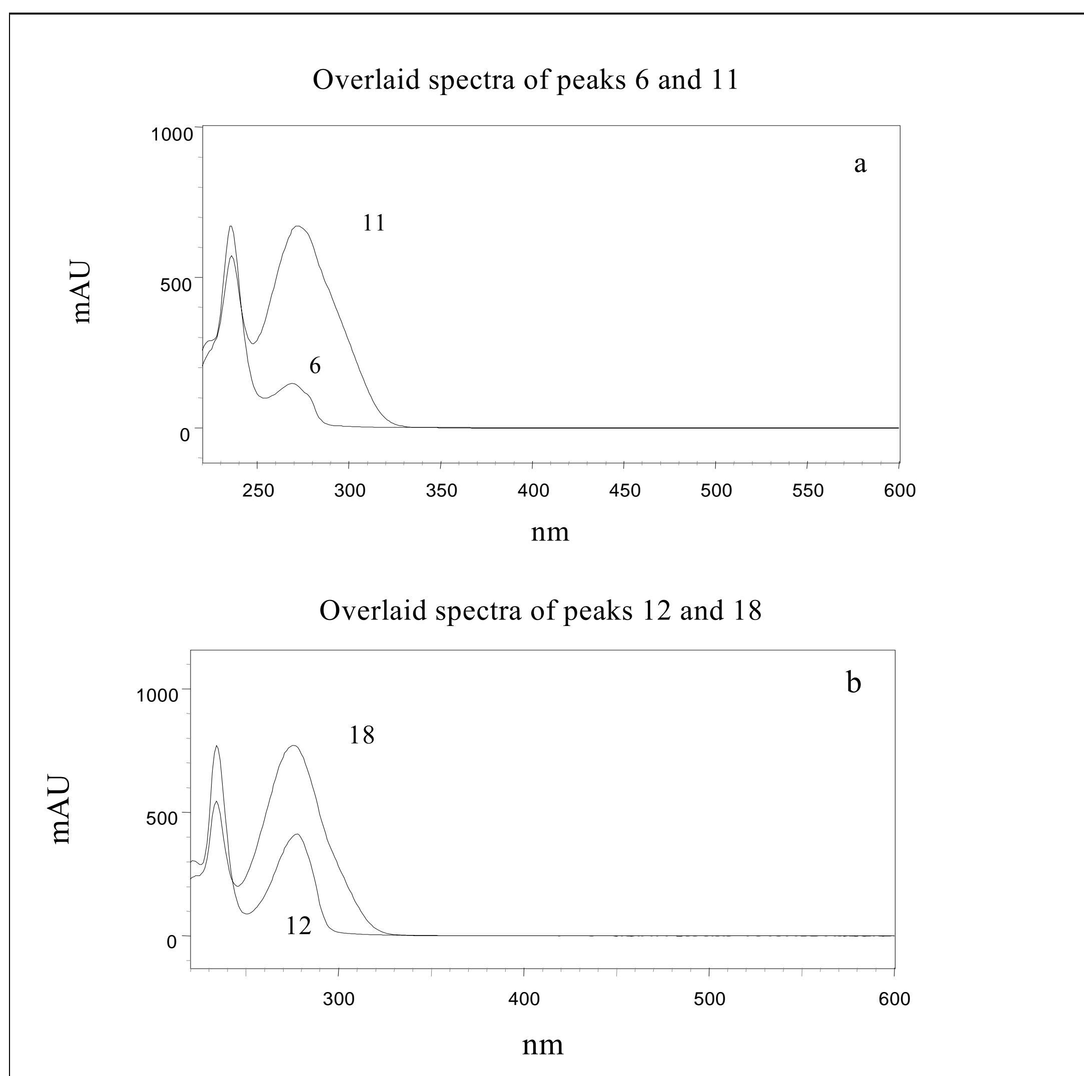


Figure 5.6 Photodiode array UV spectra of the principal green tea polyphenols: a. EGC (peak 6) and its gallate EGCG (peak 11); b. EC (peak 12) and its gallate ECG (peak 18).

The PDA spectra and retention times of peaks 13 and 14 compared satisfactorily with the PDA spectra and retention times of the authentic compounds, *p*-coumaric acid and 3-(*p*-hydroxyphenyl)-propionic acid, respectively, and were so assigned.

The PDA spectra of peaks 17 and 24 (Table 5.6) are very similar to the spectra of EGCG and ECG, respectively. Coxon *et al*. (1972) isolated a compound from tea liquor using Sephadex LH 20 column chromatography, which was equilibrated in and eluted with chloroform-methanol-light petroleum (1:2:1) solvent mixture. This compound eluted between EGCG and TFDG, and had a maximum UV absorption at 283 nm in ethanol, whereas the maximum UV absorption of EGCG is 275 nm. This compound was identified as EGCDG, with the shift in the absorbance that occurs for EGCDG relative to EGCG being caused by the presence of the extra gallate group (Coxon *et al*., 1972). Thus, peak 17 was assigned to EGCDG. In the similar manner, peak 24 was assigned to ECDG, which eluted after ECG (Tables 5.6 and 5.7).

Analysis of the chromatogram in Figure 5.2 showed that there are five peaks (16, 20, 21, 22, 23) between 18 and 30 min. These peaks possessed PDA UV/VIS spectra (Figure 5.7) characteristic of flavonol glycosides: i.e. band I between 240 and 285 nm and band II between 300 and 550 nm. This type of structure may have the following properties (Mabry *et al*., 1970; Markham, 1982):

- The substitution pattern of ring A tends to change in the position of band II, while the pattern changes of rings B and C are reflected in the position of band I;
- Introduction of additional hydroxy groups causes a red shift of band I (absorbing a longer wavelength) and a blue shift of band II (absorbing a shorter wavelength);
- Addition of sugar residues, especially to hydroxyl groups at positions 3, 5, 7 and 4′ causes a blue shift of band I but has little effect on band II. The shift of band I is thus determined by the position of glycoside linkage;
- Shifts are not affected by the nature of sugar, except that band I of flavonol 3-rhamnosides is blue-shifted (ca 10 nm) with respect to the corresponding 3-glucosides.

Bailey *et al*. (1990) compared the PDA spectra of these five peaks using HPLC and authentic compounds. Based on the HPLC elution order, retention times and UV/VIS spectra (Table 5.7), the assignment of peaks 16, and 20 to 23 to the relevant flavonoid glycosides listed in Table 5.6 is possible.

The PDA spectra of the flavonol glycosides show absorption bands at wavelengths from 220 to about 420 nm (Bailey *et al*., 1990). This range covers the wavelength, 380 nm, which was extensively used in the spectrophotometric measurements of the contents of theaflavins and thearubigins in black tea liquor (Bhatia, 1960; Roberts and Smith, 1961, 1963; Roberts, 1962; Bhatia and Ullah 1968; Takeo and Oosawa, 1976; Spiro and Siddique, 1981ab; Yao *et al*., 1993; Harbowy and Balentine, 1997; Muralidharan, 1997). Therefore, the measurements of black tea theaflavins and thearubigins using this wavelength would include some of the absorbance of flavonol glycosides, resulting in inaccuracy or errors (Cattell and Nursten, 1976, 1977; Bailey *et al*., 1990).

The oxidation products of green tea polyphenols should be the slowest eluting compounds during HPLC analysis based on the work of Bailey *et al*. (1990). However, there are no obvious peaks in the chromatogram of Figure 5.2 after 30 min of elution, suggesting that the extraction of polyphenols caused little oxidation.

5.2.1.2 Identification of phenolic compounds from Australian black tea

The phenolic compounds in black tea are usually the oxidation products or residues of the phenolic compounds of green tea or fresh tea leaves. Theaflavins are a group of characteristic phenolic compounds in black tea or the teas on the processing line. Theaflavins have long been regarded as responsible for the quality of black teas (Roberts and Smith, 1961; Hilton and Ellis, 1972; Hilton and Palmer-Jones, 1975; Palmer-Jones and Hilton, 1976; Cloughley and Ellis, 1980; Ellis and Cloughley, 1981; Owuor and Odhiambo, 1994; McDowell *et al*., 1995; Owuor and Obanda, 1995a; Owuor, 1996; Owuor and Obanda, 1996; Sud and Baru, 2000; Owuor and Obanda, 2001). In this study, the principal peaks in the chromatogram of Australian black tea liquor are shown in Figure 5.8. The compounds eluting in the first 30 min were identified as peaks 1-24 (Table 5.6).

Compounds producing the four peaks 25, 26, 27 and 28 (Figure 5.8) were identified by comparing their PDA UV/VIS spectra (Figure 5.9) and retention times with authentic compounds. The four theaflavins TF, TF3G, TF3G and TFDG were identified, respectively (Tables 5.8 and 5.9; Figure 5.7).

Three UV/VIS absorption bands were reported for the four main theaflavins (Table 5.9). It is noteworthy that the PDA absorption maxima could not be compared directly with the literature values, but the maxima in the region 267 to 273 nm (Table 5.8) were arranged in the same numerical orders as the literature values (Table 5.9).

Meanwhile, examination of Figure 5.9 indicates that the PDA UV/VIS spectral patterns of the theaflavins are very similar, with the introduction of galloyl groups into the structure having little effect; most change occurs in the retention time. These spectroscopic properties of theaflavins are also reflected in the literature data (Table 5.9), although the spectra of the theaflavins were measured in

ethanol (Coxon *et al*., 1970), methanol (Collier *et al*., 1973), or by HPLC with PDA detection (Bailey *et al*., 1990).

The UV/VIS spectra for the theaflavins (Figure 5.9), show absorption maxima in the wavelength range of 260 to 460 nm that tailed off into ca 600 nm. This suggests that theaflavins have strong absorption in a very wide visible region, in addition to that in the ultraviolet region. The absorption of theaflavins in the visible region is important for black tea because of the role it plays in determining the colour of tea liquor, one of the quality indicators for black tea. Furthermore, the main maximum absorptions of theaflavins are around 270 nm rather than ca 380 nm (Figure 5.9, Tables 5.8 and 5.9), which clearly are the second main absorption areas. Thus, the measurement of this group of compounds should be more sensitive using a wavelength at ca 270 nm rather than at ca 380 nm, particularly when HPLC with PDA detection is used.

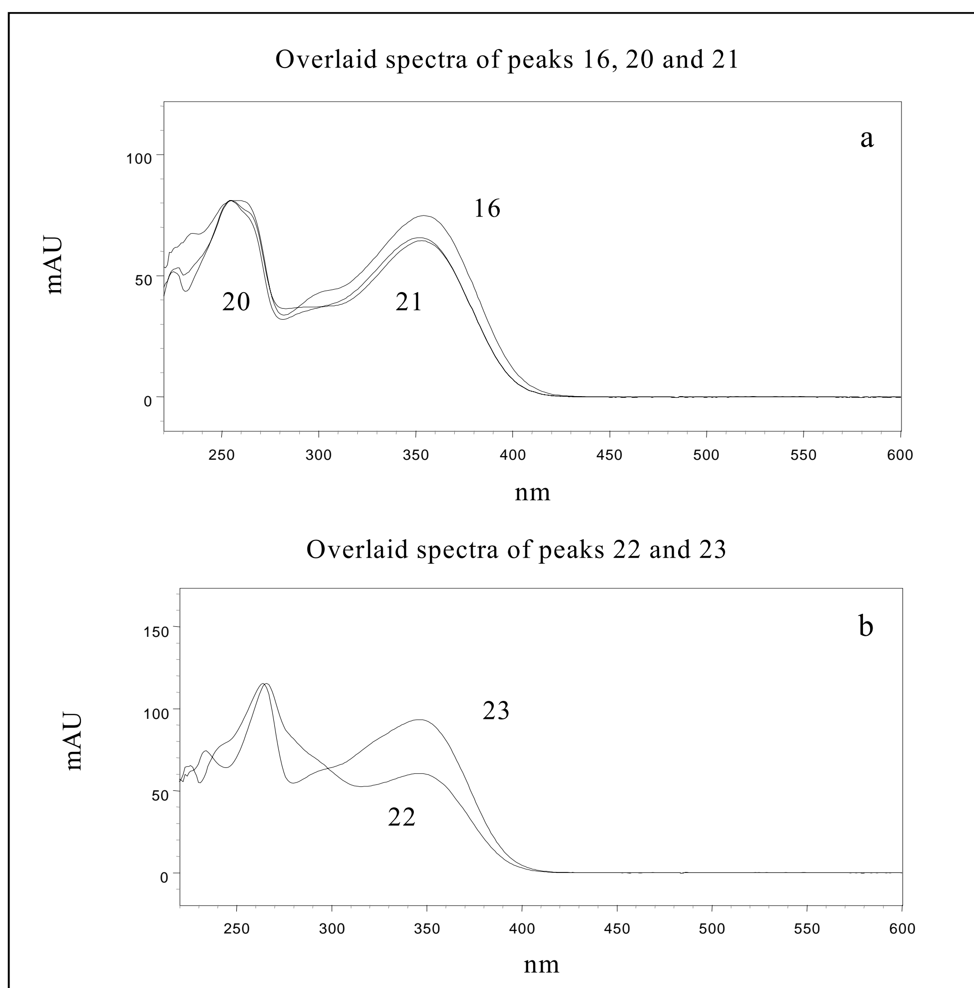


Figure 5.7 Photodiode array UV spectra of the flavonol glycosides: a. Q3RG (peak 16), Q3G (peak 20), and QG (peak 21); b. K3RG (peak 22), and KG (peak 23).

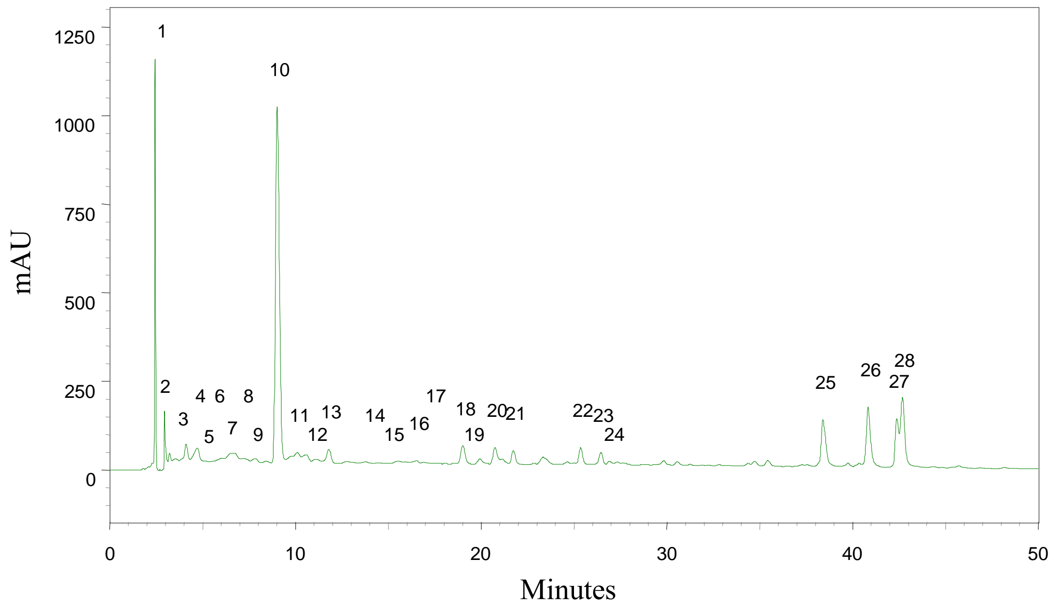


Figure 5.8 HPLC chromatogram of an Australian black tea liquor monitored at 280 nm. Mobile phase: A = 2 % acetic acid, B = 100 % acetonitrile. Linear gradient: 8 % to 31 % B over 50 min, then 100 % B over 2 min, maintained at 100 % B for 3 min, returning to 8 % B for 10 min.

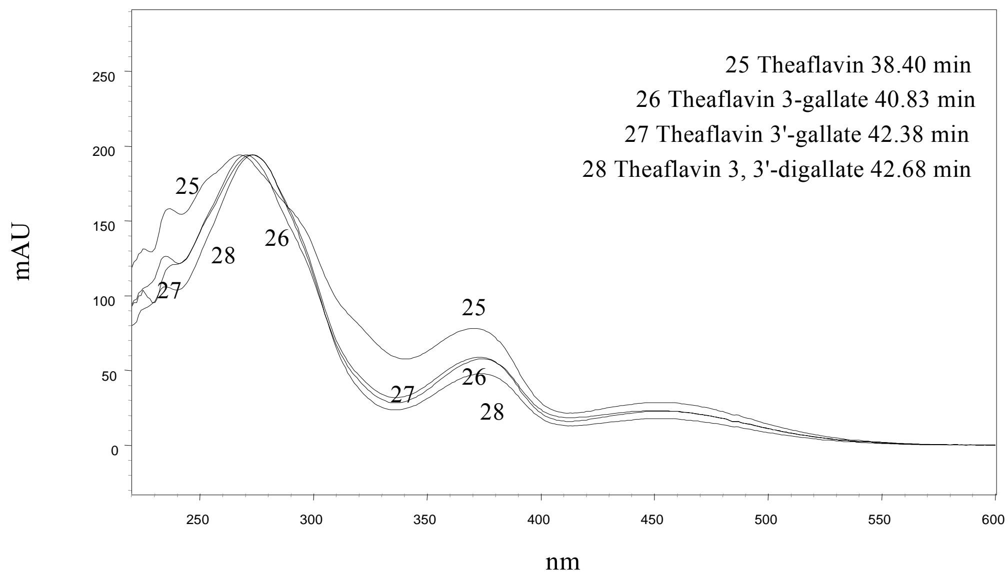


Figure 5.9 The superimposed photodiode array UV/VIS spectra of the four theaflavins in Australian black tea.

Table 5.6 UV/VIS absorption data and identity of theaflavin peaks.

Peak	Retention time (min)	Peak maxima (nm)	Identity
25	38.40	267.5, 370, 452	Theaflavin
26	40.83	271, 374.5, 452	Theaflavin 3-gallate
27	42.38	272, 373.5, 453.5	Theaflavin 3′-gallate
28	42.68	273, 373.5, 451	Theaflavin 3, 3′-digallate

Table 5.7 Literature UV/VIS absorption (peak maxima, nm) of tea theaflavins.

Compound	Reference		
	Coxon *et al*. (1970)	Collier *et al*. (1973)	Bailey *et al*. (1990)
Theaflavin	268, 379, 467	268, 378, 461	266.5, 372.5, 454.5
Theaflavin 3-gallate	275, 378, 462	272, 376, 455	270.5, 372.5, 460.5
Theaflavin 3′-gallate	275, 378, 462	278, 376, 452	274.5, 372.5, 462.5
Theaflavin 3, 3′-digallate	278, 378, 460	278, 378, 455	274.5, 372.5, 460.5

In conclusion, the results for the identification of tea polyphenols using HPLC showed that the main absorption of green tea catechins and the gallates is in the low wavelength area (ca 280 nm), representing flavanones that mostly appear in the first 20 min of elution. The flavones in tea, mostly flavonoid glycosides, elute between 20 and 30 min, with the main absorption being in the region from medium to long UV wavelengths (270 to 290 nm and 310 to 340 nm). The theaflavins of black tea have three characteristic absorption maxima ranging from UV to visible wavelengths. This study showed that the HPLC system with PDA detection is a powerful tool for monitoring the whole profile of polyphenols in tea liquor, for examining chromatographic behavior of polyphenols at different wavelengths, and for examining their spectral shifts with the changes in the analogues and analytical conditions. Thus, the HPLC-PDA method and the operation system used in this study are suitable for the analysis of tea components.

5.2.2 Optimisation of the extraction of flavonoids and other polyphenols from fresh green tea leaves

5.2.2.1 Extraction of green tea leaf components with water and re-extraction of the water extract

Researchers usually use boiling water for the extraction of polyphenols from dried green and black teas (Roberts, 1956; Wood *et al*., 1964). In an early study, Roberts and Wood (1951) boiled fresh green leaves with water to study the polyphenols in tea but found that the boiling of a tea leaf infusion resulted in epimerisation of the simple catechins and the galloyl esters. However, there is no systematic and detailed method available for the extraction of polyphenols from fresh green tea leaves, so boiling water was trialed as a method to extract fresh tea leaves in this preliminary study. Here, after blending with boiling water and concentrating to dryness in a vaccum rotary evaporator, the extracted tea solids were stored in a freezer before further analysis.

For the HPLC analysis, the extracted tea solid was re-extracted using the solvents previously used for the extraction of flavonoids from plants (Harborne, 1975; Harborne and Mabry, 1982; Harborne, 1988) and tea leaves (Roberts *et al*., 1956): chloroform, ethyl acetate, distilled water, and methanol. The levels of the main components found for this trial are shown in Table 5.10. The use of chloroform and ethyl acetate is considered unsuitable for re-extracting the dry extracts because there were only very low amounts of polyphenols extracted by these two solvents relative to water and methanol (Table 5.10). Hot distilled water extracted significantly higher ($P < 0.05$) amounts of all compounds examined, except EC, relative to ethyl acetate, while water extracted all compounds at significantly higher ($P < 0.05$) levels than did chloroform. Methanol was by far the more efficient solvent at

extracting the compounds examined since it extracted all these compounds at significantly higher ($P < 0.05$) levels from the dry extract than did chloroform, ethyl acetate, or water.

Table 5.10 Mean levels of main tea components re-extracted with different solvents from a solid water extract.

Solvent	Main compound (mg/g, dry basis)[1]				
	EGC	**EC**	**EGCG**	**Caffeine**	**ECG**
Chloroform	0.35 a	0.04 a	0.40 a	0.45 a	0.14 a
Ethyl acetate	2.46 b	1.19 b	9.49 b	0.26 a	2.59 b
Water	9.58 c	0.99 b	28.71 c	9.35 b	11.84 c
Methanol	25.20 d	2.89 c	38.68 d	14.32 c	17.91 d
LSD	1.36	0.21	2.39	0.72	1.00

[1] Means ($n = 2$) in columns followed by a common letter are not significantly different ($P > 0.05$).

Further, methanol is worthy of trial for the extraction of polyphenols from fresh green tea leaves, in a comparison with boiling water.

5.2.2.2 Comparison of the extraction of green tea leaf components using water and methanol

Fresh green tea leaves were extracted using methanol or boiling water. Both of the extractions were followed by a concentration to dryness (drying step), and both dry extracts were re-extracted with methanol prior to HPLC analysis. There were no significant differences ($P > 0.05$) in the levels of the tea polyphenols EC, EGC, ECG and EGCG in the final extracts, whether water or methanol was used for the initial extraction of the green leaves (Table 5.11). Similarly, for the principal tea alkaloid caffeine there were no significant differences ($P > 0.05$) in the levels extracted by boiling water or by methanol. The four principal catechins and catechin gallates were chosen since they usually make up 70 % of polyphenols in green tea leaves (Roberts and Wood, 1951; Roberts, 1962; Sanderson, 1972; Hilton, 1973; Millin, 1987; Graham, 1992; McDowell and Taylor, 1993; Harbowy and Balentine, 1997).

Methanol is supposed to be a suitable solvent for the extraction of phenolic compounds (Waterman and Mole, 1994), including flavonoids (Harborne, 1975; Harborne and Mabry, 1982; Harborne, 1988), from fresh plant tissues. In early studies, Bhatia and Ullah (1968) used methanol to extract polyphenols from dried green tea leaves for paper chromatographic analysis. Forrest and Bendall (1969) extracted polyphenols from dried green leaves using 50 % aqueous methanol. Ethanol was also used for the extraction of polyphenols from fresh tea leaves for paper chromatographic analysis (Roberts and Fernando, 1981), which included a heat treatment of fresh leaves immersed in ethanol and a homogenising process of the materials; and the final tea liquor was evaporated to dryness (dried) and re-extracted with methanol. The success of studies using methanol and ethanol as extracting solvent suggests that it is possible to use alcoholic solvent for extraction of polyphenols from fresh tea shoots.

Taylor and McDowell (1991) demonstrated that methanol was efficient in extracting pigments from fresh tea leaves. This extract was analysed by HPLC using an HPLC system with PDA detection, previously used by Bailey *et al*. (1990). There were twenty-eight pigments of carotenoids and chlorophylls extracted from fresh tea leaves. Thus, methanol was chosen to investigate further based on the use of methanol previously in the literature for extracting tea polyphenols, its ease of evaporation relative to water and its equal extraction efficiency to water found in this study.

Table 5.11 Mean levels of main components from fresh leaf extracted with boiling water and methanol.

Solvent	Main compound (mg/g, dry basis)[1]				
	EGC	EC	EGCG	Caffeine	ECG
Boiling water	24.16 a	15.09 a	55.13 a	29.18 a	16.47 a
Methanol	30.95 a	15.80 a	70.76 a	25.42 a	29.01 a
LSD	14.43	5.21	46.39	7.11	14.89

[1] Means (n = 2) in columns followed by a common letter are not significantly different (P > 0.05).

At the same time, consideration must give to the need for a concentration (drying) of the extracted fresh tea liquor. Concentration to dryness before storage (and subsequent HPLC analysis) was initially considered since it slows decomposition of the polyphenols. However, some concern exists as to whether the concentration to dryness (drying step) of the methanol liquor by rotary evaporation under vacuum would affect the stability of the catechins and catechin gallates, since the drying usually takes time and also needs a higher temperature. Thus, the form of the extraction method needs to be justified by a further trial.

5.2.2.3 Composition of methanol extracts of green tea leaves with and without a drying step

It is likely that oxidation occurs in a complex of fresh tea liquor, irrespective of whether it is extracted with methanol or boiling water. For the enzymatic oxidation of tea polyphenols present in tea, it would appear that substrate concentration and temperature are the critical factors that contribute to the oxidation (Harrison and Roberts, 1939; Roberts, 1941, 1949; Cloughley, 1980; Millin and Swaine, 1981). In addition, non-enzymatic oxidation of tea polyphenols has been reported during the storage of teas (Roberts and Smith, 1963; Cloughley, 1981; Roberts *et al*., 1981; Owuor and Othieno, 1988; Obanda and Owuor, 1995). This oxidation has been reported to occur rapidly in canned drinks that contain polyphenols (Chen *et al*., 2001). Even for a solution of pure catechins in water, non-enzymatic oxidation may occur spontaneously even under very low temperature, although the process may take place very slowly (Yao and Nursten, 1997, 1998). Regarding the drying of the polyphenol extracts, Degenhardt *et al*. (2000ab) used aqueous methanol to extract catechins from green tea and the solvent was evaporated under vacuum. No relevant methods are available for the extraction of polyphenols from fresh green tea leaves for HPLC analysis. Therefore, it is necessary to compare methanol extracts from fresh tea leaves that are not concentrated to dryness (drying) with methanol extracts that are first evaporated to dryness and then re-dissolved in a methanol in order to determine whether the drying (evaporation) process affects the composition of the extracted components.

The levels for two of the four principal catechins in fresh tea leaves, EGC and ECG, were not significantly different (P > 0.05) between the treatments with and without a drying step (Table 5.12). However, levels for the other two principal catechin and catechin gallate, EC and EGCG, and the tea alkaloid caffeine were significantly different (P < 0.05) between treatments, with the levels detected in methanol extracts without a drying step being significantly higher (P < 0.05) than those analysed with a drying step (Table 5.12). Thus, the HPLC analysis of tea polyphenols is better done directly on the methanol extract initially obtained, rather than that obtained after concentration to dryness and re-dissolution.

During the concentration process, EGCG decreased the most (Table 5.12). This result indicates that EGCG is a very sensitive compound in the complex and may be easily oxidised. EGCG comprises 30-40 % of the polyphenols in fresh tea leaves (Roberts and Wood, 1951; Roberts, 1962; Sanderson, 1972; Hilton, 1973; Millin, 1987; Graham, 1992; McDowell and Taylor, 1993; Balentine *et al*., 1997). Because EGCG is a major component of total tea polyphenols, its extraction needs to be maximised through the use of one single methanol extraction of fresh tea leaves (and no concentration step).

Table 5.12 Mean levels of main components in tea methanol extracts with and without concentration to dryness.

Treatment	Main compound (mg/g, dry basis)[1]				
	EGC	EC	EGCG	Caffeine	ECG
Dried	30.95 a	15.80 a	70.76 a	25.42 a	29.01 a
No drying	32.71 a	18.50 b	105.99 b	31.50 b	33.41 a
LSD	10.77	2.70	10.08	1.50	7.15

[1] Means (n = 2) in columns followed by a common letter are not significantly different (P > 0.05).

Finally, polyphenols extracted from tea leaves are best stored for long periods as solids. However, direct HPLC analysis of tea polyphenols in solution, immediately after the initial extraction, avoids any changes that may occur during a concentration step, with the obtained phenolic profile reflecting the true profile of phenolic compounds present in the fresh green tea leaves. Such a procedure was optimised in subsequent trials. All the unused tea leaves (not used for HPLC analysis) were dried, using a freeze dryer, and then stored for future use.

5.2.2.4 Optimisation of the blending time for the extraction of tea polyphenols from fresh green tea leaves

No significant (P > 0.05) differences were found for all of the main compounds extracted from fresh tea leaves by methanol with 3 to 7 min blending times, except for EGC where a significant (P < 0.05) increase in the extracted level occurred for a 5 min blending time relative to a 4 min one (Table 5.13). Overall, the results indicate that after blending for 3 min, most of the tea components were extracted by methanol. Thus, any of the blending times from 3 to 7 min would be suitable for the blending extraction, without significantly affecting the extraction rate of the principal tea catechins and catechin gallates. Considering time and efficiency, 5 min was selected as the blending time in this study.

Table 5.13 Mean levels of main components extracted with different blending time.

Time (min)	Main compound (mg/g, dry basis)[1]				
	EGC	EC	EGCG	Caffeine	ECG
3	47.10 ab	16.78 a	103.13 a	38.33 a	36.33 a
4	45.02 a	16.61 a	103.97 a	39.62 a	35.78 a
5	47.88 b	17.53 a	114.71 a	41.63 a	40.73 a
6	46.80 ab	16.84 a	113.15 a	43.63 a	39.82 a
7	46.80 ab	16.84 a	113.15 a	40.35 a	38.92 a
LSD	2.68	2.15	18.23	5.55	7.12

[1] Means (n = 4) in columns followed by a common letter are not significantly different (P > 0.05).

5.2.2.5 Test of the enzymatic activity in the methanol extracts of fresh green tea leaves

Using the peroxidase test for enzymatic activity, no enzymatic activity was detected in the solution of fresh tea leaves, produced by blending with methanol, for as short a time as 30 s. Further, there was no enzymatic activity in the methanol extracts produced by blending from 0.5 min to 7 min and, in fact, there was no enzymatic browning in a methanol extract after 1 h. Thus, the results of this test show that the blending of green tea leaves with methanol effectively inactivated the enzymatic oxidation. In conclusion, methanol is recognised as a good solvent for the extraction of tea polyphenols, because its efficiency in inhibiting enzymatic activity and its efficiency in extracting the main tea catechins and catechin gallates.

5.2.2.6 Repeatability of the final extraction method for flavonoids and other polyphenols from fresh green tea leaves

The coefficient of variance (CV) value can be used to compare the variability of two or more sets of data as a measure of relative variation (Walpole, 1990). Methanol extracts of seven sub-samples of one gross sample of fresh green tea leaves were obtained. The standard deviations (S.D.) and the coefficient of variance (CV) of the levels of the main catechins and catechin gallates, the alkaloid caffeine, and the total amount of compounds (the twenty-four compounds in Table 4.3) in the extract from green tea leaves were determined (Table 5.14). The CV % of EGCG, ECG, and the total extract were 4.12 %, 4.15 % and 4.11 %, respectively, all below 5.00 %, indicating excellent repeatability for the extraction method for flavonoids and other polyphenols. The CV % of EC and EGC were 5.09 % and 8.55 %, respectively, suggesting good repeatability for the extraction method for these two compounds.

Table 5.14 Analysis of CV of the extraction method for fresh green tea leaves.

Statistical analysis	Main compound (mg/g, dry basis)					
	EGC	**EC**	**EGCG**	**Caffeine**	**ECG**	**Total[1]**
Mean	32.54	14.98	100.85	32.10	31.03	251.97
CV %[2]	8.55	5.09	4.12	3.74	4.15	4.11

[1] Mean (n = 7) of content of all twenty-four compounds (Table 4.3) extracted.
[2] CV % (% coefficient of variation) = Standard Deviation (S.D.) × 100/Mean.

These results demonstrated that the methanol extraction method and HPLC analysis developed for the extraction of green tea leaves has acceptable repeatability.

5.2.2.7 Recovery trial for final extraction method developed for fresh green tea leaves

The five authentic compounds ECG, EGCG, GA, caffeine and coumarin were added to the fresh green tea leaves before blending as part of a recovery study. High recoveries were obtained (Table 5.15).

Table 5.15 Analysis of CV for the recovery test of some tea standards.

Statistical analysis	Recovery of standard compound				
	GA	**Caffeine**	**Coumarin**	**ECG**	**EGCG**
Mean recovery (%)	110.06	88.86	90.44	115.63	104.95
CV %[1]	3.45	4.25	1.47	5.82	8.66

[1] CV % = Standard Deviation (S.D.) × 100/Mean (n = 4).

The results from this recovery trial indicate that the recovery rate of the tested standards varied from 88.86 to 115.63 % (Table 5.15). The CV % of gallic acid (GA), caffeine and coumarin were 3.45 %, 4.25 % and 1.47 %, respectively, all below 5.00 %, indicating high recovery rates can be consistently obtained for these compounds when the developed methanol extraction method is used. The CV % of ECG and EGCG were 5.82 % and 8.66 %, respectively, again suggesting similar findings for ECG and EGCG. Since a seasonal analysis was involved and the CV % for these compounds showed a high and consistent recovery rate, the data reported in this study are actual data obtained from the analysis of pairs of samples described in the relevant sections.

In conclusion, the results of this recovery trial demonstrate that the developed method for the extraction and HPLC analysis of flavonoids and other polyphenols in green tea leaves has a high recovery rate.

5.2.2.8 Summary of the final extraction method of fresh green tea leaves

Stepwise optimisation of the developed extraction method for tea components from fresh green tea leaves from the field demonstrate that this extraction method when combined with HPLC analysis has high repeatability and good recovery for tea constituents. In addition, this same method must be used for the analysis of green leaves taken from the processing line prior to the CTC step.

5.2.3 Extraction of flavonoids and other polyphenols from tea samples taken off the black tea processing line

Boiling water is the most used solvent for the extraction of polyphenols from green and black teas. Generally, 3-5 g of dry tea was extracted with boiling water (100-200 mL) (Bailey *et al*., 1990, 1991; Kuhr and Engelhardt, 1991; Ding *et al*., 1992; Engelhardt *et al*., 2001). Alternatively, for a large quantity of tea (20 g), boiling water (1000 mL) was used for the specific extraction of theaflavins (Degenhardt *et al*., 2000ab, 2001). Other descriptions of the extraction method for HPLC analysis of tea polyphenols involved the use of 100 % (v/v) methanol and 70 % (v/v) aqueous methanol for successive extraction of tea flavonoids, mainly flavonoid glycosides (Finger *et al*., 1991ab, 1992, 1994; Kuhr and Engelhardt, 1991; Ding *et al*., 1992; Engelhardt *et al*., 1992, 1993; Degenhardt *et al*., 2000ab, 2001). These methanol extraction methods involved 1.5-5 g of tea (black, green, and/or oolong tea) being extracted with 200-250 mL methanol and the 2 × 75-100 mL 70 % (v/v) aqueous methanol using a rotary evaporator without vaccum for 15 min (Finger *et al*., 1991ab, 1992, 1994; Kuhr and Engelhardt, 1991; Ding *et al*., 1992; Engelhardt *et al*., 1992). Alternatively, 16 g black tea was extracted with 70 % (v/v) aqueous methanol (Degenhardt *et al*., 2000ab, 2001), followed by two successive extractions with 70 % (v/v) aqueous methanol. All those methods were for the extraction of catechins and catechin gallates or flavonoid glycosides from dry teas.

The method for the extraction of the samples from the black tea processing line was designed with reference to the infusion kinetics of tea polyphenols (Yao *et al*., 1992, 1993). Namely, the sample was extracted with 100 % (v/v) methanol (100 mL), followed by two successive extractions using 70 % (v/v) aqueous methanol (2 × 50 mL) each. The quantity of samples used was about 4-5 g of tea on a dry weight basis, in accordance with the method used for extraction of tea components from fresh leaves. This method was not optimised as it was based on the method of the other tea researchers (Finger *et al*., 1991ab, 1992, 1994; Kuhr and Engelhardt, 1991; Ding *et al*., 1992; Engelhardt *et al*., 1992; Degenhardt *et al*., 2000ab, 2001). The developed method is only suitable for the analysis of polyphenols in samples taken from the black tea processing line from the CTC step onwards.

5.2.4 Moisture analysis

No published standard method was available for the measurement of the moisture content in freshly harvested green tea leaves or teas taken from a processing line. There are two international standards for the measurement of moisture content of made tea, such as green and black teas (ISO 1573 and ISO 9768). These methods recommend the use of an oven at a temperature of 103 °C and measuring the loss of mass. In the AOAC Official Methods (Cunniff, 1995), it was recommended that the method "No. 925.19 Moisture in Tea" would be referred to another AOAC method "No. 934.01 Moisture in Animal Feed". The latter AOAC method recommended using a vaccum oven with a temperature at 95-100 °C or at ≤ 70 °C for feeds with high molasses content.

For the selection of the measurement of the moisture content in various tea samples, quite a few factors had to be taken into consideration. Firstly, too high temperature might decompose the high concentration of polyphenols in the tea leaf. Secondly, the method should be consistent for the analysis for all the samples from the different stages of black tea processing. Accordingly, in this study, the measurement of moisture content for the tea samples from the field and factory involved use of a vacuum oven (−65 kPa) and a temperature of 75 °C. This method was used to determine the moisture content of all the tea samples, so all the results in this study could be presented on a dry weight basis.

5.3 Analysis of flavonoids and other phenolic compounds in fresh Australian grown tea leaves

5.3.1 Phenolic compounds in hand plucked fresh leaves

5.3.1.1 EGCG

The mean levels of EGCG content in hand plucked fresh tea leaves showed no significant difference ($P > 0.05$) among the locations and points. The EGCG levels showed a gross mean of 108.98 mg/g (Table 5.16).

Table 5.16 Mean (n=2) content of EGCG content in fresh leaves at different sampling points.

Locations	EGCG, mg/g[1]	Standard Error (±)
Paddock A1	109.31	2.21
Paddock A3	111.55	2.10
Paddock B1	109.08	2.10
Paddock B3	109.34	2.21
Paddock C1	106.19	2.55
Paddock C2	108.41	2.69
Gross mean	108.98	1.73
CV %	7.47	
P-value	0.74	

[1] Dry weight basis.

In an early study, Nakagawa and Torri (1964b) showed that level of EGCG content was 88.1 mg/g and 113.1 mg/g in the fresh leaves of *Camellia sinensis* var. *sinensis* and var. *assamica* grown in Japan, respectively. These levels of EGCG in the *assamica* variety is very close to the level of EGCG found in the fresh leaves in Australia grown tea of a similar variety. Chu (1997) found that the level of EGCG content was at 121.0 mg/g for green tea made of *Camellia sinensis* var. *assamica*, which is also of a similar level to that measured for the Australian green tea leaves in this study. Obanda *et al*. (1997ab) studied EGCG content in green tea leaves using a similar HPLC analytical method to this current study. These researchers have found that the EGCG content is clonal dependent, ranging 60.6-117.5 mg/g, with the higher level similar to the mean level of EGCG in green leaves of Australian grown tea.

The high levels of EGCG in green tea leaves have been recognised as an indicator of good quality for the resulting black tea (Bokuchava and Skobeleva, 1969; Biswas *et al*., 1973; Graham, 1992; Obanda *et al*., 1997ab). Thus, results of this current study suggest that the mean level of EGCG in Australian grown tea shoots is relatively high and similar to the levels in tea grown in other countries, and is likely to produce good quality black tea.

5.3.1.2 ECG

Regardless of locations and sampling points within a location, the mean levels of ECG content in hand plucked flushes showed no significant difference ($P > 0.05$). The ECG levels showed a gross mean of 36.60 mg/g (Table 5.17). This compares favourably with the study of Nakagawa and Torri (1964b) who showed that level of ECG content was 25.8 mg/g and 40.3 mg/g in the fresh leaves of *Camellia sinensis* var. *sinensis* and var. *assamica*, respectively. Chu (1997) showed that the level of ECG in green tea leaf was 33.5 % of dry mass in *Camellia sinensis* var. *assamica*, which is similar to or slightly smaller than the level of ECG found in the Australian grown green tea leaves (Table 5.17).

Table 5.17 Mean (n=2) content of ECG content in fresh leaves at different sampling points.

Locations	ECG, mg/g[1]	Standard Error (±)
Paddock A1	35.97	0.91
Paddock A3	36.39	0.86
Paddock B1	38.68	0.86
Paddock B3	37.46	0.91
Paddock C1	34.92	1.05
Paddock C2	36.17	1.10
Gross mean	36.60	1.30
CV %	9.13	
P-value	0.10	

[1] Dry weight basis.

On comparing the overall mean content of ECG to EGCG in hand plucked tea flushes, the level of EGCG was found to be three times of that of ECG (Tables 5.16 and 5.17). This result suggests that the ratio of EGCG to ECG could be more or less fixed in the same variety, supporting the findings of Bhatia (1961, 1963, and 1964).

As for EGCG, ECG in fresh green tea leaves has also been recognised as an indicator of good quality for the resulting black tea (Bokuchava and Skobeleva, 1969; Biswas *et al*., 1971; Graham, 1992; Obanda *et al*., 1997ab). Thus, the ECG results of the present study also suggest that the tea flushes grown in Australia are comparable to those grown in other countries with regard to ECG levels, and are likely to produce good quality black tea.

5.3.1.3 EGC

The mean levels of EGC content (Table 5.18) in hand plucked flushes showed no significant differences ($P > 0.05$) regardless of locations and sampling points within a location.

Table 5.18 Mean (n=2) content of EGC content in fresh leaves at different sampling points.

Locations	EGC, mg/g[1]	Standard Error (±)
Paddock A1	46.55	1.18
Paddock A3	47.45	1.12
Paddock B1	46.82	1.12
Paddock B3	47.11	1.18
Paddock C1	44.70	1.36
Paddock C2	44.05	1.43
Gross mean	46.11	1.40
CV %	9.47	
P-value	0.37	

[1] Dry weight basis.

EGC usually occurs in green tea leaf at a similar level to ECG, ranging 30-60 mg/g dry weight (Sanderson, 1972, Hilton, 1973; Millin, 1987). The content of EGC measured in the hand plucked green tea leaves in the present study averaged 46.11 mg/g (Table 5.18), which is slightly higher than the level of ECG (Table 5.17) and much smaller than that of EGCG (Table 5.2). Nakagawa and Torri (1964b) found the level of EGC was 36.6 mg/g and 48.0 mg/g in the fresh leaves of *Camellia sinensis* var. *sinensis* and var. *assamica*, respectively. This level of EGC in the *assamica* variety is similar to that of the green leaves in Australian grown tea (Table 5.18), which also belongs to *assamica* variety,

suggesting this compound could be relatively consistent in a same variety regardless of the geographical locations. This similarity or consistency is supported by the research of Obanda *et al.* (1997ab) who found that the EGC content is also clonal dependent, with levels ranging 23.9-82.3 mg/g in different clones.

The high levels of EGC in green tea leaves have long been recognised as an indicator of good quality for the resultant black tea (Roberts and Smith, 1961, 1963; Roberts 1962; Bokuchava and Skobeleva, 1969; Hilton and Palmer-Jones, 1973; Obanda *et al.*, 1997ab). Thus, the EGC results of the current study suggest that green leaves harvested from Australian grown tea are comparable to those grown in other countries with respect to EGC levels, and are likely to produce good quality black tea.

5.3.1.4 Total catechins

No matter which location or sampling point within a location, the mean content of total catechins (C, EC, GC and EGC) in hand plucked flushes showed no significant differences ($P > 0.05$). The gross mean of total catechins in fresh green tea leaves was 80.38 mg/g (Table 5.19).

On comparing the levels of EGC (Table 5.18) and the levels of total catechins (Table 5.19), it can be seen that EGC represents more than 50 % of total catechins. Because the total catechins and EGC in fresh leaves do not vary significantly ($P > 0.05$) according to the location on the tea farm of Glen Allyn Tea Estates, then the other three catechins may similarly not vary with location. Generally, the main catechins EC and EGC occur at higher levels in fully developed tea shoots than younger shoots (Nakagawa and Torri, 1964b). No literature data are available for comparison of the total catechins in fresh green tea leaves.

Table 5.19 Mean (n=2) content of total catechins in fresh leaves at different sampling points.

Locations	Total catechins, mg/g[1]	Standard Error (±)
Paddock A1	79.83	1.74
Paddock A3	82.43	1.65
Paddock B1	82.47	1.65
Paddock B3	82.44	1.74
Paddock C1	77.18	2.01
Paddock C2	77.91	2.11
Gross mean	80.38	2.43
CV %	7.98	
P-value	0.17	

[1] Dry weight basis.

According to the studies of Roberts (1962), Takino *et al.* (1964), Ferretti *et al.* (1968), Bryce *et al.* (1970) and Coxon *et al.* (1970abc), the four catechins EC, C, EGC, GC are responsible for the formation of seven out of ten theaflavins and related compounds. Thus, high levels of total catechins in the fresh green tea flushes could be an indicator for producing good quality of the resultant black tea.

5.3.1.5 Total catechin gallates

No significant differences ($P > 0.05$) in the mean levels of total catechin gallates in hand plucked tea flushes were observed between locations or between sampling points within a location. The gross mean for the total catechin gallates in fresh tea leaves was 154.76 mg/g (Table 5.20).

Table 5.20 Mean (n=2) content of total catechin gallates in fresh leaves at different sampling points.

Locations	Total catechin gallates mg/g[1]	Standard Error (±)
Paddock A1	154.65	3.22
Paddock A3	157.53	3.06
Paddock B1	156.60	3.06
Paddock B3	155.99	3.22
Paddock C1	149.76	3.71
Paddock C2	154.05	3.91
Gross mean	154.76	2.76
CV %	7.65	
P-value	0.69	

[1] Dry weight basis.

Of the total catechin gallates in the fresh tea leaves (Table 5.20), EGCG contributes about 70 % (Table 5.16) and ECG 20 % (Table 5.17) of the total content. Thus, EGCG and ECG dominate the catechin gallate group of compounds. Thus, their pattern may determine the distribution pattern of the total catechin gallates.

No literature data are available for comparison of the total catechin gallates in fresh green tea leaves. Generally, the galloyl catechins occur at higher levels in young tea shoots (Nakagawa and Torri, 1964b). According to the studies of Roberts (1962), Takino *et al*. (1964), Ferretti *et al*. (1968), Bryce *et al*. (1970) and Coxon *et al*. (1970abc), the catechin gallates are responsible for the formation of five out of ten theaflavins, including three principal black tea theaflavin gallates (TF3G, TF3′G and TFDG). Thus, high levels of total catechin gallates in the fresh green tea flushes could be an indicator for producing good quality of the resultant black tea.

5.3.1.6 Combined catechins/catechin gallates

Regardless of locations and sampling points within a location, the mean levels of the combined catechins/catechin gallates in hand plucked tea flushes showed no significant differences ($P > 0.05$). For the combined catechins/catechin gallates, total catechin gallates represent about 65 % of total content (Tables 5.20 and 5.21). Thus, the gallates such as ECG and EGCG may control the general distribution patterns of the combined catechins/catechin gallates due to their prominence in green tea leaves in this study.

Table 5.21 Mean (n=2) content of combined catechins/catechin gallates in fresh leaves at different sampling points.

Locations	Combined catechins and catechin gallates, mg/g[1]	Standard Error (±)
Paddock A1	234.48	4.05
Paddock A3	239.96	3.85
Paddock B1	239.07	3.85
Paddock B3	238.43	4.05
Paddock C1	226.94	4.67
Paddock C2	231.95	4.92
Gross mean	235.14	5.05
CV %	6.35	
P-value	0.28	

[1] Dry weight basis.

The combined catechins/catechin gallates in green tea leaves are also referred to as the total flavanols (Obanda *et al*., 1997ab). The concentration of these compounds are clonal dependent, ranging 128.1-226.0 mg/g in different clones grown in Kenya (Obanda *et al*., 1997ab). In var. *sinensis*, the content of total flavanols is 161.2 mg/g, while in var. *assamica*, the content of total flavanols is 218.5 mg/g (Nakagawa and Torri, 1964b). These levels are lower than that found in the present study (235.14 mg/g) of Australian grown tea (Table 5.21).

Total flavanols contribute 70 % of total phenolic compounds in fresh green tea leaves (Sanderson 1972; Hara *et al*., 1995). The content of these compounds in fresh green tea leaves significantly ($P < 0.05$) correlates to the quality of resultant black tea (Obanda *et al*, 1992), with lower total flavanols producing lower quality black tea. Thus, the high level of total flavanols present in the fresh green shoots of Australian grown tea found in this study suggest there is potential for the Australian tea factory to produce high quality black tea, of comparable quality to other world teas, if the black manufacturing process is comparable to processes in other countries.

5.3.1.7 Total phenolic compounds

Regardless of locations and sampling points within a location, the mean levels of the total phenolic compounds in hand plucked tea flushes showed no significant differences ($P > 0.05$); a gross mean of 274.44 mg/g was found (Table 5.22). On comparing the results of total phenolic compounds (Table 5.22) with those of the combined catechins/catechin gallates (Table 5.21), it is clear that the combined catechins/catechin gallates represent about 85 % of total phenolic compounds in the fresh leaves. Thus, the catechins and catechin gallates determine the general distribution pattern of the total phenolic compounds in the fresh leaves of Australian grown tea.

The level of total phenolic compounds in fresh green tea leaves has been estimated at about 300 mg/g of the dry mass (Millin and Rustige, 1967; Sanderson, 1972; Dev Choudhury and Bajaj, 1980; Graham, 1992; McDowell and Taylor, 1993). However, the actual content of total phenolic compounds is hard to determine due to their diverse constituents. Hilton (1973) suggested that the content of polyphenols in young tea shoots might be over 200 mg/g of the dry weight of the bud and first three leaves. The results of this present study (Table 5.22) show that the total phenolic compounds measured in the fresh leaves of Australian grown tea is relatively high, at 274.44 mg/g of the dry mass (Table 5.22), and comparable to levels in previously studied teas.

Table 5.22 Mean (n=2) content of total phenolic compounds in fresh leaves at different sampling points.

Locations	Total phenolic compounds, mg/g[1]	Standard Error (±)
Paddock A1	273.69	4.68
Paddock A3	279.34	4.45
Paddock B1	277.96	4.45
Paddock B3	278.34	4.68
Paddock C1	265.12	5.39
Paddock C2	272.20	5.69
Gross mean	274.44	5.37
CV %	6.28	
P-value	0.37	

[1] Dry weight basis.

Obanda *et al*. (1992) showed that total phenolic compounds in fresh green tea leaves correlated significantly with the quality of resulting black tea. Dev Choudhury and Goswami (1983) suggested that leaf phenolic composition could be the important chemical components for producing black teas with good liquor characteristics. This is because, firstly, the phenolic compounds undergo fermentation to form compounds that are characteristic of black tea (Harbowy and Balentine, 1997);

and secondly, the residue of the phenolic compounds in black tea contribute to the taste (Bailey *et al*., 1990) and thus affect the liquor quality. Therefore, the high levels of phenolic compounds in Australian grown green tea leaves suggest it is possible to produce a good quality black tea, if the black tea manufacturing process is optimal.

5.3.1.8 Summary of phenolic compounds in fresh tea leaves

The main individual phenolic compounds ECG, EGC and EGCG of fresh tea leaves showed no significant differences (P > 0.05) among the sampling locations and points. The grouped phenolic compounds, total catechins, total catechin gallates, combined catechins/catechin gallates, and total phenolic compounds comprise mainly ECG, EGCG and/or EGC. On comparing the levels of these compounds in the fresh green leaves of Australian grown tea to those of the teas grown in other countries, the findings of this present study can be summarised below:

- EGCG shows generally a close level to the high levels in fresh tea leaves reported previously (Nakagawa and Torri, 1964b; Chu, 1997; Obanda *et al*., 1997ab).
- The level of ECG in Australian fresh green tea leaves is similar to or slightly higher than that found in the fresh green tea leaves grown in other countries (Chu, 1997).
- The level of EGC in Australian fresh green tea leaves is close to the high levels of EGC in the fresh green tea leaves found in other countries (Nakagawa and Torri, 1964b; Obanda *et al*., 1997ab).
- No data are available to compare the total catechins and the total catechin gallates in fresh green tea leaves.
- The level of total flavanols (combined catechins/catechin gallates) in the fresh shoots of Australian grown tea is higher than that of the teas grown in other countries (Nakagawa and Torri, 1964b; Obanda *et al*., 1997ab).
- The total phenolic compounds measured in the fresh green leaves of Australian grown tea are relatively high, averaging 274.44 mg/g of the dry mass. This level is close to or higher than the levels measured in fresh green teas in the other countries (Millin and Rustige, 1967; Sanderson, 1972; Dev Choudhury and Bajaj, 1980; Graham, 1992; McDowell and Taylor, 1993).

Generally, levels of the individual and grouped phenolic compounds in Australian grown fresh green tea leaves are relatively high, suggesting these leaves could be processed to produce a high quality black tea (Roberts, 1962; Nakagawa and Torri, 1964b; Takino *et al*., 1964; Obanda *et al*., 1992, 1997ab), if the appropriate black tea manufacturing process is used in Australia.

Finally, because the levels of flavonoids and other polyphenols in green leaves do not significantly vary (P > 0.05) across the farm of Glen Allyn Tea Estates, when the results are analysed for seasonal variations below, the overall mean for all locations/sampling points are considered for each compound.

5.3.2 Seasonal variations of phenolic compounds in hand plucked fresh tea leaves

5.3.2.1 EGCG

The EGCG content showed a significant effect (P < 0.05) of harvest date, with differences found between the EGCG of samples plucked in the warmer months and those in the cooler months (Table 5.23). In 2000, the EGCG contents of fresh tea leaves harvested during the warmer months of 12 April and 9 May 2000 were significantly higher (P < 0.05) than those for leaves harvested during the cooler months of July to September (Table 3.2, Figure 5.10), which themselves showed the lowest EGCG contents. In addition, for samples plucked during the cooler months (from July to September), there were no significant differences (P > 0.05) in the contents of EGCG. There was a trend of a slight rise in EGCG content among the samples harvested from October 2000 to January 2001 (Figure 5.10), when temperature were on the rise again (Table 3.2). During 2001, there were no obvious changes in the EGCG levels. Thus, it appeared that EGCG reached the highest levels during the warmer months of 2000 (April, May and December) and 2001 (January to February), and the lowest levels during the cooler period from July to September 2000 (Tables 3.2 and 5.23), suggesting that the synthesis of

EGCG in tea shoots could be temperature sensitive or dependent. Similar seasonal effects were reported in northeastern Indian tea (Bhatia and Ullah, 1968; Singh *et al.*, 1999), in which EGCG content rose in warm seasons and fell in cold seasons, and in Japan, where the EGCG content of green teas was found to be 88.0 mg/g of the dry mass in spring and 122.0 mg/g in summer (Chu and Juneja, 1997).

The increase of EGCG in summer months was suggested by Bockuchava and Skobeleva (1969) to be due to the active synthesis of EGCG in the plant tissues. Therefore, the seasonal pattern of EGCG in fresh leaves of the Australian grown tea (Table 5.23, Figure 5.10) suggests that the synthesis of EGCG is more active during the warmer months, while this physiological process becomes less active during the cooler months. It has also been suggested that the active synthesis of EGCG may relate to the length of daytime or stronger sunlight during the summer months (Harbowy and Balentine, 1997).

Table 5.23 Mean content of EGCG in hand plucked tea leaves.

Harvest date	EGCG[1] mg/g, dry basis	Harvest date	EGCG[1] mg/g, dry basis
12 April 2000	120.52 gh	27 December 2000	112.93 efg
9 May 2000	128.63 h	15 January 2001	113.65 efg
19 July 2000	91.39 a	1 February 2001	121.41 gh
23 August 2000	91.31 a	22 February 2001	112.14 defg
28 September 2000	96.12 ab	19 March 2001	108.63 cde
23 October 2000	100.82 bc	9 April 2001	118.38 fg
13 November 2000	103.09 bcd	2 May 2001	104.72 bcde
6 December 2000	110.97 def		
LSD	9.39	LSD	9.39

[1] Means (n=6) in table followed by a common letter are not significantlydifferent (P > 0.05).

EGCG has been proposed as a quality indicator for the processing of black tea (Bhatia, 1962, 1963; Roberts, 1962; Obanda *et al.*, 1997ab). Thus, the results of this experiment for EGCG levels in fresh leaves may indicate that in Australia, there is potential to produce higher quality black tea during the warmer months of January and February.

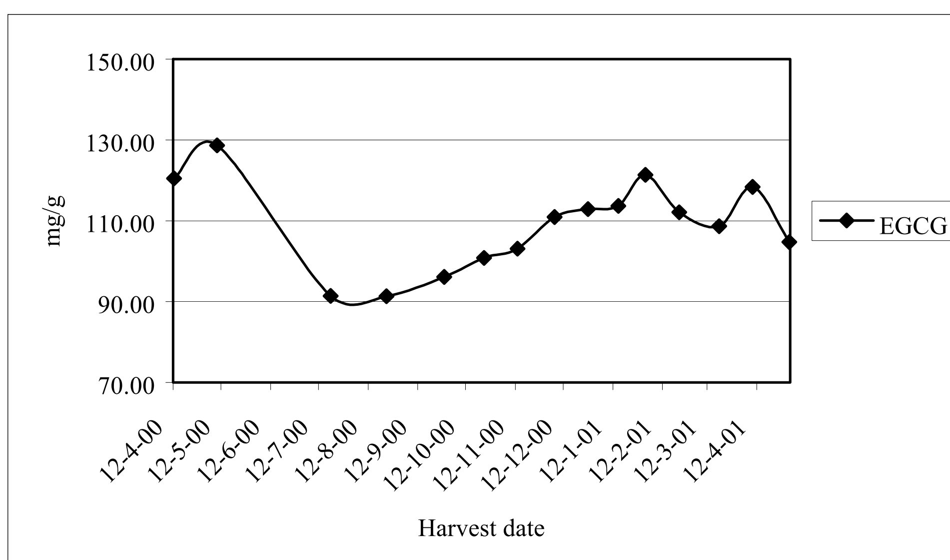


Figure 5.10 A seasonal variation of EGCG in hand plucked tea leaves.

5.3.2.2 ECG

The ECG content in fresh tea leaves showed a significant effect (P < 0.05) of harvest date. However, no significant differences (P > 0.05) in the ECG contents were observed among the samples harvested during the warmer months from December 2000 to May 2001 (Table 5.24). Further, no significant differences (P > 0.05) in the ECG contents were observed among the samples collected from August to November 2000, which themselves were significantly lower (P < 0.05) than the levels in samples harvested from 27 December 2000 to 2 May 2001, except for the 23 October harvest which was not significantly different (P > 0.05) (Table 5.24). ECG levels significantly peaked (P < 0.05) during the 9 May 2000 harvest (Table 5.24, Figure 5.11), similar to EGCG levels (Table 5.23, Figure 5.10).
This seasonal pattern of ECG in the fresh leaves (Figure 5.11) showed some a similarity to the pattern for EGCG (Figure 5.10), indicating that ECG may generally be synthesised in the same synthetic pathway as EGCG in tea. In addition, lower levels of ECG (Table 5.24) occurred in the cooler months (July to September 2000), in common with those of EGCG (Table 5.23). This was in agreement with findings of Bokuchava and Skobeleva (1969). The seasonal variation of ECG content in the *Camellia sinensis* var. *sinensis* was 28.0 mg/g in spring and 41.0 mg/g in summer (Chu and Juneja, 1997), which is slightly lower than the contents found in Australian grown fresh tea leaves (Table 5.24), of 31.56 mg/g (cooler month) and 44.26 mg/g (warmer month). This difference may be due to clonal difference, since Australian tea belongs to *Camellia sinensis* var. *assamica* (Chudleigh, 1999). This clonal difference is supported by the finding of Obanda *et al*. (1997ab) who showed that the level of ECG ranged 18.3-49.1 mg/g in green leaves from various clones. Nevertheless, the seasonal trend is clear from the results of Chu and Juneja (1997) and the present study; warmer months favour synthesis of ECG in the fresh tea leaves and cooler months do not.

Table 5.24 Mean content of ECG in hand plucked tea leaves.

Harvest date	ECG[1] mg/g, dry basis	Harvest date	ECG[1] mg/g dry, basis
12 April 2000	34.50 abcd	27 December 2000	37.89 cde
9 May 2000	44.26 f	15 January 2001	37.48 cde
19 July 2000	35.16 abcd	1 February 2001	40.62 ef
23 August 2000	31.56 a	22 February 2001	37.33 cde
28 September 2000	33.51 ab	19 March 2001	37.55 cde
23 October 2000	34.29 abc	9 April 2001	38.04 de
13 November 2000	33.37 ab	2 May 2001	37.32 cde
6 December 2000	36.10 bcd		
LSD	3.71	LSD	3.71

[1] Means (n=6) in table followed by a common letter are not significantly different (P > 0.05).

The biosynthesis of phenolic compounds can be effectively induced by sunlight (Harbowy and Balentine, 1997). That is why in shaded tea flushes the concentrations of polyphenols are much lower by comparison (Mahanta and Baruah, 1992). Therefore, the differences in ECG and EGCG levels between fresh leaves harvested in cooler and warmer months in Australia may not be a temperature but a day length effect. However, the induction of the biosynthesis of ECG and EGCG by light has not been elucidated as yet.

Forrest and Bendall (1969) demonstrated that ECG and EGCG do not occur in the embryo of tea seeds. They are synthesised during the germination and development of tea shoots. For the catechin related compounds, including gallated catechins, only catechin and epicatechin are detectable in the embryo of tea seeds (Forrest and Bendall, 1969). Catechin gallates are formed during the germination, with ECG predominating. EGCG increases rapidly after the emergence of the plumule and becomes dominant from the occurrence of the leaf development (Forrest and Bendall, 1969). However, the results of this Australian study and others where the level of EGCG in the fresh leaves was three times

the level of ECG may imply that the synthesis of EGCG is more active and surpasses the synthesis of ECG during the growth of tea shoots.

Another possibility is that ECG may be formed by the gallated esterification of EC, whereas EGCG may be formed from EGC during the development of tea shoots (Harbowy and Balentine, 1997). Further studies are necessary to elucidate the flavonoid biosynthesis in tea.

ECG in freshly harvested green tea leaves has been proposed as a quality indicator for the processing of black tea (Bhatia, 1963; Bhatia and Ullah, 1968; Singh *et al*., 1999). Along with EGCG, the active synthesis of ECG in summer seasons may explain the increase in total flavanols and other polyphenols observed in tea flushes (Bockuchava and Skobeleva, 1969). In conclusion, the observed seasonal pattern of ECG formation in Australian grown fresh tea leaves suggests that tea harvested during the warmer months of January and February in Australia may produce better quality black tea.

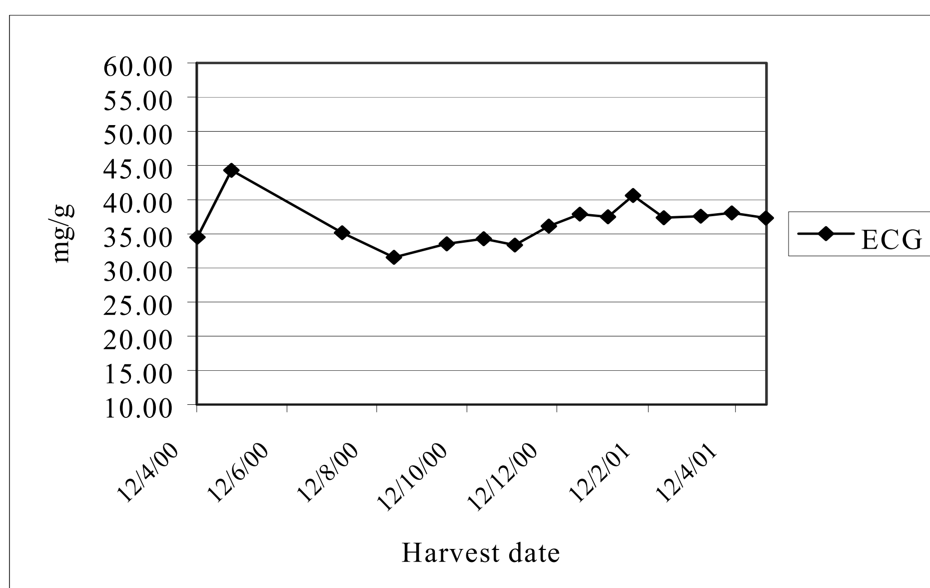


Figure 5.11 A seasonal variation of ECG in hand plucked tea leaves.

5.3.2.3 EGC

The seasonal profiles of EGC content in the fresh tea leaves showed that there was a significant effect ($P < 0.05$) due to harvest date. There was no significant difference ($P > 0.05$) in the EGC content in the fresh leaves among the samples harvested from 12 April to 13 November 2000 (which involves some of the cooler months) and from 6 December 2000 to 22 February 2001 (Table 5.25). Overall, there were significantly higher ($P < 0.05$) levels of the EGC content in fresh leaves harvested during the months from April to October 2000 (mean = 50.55 mg/g) than in the months of January to May 2001 (mean = 42.19 mg/g), with the latter months showing a decline in EGC levels (Table 5.25, Figure 5.12). Thus, the only useful trend from this data (Table 5.25, Figure 5.12) is that the levels of EGC were highest and constant during the cooler months of July to October 2000 and then slowly decreased as the temperature and day length increased into the warmer months of January 2001 to April 2001.

The seasonal pattern of EGC in tea flushes was different from those of ECG and EGCG (Figures 5.10, 5.11 and 5.12). While the gallates ECG and EGCG levels tended to decrease during the cooler months and increase during the warmer months, EGC behaved in a different fashion. Little change in EGC

levels occurred during the cooler months, and then a decrease from the constant cooler months levels occurred as the temperature rose throughout the warmer months (November 2000 to April 2001). These findings for ECG and EGCG show some similarities to the findings of Bokuchava and Skobeleva (1969) who reported that the formation of ECG and EGCG decreased sharply during the winter. However, these authors found that the formation of EGC rose during the winter months, which is not in agreement with this study in Australia, where EGC levels remain constant but higher in the cooler months.

Table 5.25 Mean content of EGC in hand plucked tea leaves.

Harvest date	EGC[1] mg/g, dry basis	Harvest date	EGC[1] mg/g dry, basis
12 April 2000	47.65 defg	27 December 2000	41.99 bc
9 May 2000	51.79 g	15 January 2001	45.63 cde
19 July 2000	50.65 fg	1 February 2001	46.45 cdef
23 August 2000	50.58 efg	22 February 2001	41.79 bc
28 September 2000	50.28 efg	19 March 2001	35.49 a
23 October 2000	52.33 g	9 April 2001	39.82 ab
13 November 2000	47.47 defg	2 May 2001	43.94 bcd
6 December 2000	45.83 cdef		
LSD	5.00	LSD	5.00

[1] Means (n=6) in table followed by a common letter are not significantly different (P > 0.05)

The Australian data shows EGC levels in fresh leaves are highest and constant during the cooler months as the plant continues to grow. This result agrees with findings of Hilton (1973) who found that EGC levels in fresh tea shoots harvested in central Africa during the cold seasons were higher than in those harvested in warmer seasons.

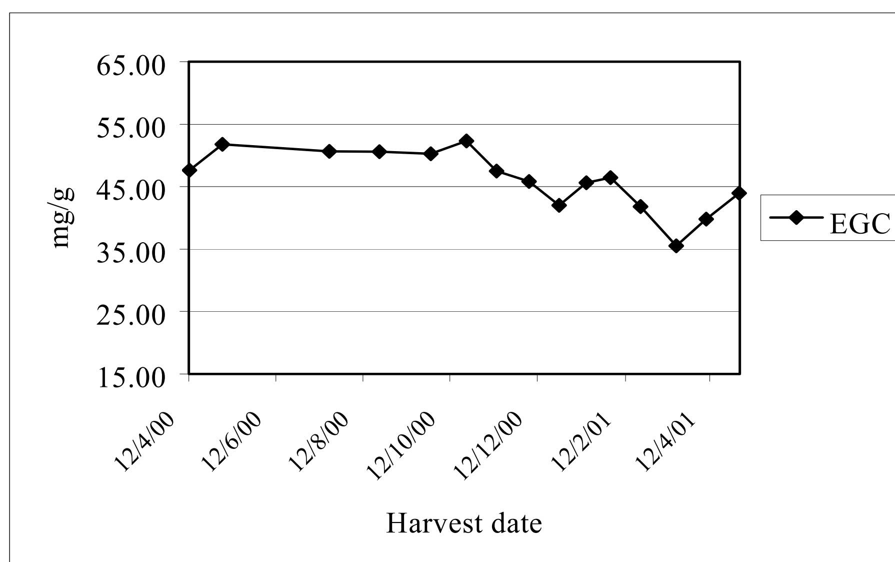


Figure 5.12 A seasonal variation of EGC in hand plucked tea leaves.

In Australia grown tea, the lower EGC content in fresh leaves during the summer months may imply that the active biosynthesis of EGCG consumes large amounts of EGC, thus the content of EGCG in fresh leaves is higher in summer, whereas the content of EGC remains lower. However, the

biosynthesis of EGCG would slow down during the winter, which allows the accumulation of EGC in the fresh leaves. This is a possible explanation as to why the content of EGC is higher in winter than in summer. Further studies are necessary to confirm the relationship between EGC and EGCG in fresh tea leaves, and how seasonal variations occur.

The level of EGC in tea shoots has been found to positively relate to the total level of theaflavins in the resulting black tea (Hilton, 1972; Bhatia and Ullah, 1968). Thus, EGC could be an important catechin in the determination of black tea quality since theaflavins have been regarded as the quality indicator for black tea (Ellis and Cloughley, 1981; Hazarika *et al*., 1984b). ECG (Bhatia, 1963; Bhatia and Ullah, 1968; Obanda *et al*., 1997ab; Singh *et al*., 1999) and EGCG (Bhatia, 1963; Obanda *et al*., 1997ab) were also found to positively relate to the quality of black tea. In addition, Roberts (1958, 1962) suggested that both EGC and EGCG in tea flushes are important quality indicators for resulting black tea. However, based on the findings in this study, it is unlikely that the maximum levels of all three flavanols would be attained at the same period of harvest, since there exist opposite seasonal trends of EGC, EGCG and ECG. Therefore, on the balance of the literature data and the findings of this study, the catechin gallates clearly override the catechins in the green tea leaves and hence are more important than the catechins in determining the quality of the resulting black tea.

5.3.2.4 Total catechins

There was a significant effect ($P < 0.05$) due to the harvest date for the total catechins in fresh tea leaves. Among the fresh leaves harvested during the cooler months from 19 July to 28 September 2000, no significant differences ($P > 0.05$) in the levels of total catechins were observed (Table 5.26). However, the levels of total catechins in the tea flushes harvested during these cooler months (July to September 2000) were significantly higher ($P < 0.05$) than those in tea fresh leaves harvested during the warmer months from 22 February to 9 April 2001, which themselves were not significantly different ($P > 0.05$) from each other.

Again, as for the EGC, there is a peak in the levels of total catechins during the cooler months, and then a gradual reduction as the temperature rises into the warmer months (Figure 5.13). This effect is not unexpected since EGC represents more than 50 % of the total catechins (Tables 5.25 and 5.26). During the period from 23 October 2000 to 2 May 2001, the variation of the levels of total catechins in fresh leaves (Figure 5.13) mirrors the variation in the levels of EGC (Figure 5.12) for the same reason. Thus, the variations in the other catechins (C, EC and GC) are likely to mirror that of EGC. In conclusion, in Australian grown tea, the seasonal variation of EGC and thus the total catechins is that the highest levels are produced during the cooler months and the lowest levels during the warmer months.

Table 5.26 Mean content of total catechins in hand plucked tea leaves.

Harvest date	CS[1] mg/g, dry basis	Harvest date	CS[1] mg/g, dry basis
12 April 2000	75.25 bcd	27 December 2000	75.83 bcde
9 May 2000	80.28 def	15 January 2001	82.69 efg
19 July 2000	91.96 h	1 February 2001	83.05 efg
23 August 2000	89.86 gh	22 February 2001	72.34 abc
28 September 2000	87.20 fgh	19 March 2001	66.21 a
23 October 2000	91.42 h	9 April 2001	71.29 ab
13 November 2000	82.67 efg	2 May 2001	76.61 bcde
6 December 2000	78.98 cde		
LSD	7.39	LSD	7.39

[1] Means (n=6) in table followed by a common letter are not significantly different ($P > 0.05$)

The findings are in agreement with those of Hilton *et al*. (1973) who reported that tea shoots contained higher total catechins during the slow growth colder conditions, with EGC being affected the most by

the cold seasons. This similarity between the content of EGC and that of total catechins could be explained by the fact that winter seasons favour the accumulation of catechins EGC and EC (Bokuchava and Skobeleva, 1969), which together most affect the content of total catechins in fresh tea leaves.

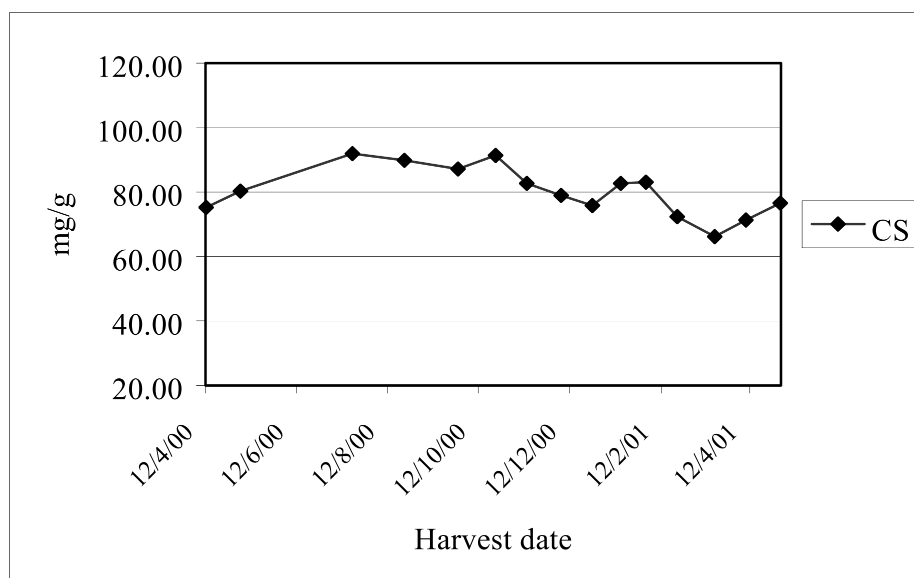


Figure 5.13 A seasonal variation of total catechins in hand plucked tea leaves.

5.3.2.5 Total catechin gallates

There was a significant effect ($P < 0.05$) of harvest date on the content of total catechin gallates in fresh tea leaves among the samples collected. During the cooler months (19 July to 28 September 2000), the levels of total catechin gallates in the fresh tea leaves were significantly lower ($P < 0.05$) than the levels in the leaves harvested in the warmer months (12 April and 9 May 2000; 6 December to 2 May 2001) (Table 5.27). From low levels during July to September 2000 harvests, the level of total catechin gallates in fresh leaves gradually increased as the temperatures increased into the warmer months (Figure 5.14).

The contents of total catechin gallates (Tables 5.27 and Figures 5.14) showed a very similar seasonal pattern to that of ECG (Table 5.24, Figure 5.11) and in particular to that of EGCG (Tables 5.23, Figures 5.10) in the fresh leaves. The reason why total catechin gallates show a similar seasonal pattern to that of EGCG is because EGCG represents about 70 % of the total catechin gallates in the tea flushes (Tables 5.23 and 5.27), thus quantitatively dominates the seasonal pattern of these grouped gallates. In addition, ECG, representing more than 20 % of total catechin gallates in tea fresh leaves, also showed a similar seasonal pattern as EGCG. In conclusion, for Australian grown tea, the seasonal pattern for the levels of total catechin gallates in fresh leaves is similar to those of EGCG and ECG, with lower levels in cooler months and higher levels in warmer months.

As discussed previously, the biosynthesis of EGCG and ECG is active during the warm months (Bockuchava and Skobeleva, 1969), which thus contribute higher contents of the total catechin gallates in the tea flushes harvested in the warmer months. The lower content of total catechin gallates in winter may be due to a corresponding less active biosynthesis of ECG and EGCG during the cooler months. Moreover, the similarities among the seasonal patterns of EGCG, ECG and total catechin gallates may suggest that the other minor catechin gallates (CG, ECDG, GCG and EGCDG) could have similar seasonal patterns to EGCG and ECG.

In addition, the cooler months (July to September) in 2000 coincided with a lower record of rainfall, while the warmer months (November 2000 to April 2001) recorded a higher rainfall (Table 3.2).

Thus, it is possible that the rainfall may contribute to the decrease and/or the increase pattern of total catechin gallates. However, more information is required to better relate the rainfall to the seasonal variations of individual or total catechin gallates, such as the irrigation and other agronomic management conducted during these dry months. These agronomic activities would be better studied under a controlled system for trials. Therefore, further investigation into the effect of rainfall and irrigation on the individual and grouped flavanols in tea shoots would be useful for fully elucidating the factors that affect the quality of Australian grown and made tea.

Table 5.27 Mean content of total catechin gallates in hand plucked fresh leaves.

Harvest date	CGS[1] mg/g, dry basis	Harvest date	CGS[1] mg/g, dry basis
12 April 2000	163.75 efg	27 December 2000	159.91 efg
9 May 2000	183.83 h	15 January 2001	161.16 efg
19 July 2000	132.30 ab	1 February 2001	173.51 gh
23 August 2000	128.64 a	22 February 2001	160.19 efg
28 September 2000	136.90 ab	19 March 2001	157.03 def
23 October 2000	143.17 bc	9 April 2001	167.36 fg
13 November 2000	144.65 bcd	2 May 2001	152.39 cde
6 December 2000	156.63 cdef		
LSD	13.68	LSD	13.68

[1] Means (n=6) in table followed by a common letter are not significantly different (P > 0.05)

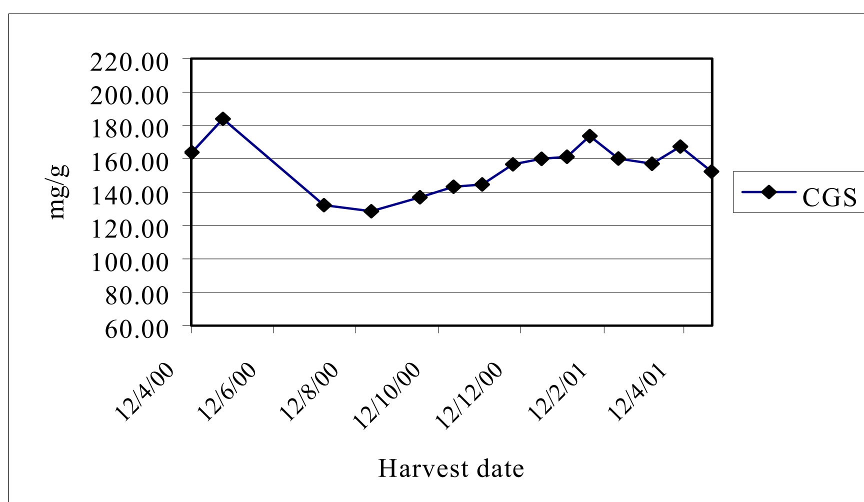


Figure 5.14 A seasonal variation of total catechin gallates in hand plucked tea leaves.

5.3.2.6 Combined catechins/catechin gallates

There is a group of compounds comprising all flavanol compounds determined as combined catechins/catechin gallates. Together, they are also referred to as flavanols or total flavanols (Hilton, 1972, 1973 and Hilton *et al*., 1973). The combined levels of catechins and catechin gallates in tea flushes showed a significant effect (P < 0.05) due to harvest date. However, there was no significant difference (P > 0.05) in the contents of combined catechins/catechin gallates among the samples harvested from 19 July to 6 December 2000, from 23 October 2000 to 15 January 2001, and from 22 February to 2 May 2001 (Table 5.28, Figure 5.15). There are two peaks in the combined levels of these compounds; one on 9 May 2000 and another on 1 February 2001 (Table 5.28).

Table 5.28 Mean content of combined catechins/catechin gallates (CCS) in hand plucked tea leaves.

Harvest date	CCS[1] mg/g, dry basis	Harvest date	CCS[1] mg/g, dry basis
12 April 2000	239.00 bc	27 December 2000	235.74 bc
9 May 2000	264.11 e	15 January 2001	243.85 cd
19 July 2000	224.25 ab	1 February 2001	256.56 de
23 August 2000	218.49 a	22 February 2001	232.53 abc
28 September 2000	224.10 ab	19 March 2001	223.24 ab
23 October 2000	234.60 abc	9 April 2001	238.65 bc
13 November 2000	227.32 abc	2 May 2001	228.99 abc
6 December 2000	235.61 abc		
LSD	17.21	LSD	17.21

[1] Means (n=6) in table followed by a common letter are not significantly different (P > 0.05)

The trend in the data (Figure 5.15) is that the lowest level of combined catechins/catechin gallates in the fresh tea leaves occurred during the cooler months from 19 July to 28 September 2000, after which the levels rose again through the warmer months. This result is very similar to those of EGCG and total catechin gallates (Tables 5.23 and 5.27). This similarity of seasonal pattern reveals that catechin gallates (and EGCG in particular), representing about 65 % of the combined catechins/catechin gallates, dominate the seasonal distribution pattern of flavanols in the tea flushes (Tables 5.27 and 5.28).

The contents of combined catechins/catechin gallates were not significantly different (P > 0.05) among the samples harvested from 19 July to 6 December 2000, suggesting that the differentiation of the combined catechins/catechin gallates between cooler and warmer months is complex. This is because the seasonal patterns of the catechins were the reverse of the patterns for the catechin gallates. Thus, when those two groups of compounds were added together as the combined catechins/catechin gallates, any seasonal differences cancelled out.

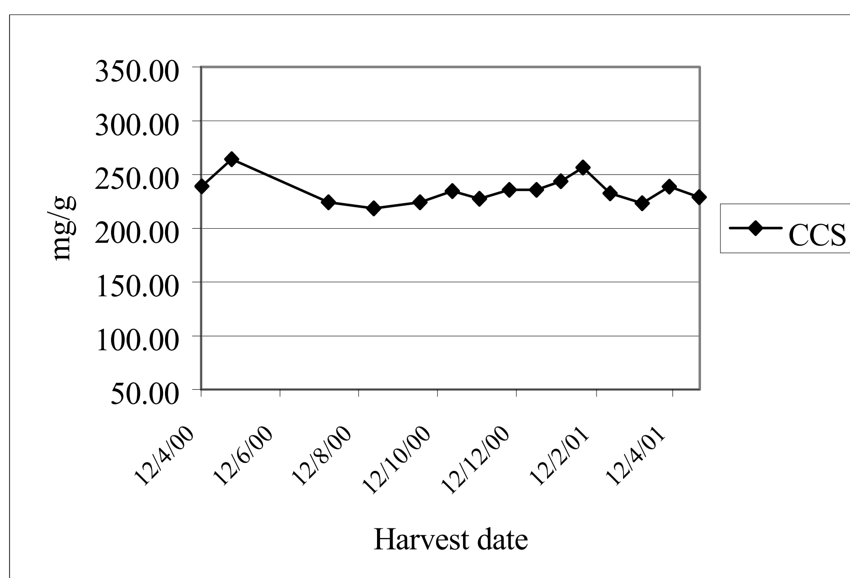


Figure 5.15 A seasonal variation of the combined catechins and catechin gallates in hand plucked tea leaves.

The results of this study indicate that the combined catechins/catechin gallates could only represent a general pattern for flavanols in tea flushes, and do not precisely reveal the true seasonal patterns of individual compounds or grouped catechins or catechin gallates. This study has shown that the variations of the individual compounds ECG, EGC and EGCG better predict the seasonal variations in fresh tea shoots than does the combined flavanol content. This is a significant result since ECG, EGC and EGCG are considered to be quality indicators for black tea processing. Therefore, the variations of ECG, EGCG and EGC levels in fresh tea leaves may be used as indicators of the likely variation that may occur in black tea quality.

5.3.2.7 Total phenolic compounds

Total phenolic compounds in this study include all the catechins, catechin gallates, phenolic acids and flavonoid glycosides. The total phenolic compounds in fresh tea leaves showed a significant effect ($P < 0.05$) of harvest date. However, there was no overall trend in the data (Table 5.29, Figure 5.16), particularly not between the cooler and warmer months as observed earlier for other tea components.

Table 5.29 Mean content of total phenolic compounds (TPS) in hand plucked tea leaves.

Harvest date	TPS[1] mg/g, dry basis	Harvest date	TPS[1] mg/g, dry basis
12 April 2000	275.29 ab	27 December 2000	275.47 ab
9 May 2000	304.73 c	15 January 2001	286.22 bc
19 July 2000	264.22 a	1 February 2001	300.14 c
23 August 2000	258.23 a	22 February 2001	268.69 ab
28 September 2000	260.75 a	19 March 2001	262.88 a
23 October 2000	274.64 ab	9 April 2001	277.53 ab
13 November 2000	264.61 a	2 May 2001	268.28 ab
6 December 2000	274.95 ab		
LSD	19.88	LSD	19.88

[1] Means (n=6) in table followed by a common letter are not significantly different ($P > 0.05$)

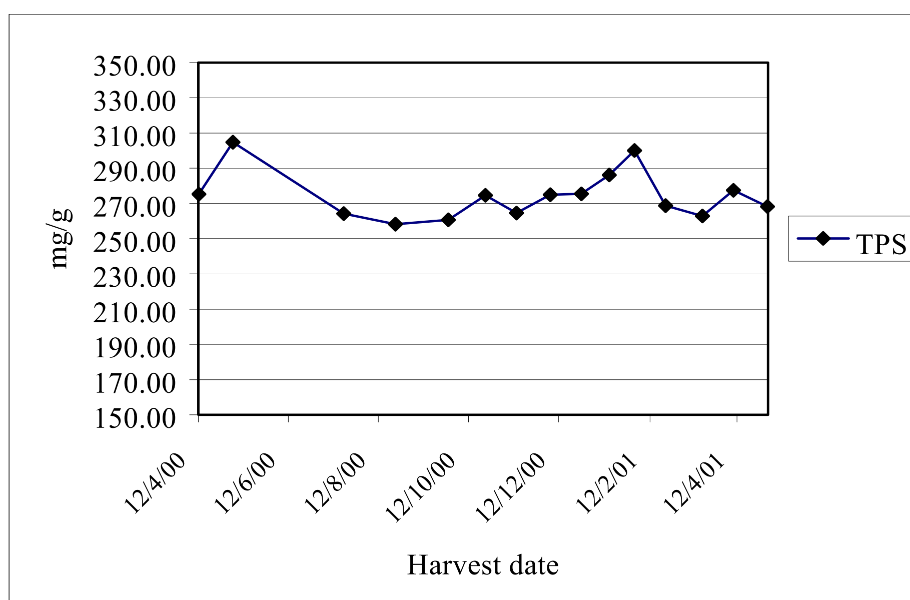


Figure 5.16 A seasonal variation of total phenolic compounds in hand plucked tea leaves.

Thus, the seasonal variations of phenolic compounds are better measured using the variations of catechins and catechin gallates.

5.3.2.8 Summary of seasonal variations of phenolic compounds in hand plucked fresh tea leaves

There are generally significant differences ($P < 0.05$) between the levels of EGCG, ECG, EGC, total catechins or total catechin gallates in hand plucked fresh leaves harvested in cooler and warmer months. Levels of EGCG, ECG and total catechin gallates in fresh leaves were lowest during the cooler months from 19 July to 28 September 2000, then increased throughout the warmer months from October to December 2000, and thereafter, remained constant during the warmer month up to May 2001. The levels of EGC and total catechins were highest during the cooler months from 19 July to 28 September 2000, thereafter, the levels decreased throughout the warmer months from November 2000 to May 2001. Thus, catechins show in general a different seasonal pattern from that of the catechin gallates. The combined catechins/catechin gallates, and total phenolic compounds show closer seasonal pattern to those of the dominant EGCG and catechin gallates.

The results of this study on Australian grown tea leaves demonstrate that EGCG and ECG are dominant flavanols present in tea green leaves, and their levels better reflect seasonal variations. The results of this study further suggest that fresh leaves harvested during the warmer months (December to May) when the levels of EGCG and ECG are highest should provide the basis for producing the best quality black tea in Australia.

5.3.3 Seasonal variations of phenolic compounds in mechanically harvested green tea leaves

5.3.3.1 EGCG

A significant effect ($P < 0.05$) of harvest date was found for the EGCG content in the mechanically harvested green tea leaves. There were no obvious trends in the EGCG data except a gradual increase in levels occurred as the temperature increased, although the levels fluctuated up and down (Table 5.30, Figure 5.17). There was a significant decrease ($P < 0.05$) in levels between 9 May and 19 July 2000, when the temperatures (Table 3.2) began to drop (Table 5.30). Clearly, mechanically harvested green leaves are inferior to hand plucked samples when seasonal variations need to be observed.

However, the content of EGCG in the green leaves harvested on 23 August 2000 was significantly different ($P < 0.05$) from those harvested on 19 July and 28 September 2000 (Table 5.30). This variation under similar cooler weather conditions is unusual. The reason for this phenomenon may be explained as follows.

An unusual frost occurred in early June 2000 on the tea farm. This frost caused most of the tips of tea shoots to burn. The burnt tea shoots and some good tea shoots were harvested but dumped because they were unsuitable for black tea processing (Benson, 2001). However, some parts of the paddocks might not have been burnt and may have been left over for the next round of harvest in July 2000. The tea trees with burnt shoots would need time to recover physiologically to normal growth. During the cold weather of June 2000, the growth of tea shoots possibly resulted in some banjhi leaves (leaves without obvious bud) rather than normal flushes (one bud with a few adjoining leaves). The tea shoots unaffected by the frost in June 2000 would have grown slowly in this unusually cold month and probably formed banjhi leaves (with lower EGCG levels) during July 2000. Thus, the EGCG levels were very low in the green leaves harvested mechanically on 19 July 2000. The hand plucked samples on the 19 July 2000 would not have included the banjhi leaves and thus a higher EGCG level was observed (Table 5.9).

Table 5.30 Mean content of EGCG in mechanically harvested tea leaves.

Harvest date	EGCG[1] mg/g, dry basis	Harvest date	EGCG[1] mg/g, dry basis
12 April 2000	106.95 cd	27 December 2000	94.91 bc
9 May 2000	105.33 cd	15 January 2001	109.88 d
19 July 2000	74.41 a	1 February 2001	110.10 d
23 August 2000	98.48 cd	22 February 2001	98.15 cd
28 September 2000	82.30 ab	19 March 2001	100.33 cd
23 October 2000	83.00 ab	9 April 2001	106.60 cd
13 November 2000	102.38 cd	2 May 2001	99.50 cd
6 December 2000	99.05 cd		
LSD	13.53	LSD	13.53

[1] Means (n=3) in table followed by a common letter are not significantly different ($P > 0.05$)

The peak value of EGCG in the green leaves that were mechanically harvested in August 2000 (Figure 5.17) may be due to the stimulation from the slow growing in July 2000 because of the frost in June 2000. During the period of frosts, the tea plant should have accumulated enough nutrients for the growth of new shoots. However, the abrupt cold weather and the burn damage on the tips of the tea shoots would stop this physiological process. Thereafter, the tea plants readjusted their physiological processes to recover from this damage. This period took about two months from June to July 2000, after which the tea shoots started to grow faster than normally because of the accumulation of nutrients. This fast growth resulted in more young leaves being mechanically harvested and resulted in a significantly higher ($P < 0.05$) level of EGCG for the 23 August 2000 harvest than in say September/October 2000 harvests. After this period, based on EGCG levels the tea plants returned to normal growth in November 2000.

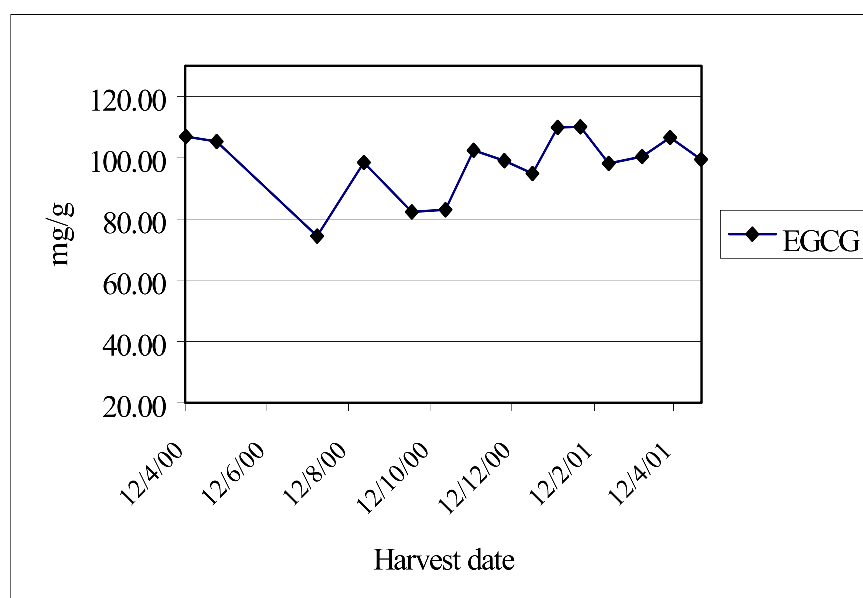


Figure 5.17 A seasonal variation of EGCG in mechanically harvested green leaves.

5.3.3.2 ECG

The contents of ECG in mechanically harvested green tea leaves showed a significant effect ($P < 0.05$) of harvest date. Again, as for EGCG, there was no obvious trend in the data except a slight increase as

the temperatures increased, with levels varying up and down (Table 5.31, Figure 5.18). The differences in ECG levels observed in the hand plucked fresh leaves harvested in warmer and cooler months were not observed for the mechanically harvested leaves.

Table 5.31 Mean content of ECG in mechanically harvested tea leaves.

Harvest date	ECG[1] mg/g, dry basis	Harvest date	ECG[1] mg/g, dry basis
12 April 2000	34.10 bcdef	27 December 2000	32.00 abcd
9 May 2000	35.88 cdef	15 January 2001	37.58 ef
19 July 2000	27.67 a	1 February 2001	39.24 f
23 August 2000	35.61 cdef	22 February 2001	34.03 bcdef
28 September 2000	30.47 abc	19 March 2001	35.82 cdef
23 October 2000	29.97 ab	9 April 2001	35.82 cdef
13 November 2000	35.98 def	2 May 2001	35.62 cdef
6 December 2000	32.72 abcde		
LSD	5.50	LSD	5.50

[1] Means (n=3) in table followed by a common letter are not significantly different (P > 0.05)

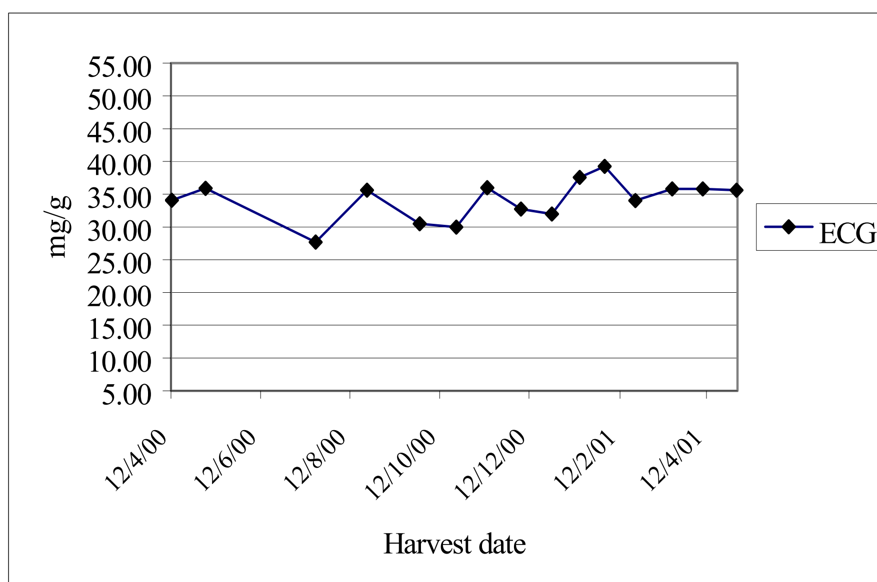


Figure 5.18 A seasonal variation of ECG in mechanically harvested tea leaves.

5.3.3.3 EGC

The content of EGC in mechanically harvested green tea leaves showed a significant effect ($P < 0.05$) of harvest date. The trend in the data was that there was a significant increase ($P < 0.05$) in EGC levels in the mechanically harvested green leaves between the harvests of April/May 2000 and those from July to November 2000, with the EGC levels gradually falling from 28 September 2000 until 19 March 2001 (Table 5.32, Figure 5.19). This large increase in EGC levels in the mechanically harvested green tea leaves between harvest dates on 9 May 2000 and 19 July 2000 was not observed for the hand plucked leaves between the same harvest dates.

Table 5.32 Mean content of EGC in mechanically harvested tea leaves.

Harvest date	EGC[1] mg/g, dry basis	Harvest date	EGC[1] mg/g, dry basis
12 April 2000	33.10 a	27 December 2000	46.76 de
9 May 2000	39.03 abc	15 January 2001	46.22 d
19 July 2000	47.78 def	1 February 2001	47.71 def
23 August 2000	53.03 ef	22 February 2001	38.07 abc
28 September 2000	53.97 f	19 March 2001	36.79 ab
23 October 2000	47.94 def	9 April 2001	41.98 bcd
13 November 2000	48.29 def	2 May 2001	44.01 cd
6 December 2000	46.21 d		
LSD	6.56	LSD	6.56

[1] Means (n=3) in table followed by a common letter are not significantly different (P > 0.05)

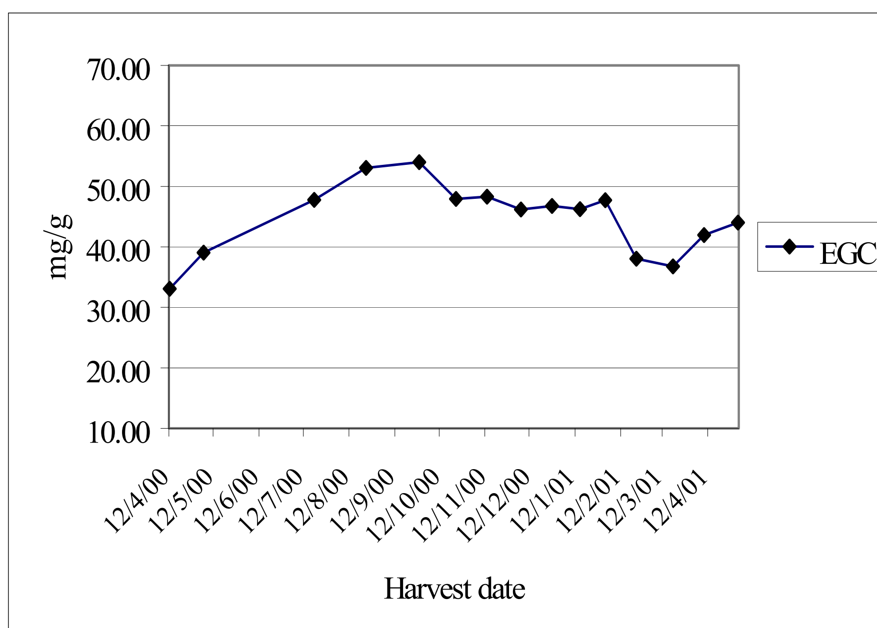


Figure 5.19 A seasonal variation of EGC in mechanically harvested tea leaves.

However, the decrease in EGC levels that occurred for the mechanically harvested green tea leaves during the warmer months is the same as that observed earlier for the hand plucked fresh tea leaves. A significant decrease ($P < 0.05$) in EGC levels was observed from the 1 February to 22 February 2001 harvests for the mechanically harvested tea leaves, while a significant decrease ($P < 0.05$) was observed in hand plucked fresh leaves from 22 February to 19 March 2001 harvests. Although there was a harvest time difference, this result suggests that the decrease in EGC levels in the tea leaves during this period could be a real trend.

5.3.3.4 Total catechins

There was a significant effect ($P < 0.05$) of harvest date for the levels of total catechins in the mechanically harvested green tea leaves. The content of total catechins in the green leaves showed a significant increase ($P < 0.05$) from April/ May 2000 to 19 July 2000, and from 19 July to 23 August 2000, after which it remained constant until 13 November 2000 (Table 5.33, Figure 5.20). The trend was for an increase in total catechin levels in the mechanically harvested green tea leaves during the cooler months. In addition, the content of total catechins in the green leaves were significantly lower ($P < 0.05$) in the warmer months from 22 February to 2 May 2001 (Table 5.33). This seasonal pattern of total catechins for the mechanically harvested green tea leaves was similar to that discussed earlier for the hand plucked fresh tea leaves on the same harvest dates.

Table 5.33 Mean content of total catechins in mechanically harvested tea leaves.

Harvest date	CS[1] mg/g, dry basis	Harvest date	CS[1] mg/g dry basis
12 April 2000	56.64 a	27 December 2000	77.17, de
9 May 2000	63.56 ab	15 January 2001	81.05 efg
19 July 2000	75.64 de	1 February 2001	79.03 ef
23 August 2000	88.51 fg	22 February 2001	65.20 abc
28 September 2000	89.24 g	19 March 2001	63.69 ab
23 October 2000	80.28 efg	9 April 2001	68.53 bcd
13 November 2000	81.36 efg	2 May 2001	72.64 bcde
6 December 2000	73.84 cde		
LSD	10.02	LSD	10.02

[1] Means (n=3) in table followed by a common letter are not significantly different ($P > 0.05$)

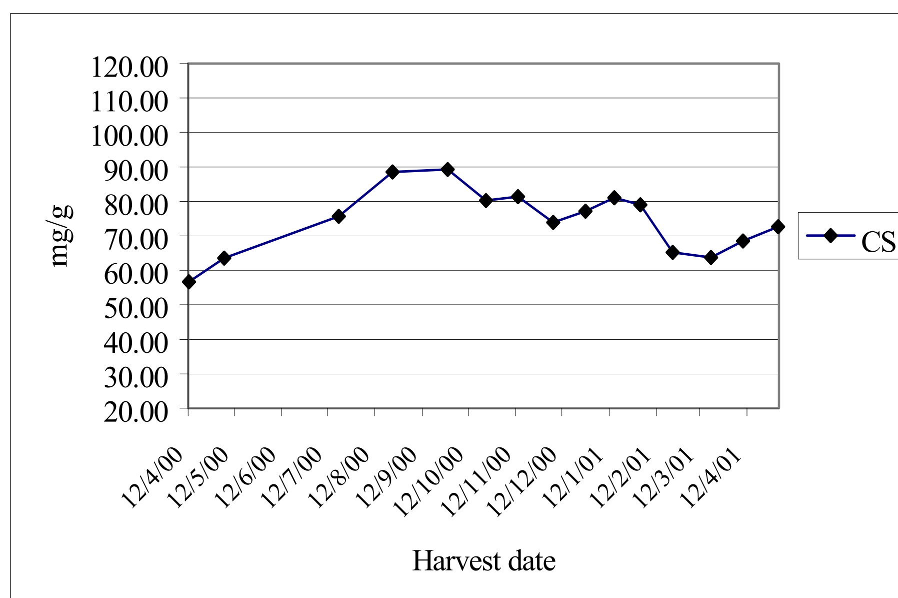


Figure 5.20 A seasonal variation of total catechins in mechanically harvested green leaves.

The seasonal distribution pattern of total catechins is very similar to that of EGC in the mechanically harvested tea leaves (Figures 5.19 and 5.20). This is because EGC represents about 60 % of total catechins in the mechanically harvested green leaves (Tables 5.32 and 5.33). Thus, the seasonal

distribution pattern of EGC may be used to represent the seasonal pattern of total catechins in the green leaves that are harvested mechanically.

The levels of total catechins ranged 56.64-89.24 mg/g (dry weight basis) in mechanically harvested green leaves (Table 5.33), which is lower than the levels of total catechins in the hand plucked fresh leaves (Table 5.26). This is because there were more mature tea shoots in the mechanically harvested green leaves than in the hand plucked samples. Baruah *et al*. (1986) found that the biosynthesis of catechins including EGC is less active in the mature tea shoots than in the tender tea shoots.

5.3.3.5 Total catechin gallates

The levels of total catechin gallates in mechanically harvested tea flushes showed a significant effect ($P < 0.05$) of harvest date. In general, total catechin gallates in the green leaves showed lower levels in cooler months (July, September and October 2000) and higher levels in warmer months (November 2000 to May 2001) (Table 5.34, Figure 5.21).

The total catechin gallates in the mechanically harvested green leaves showed a peak value in the sample harvested on 23 August 2000, which was significantly higher ($P < 0.05$) than that in the samples harvested in the other cooler months of 19 July and 28 September 2000 (Table 5.34 and Figure 5.21). This is similar to the result for EGCG content in green leaves (Figure 5.16), as discussed in Section 5.3.3.1. Thus, the general seasonal pattern of total catechin gallates could be represented by that of EGCG since the content of EGCG represents about 70 % of total catechin gallates in the mechanically harvested green leaves. This seasonal distribution pattern of total catechin gallates may also suggest that all the other minor catechin gallates in the mechanically harvested green leaves could have a similar pattern over the seasons as that of the EGCG.

Table 5.34 Mean content of total catechin gallates (CGS) in mechanically harvested tea leaves.

Harvest date	CGS[1] mg/g, dry basis	Harvest date	CGS[1] mg/g, dry basis
12 April 2000	147.68 de	27 December 2000	132.84 bcd
9 May 2000	149.13 de	15 January 2001	156.12 e
19 July 2000	106.27 a	1 February 2001	157.17 e
23 August 2000	139.82 de	22 February 2001	139.82 de
28 September 2000	117.64 ab	19 March 2001	144.59 de
23 October 2000	118.44 abc	9 April 2001	150.68 de
13 November 2000	145.60 de	2 May 2001	143.04 de
6 December 2000	137.81 cde		
LSD	20.03	LSD	20.03

[1] Means (n=3) in table followed by a common letter are not significantly different ($P > 0.05$)

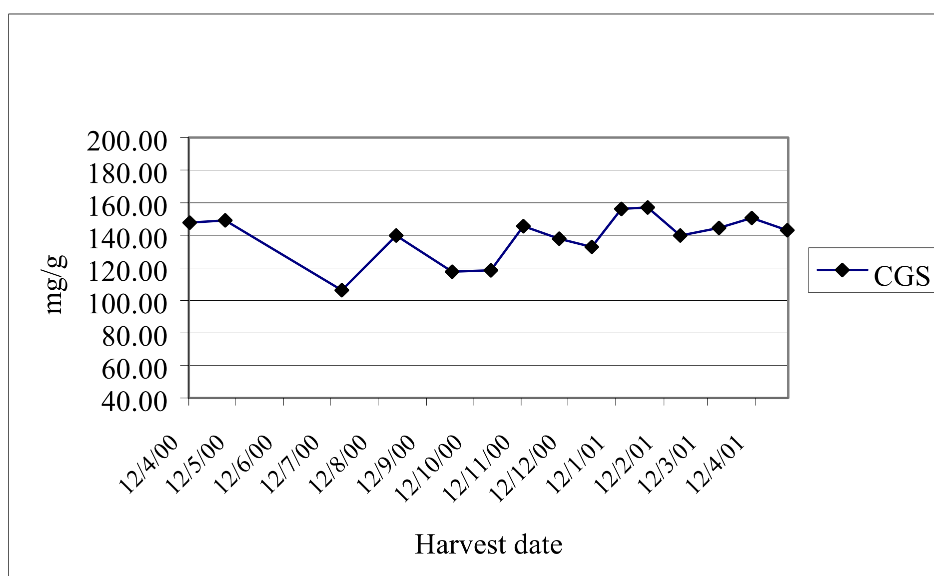


Figure 5.21 A seasonal variation of total catechin gallates in mechanically harvested tea leaves.

5.3.3.6 Combined catechins/catechin gallates

The levels of the combined catechins/catechin gallates in mechanically harvested green leaves showed a significant harvest date effect (P < 0.05) (Table 5.35 and Figure 5.22).

Table 5.35 Mean content of the combined catechins/catechin gallates (CCS) in mechanically harvested tea leaves.

Harvest date	CCS[1] mg/g, dry basis	Harvest date	CCS[1] mg/g, dry basis
12 April 2000	204.31 abc	27 December 2000	210.01 bcd
9 May 2000	212.70 bcde	15 January 2001	237.17 f
19 July 2000	181.90 a	1 February 2001	236.20 ef
23 August 2000	228.33 def	22 February 2001	205.02 abcd
28 September 2000	206.88 bcd	19 March 2001	208.28 bcd
23 October 2000	198.72 ab	9 April 2001	219.21 bcdef
13 November 2000	226.96 cdef	2 May 2001	215.68 bcdef
6 December 2000	211.66 bcd		
LSD	23.92	LSD	23.92

[1] Means (n=3) in table followed by a common letter are not significantly different (P > 0.05)

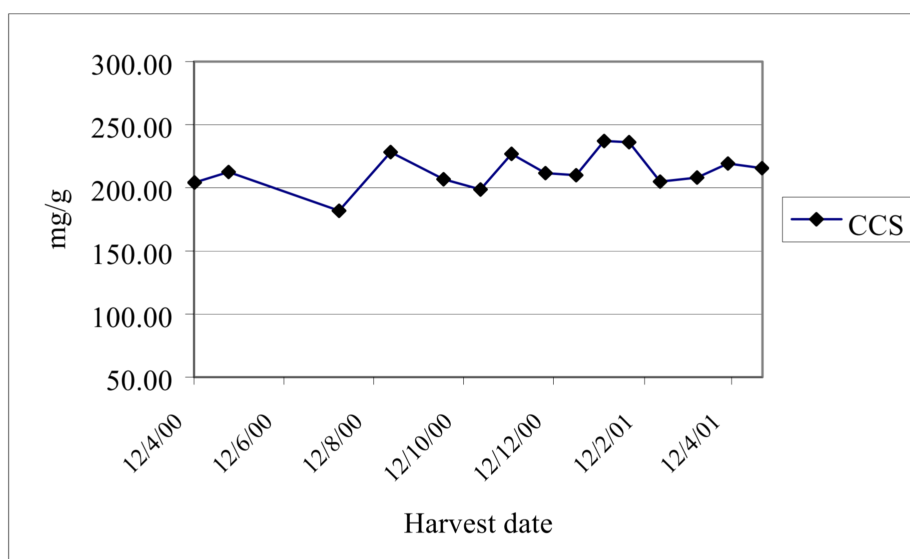


Figure 5.22 A seasonal variation of combined catechins/catechin gallates in mechanically harvested tea leaves.

The significant differences ($P < 0.05$) in the content of the combined catechins/catechin gallates occurring among the harvests of winter months might be an unusual result caused by the unusual weather change in June 2000, as discussed in Section 5.3.3.1. However, there was no general trend in the data for the combined catechins/catechin gallates with levels rising and falling, suggesting mechanically harvested tea leaves and this type of data are not useful for determining seasonal variation.

5.3.3.7 Total phenolic compounds

There was a significant harvest date effect ($P < 0.05$) for the levels of total phenolic compounds in the mechanically harvested tea leaves. The content of total phenolic compounds in the green leaves harvested on 23 August 2000 showed a significant difference ($P < 0.05$) from those harvested on 19 July and 28 September 2000 (Table 5.36 and Figure 5.23). This is very similar to the results of EGCG and total catechin gallates discussed earlier. Thus, the significant higher content ($P < 0.05$) of total phenolic compounds in the green leaves that were mechanically harvested on 23 August 2000 could be due to the same reasons as discussed for EGCG and total catechin gallates. Overall, as for the combined catechins/catechin gallates, there was no trend in the data with levels rising and falling throughout harvest periods (Figure 5.23).

The contents of total phenolic compounds (Table 5.36) are comprised of more than 50 % of catechin gallates (Table 5.34) and about 80 % of combined catechins and catechin gallates (Table 5.35). Thus, the catechins and catechin gallates dominated the seasonal patterns of phenolic compounds in the green leaves.

The contents of total catechins in the mechanically harvested tea leaves (56.64-89.24 mg/g, Table 5.33) were lower than the contents of the total catechin gallates (106.27-157.17 mg/g, Table 5.34). Thus, the seasonal patterns of phenolic compounds were dominated by the seasonal distribution patterns of the total catechin gallates.

Table 5.36 Mean content of total phenolic compounds (TPS) in mechanically harvested tea leaves.

Harvest date	TPS[1] mg/g, dry basis	Harvest date	TPS[1] mg/g, dry basis
12 April 2000	229.65 ab	27 December 2000	236.99 bcd
9 May 2000	240.71 bcde	15 January 2001	273.11 f
19 July 2000	206.98 a	1 February 2001	267.11 ef
23 August 2000	265.36 def	22 February 2001	233.39 abc
28 September 2000	231.96 abc	19 March 2001	239.64 bcde
23 October 2000	226.45 ab	9 April 2001	248.15 bcdef
13 November 2000	260.07 cdef	2 May 2001	245.34 bcdef
6 December 2000	237.99 bcde		
LSD	29.73	LSD	29.73

[1] Means (n=3) in table followed by a common letter are not significantly different (P > 0.05)

Furthermore, since EGCG was the dominant compound of the total catechin gallates (contributing about 70 % of the total amount) in the green leaves, the seasonal patterns of total phenolic compounds could be monitored by analysing the EGCG levels in the mechanically harvested green leaves.

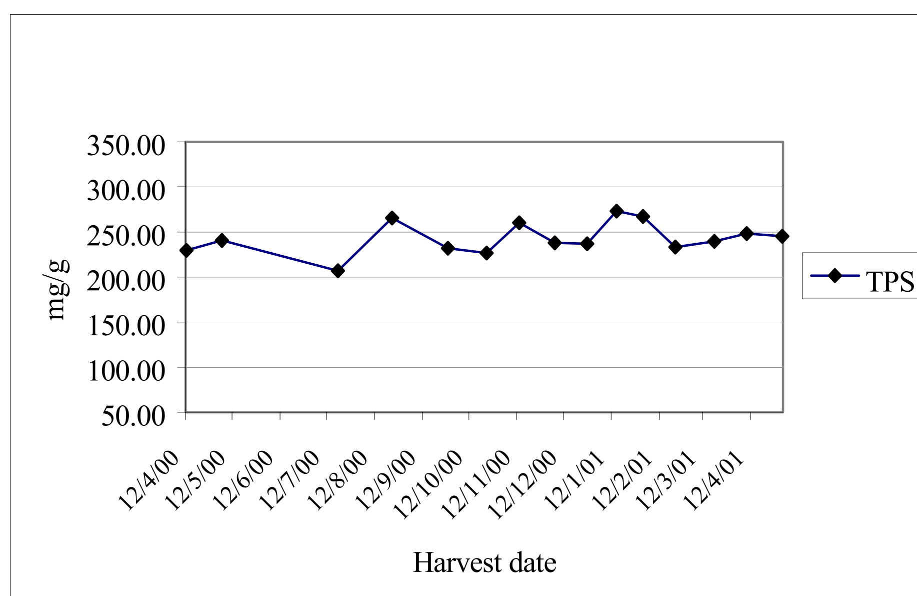


Figure 5.23 A seasonal variation of total phenolic compounds in mechanically harvested tea leaves.

5.3.3.8 Summary of seasonal variations of phenolic compounds in mechanically harvested green tea leaves

The contents of EGCG, ECG, EGC, total catechins or total catechin gallates in green tea leaves harvested mechanically showed no real trends with levels increasing and decreasing widely. The distinction between the cooler and warmer months was less obvious than for the hand plucked samples. In contrast, EGC and total catechins showed peak levels during the cooler months from 19 July to 28 September 2000 and lower levels on 12 April and 9 May 2000, and from 22 February to 2 May 2001. Thus, these two groups of compounds in the mechanically harvested tea leaves showed

different seasonal patterns controlled by the biosynthesis system in tea leaves, as discussed earlier for the hand plucked fresh leaves.

Since catechin gallates including EGCG and ECG represented about 70 % of combined catechins/catechin gallates, and 60 % of total phenolic compounds, the general seasonal trend of phenolic compounds in the green leaves were mainly affected by the distributions of catechin gallates.

5.3.4 Comparison of seasonal variations of phenolic compounds in tea leaves with different harvest methods

5.3.4.1 EGCG

The levels of EGCG at all harvests were lower in mechanically harvested samples than in hand plucked ones except for the harvest on 23 August 2000 (Tables 5.23 and 5.30). The seasonal distribution of EGCG in green leaves from these two different harvest methods showed different patterns. Since the Bin data from the mechanically harvested green leaves generally showed much more variation than the Field data from hand plucked fresh leaves, no direct statistical comparison of the Bin and Field data was carried out. In hand plucked fresh leaves (shown as the Field leaves), the distribution pattern of the content of EGCG showed a regular trend from higher levels in warmer months (April/May 2000) to lower levels in the cooler months (July to September 2000) and higher again in warmer months (October 2000 to May 2001). In the mechanically harvested green leaves (shown as the Bin leaves in Figure 5.30), the content of EGCG showed a different distribution pattern.

Records of fertiliser application to the farm for Glen Allyn Tea Estates showed that in June 2000 after a harsh frost occurred urea was applied to the tea bushes (Table 3.1). However, the cold weather stopped the tea shoots growing quickly enough to consume the fertiliser, which can be estimated from the comparison of the records for the production of green tea leaves for July-September 2000 (Table 5.37). In December 2000 and February 2001, there were two peak levels for the production of green leaves (Table 5.37), which were just one month after the fertiliser application in November 2000 and January 2001, respectively (Table 3.1). This coincided with a significant increase ($P < 0.05$) in EGCG levels between December 2000 and February 2001 for samples collected by the hand plucking harvesting method (Table 5.23), but not all the mechanical harvest method (Table 5.30). Thus, appropriate application of fertilisers may aid in the growth of tea shoots and induce the active synthesis of EGCG.

On comparing the levels of EGCG in the samples harvested on 23 August 2000 for both hand plucked and mechanically harvested leaves, it was noted that the mechanically harvested tea leaves had a higher content than that of hand plucked leaves (Figure 5.24). Since the younger tea shoots usually contain a higher concentration of EGCG (Baruah *et al*., 1986), this result may indicate that the mechanically harvested green leaves of 23 August 2000 might have been more tender than the hand plucked fresh leaves at the same period. Part of the possible reasons has been given in Section 5.3.3.1.

Owuor and Odhiambo (1994) found that the fast growing of tea shoots and increases in production of tea leaves could be due to the effect of the application of fertilisers. An increase in EGCG content with an increase of production of green leaves following the application of fertilisers may be due to the fast growth of tea shoots containing more tender tips. As a result, the increase in the young shoots in the mechanically harvested tea leaves may tend to bring the content of EGCG close to the level of EGCG in the hand plucked fresh tea leaves. It is also possible that appropriate application of fertilisers may enhance the synthetic capacity of EGCG in the young shoots during active growth.

However, high rates of nitrogen fertilisers applied on tea trees have been proved to be detrimental to the quality of black teas (Hilton *et al*., 1973; Owuor and Odhiambo, 1994). Thus, it would be useful to investigate the effect of applications of fertilisers on the production and the quality of Australian grown and made teas.

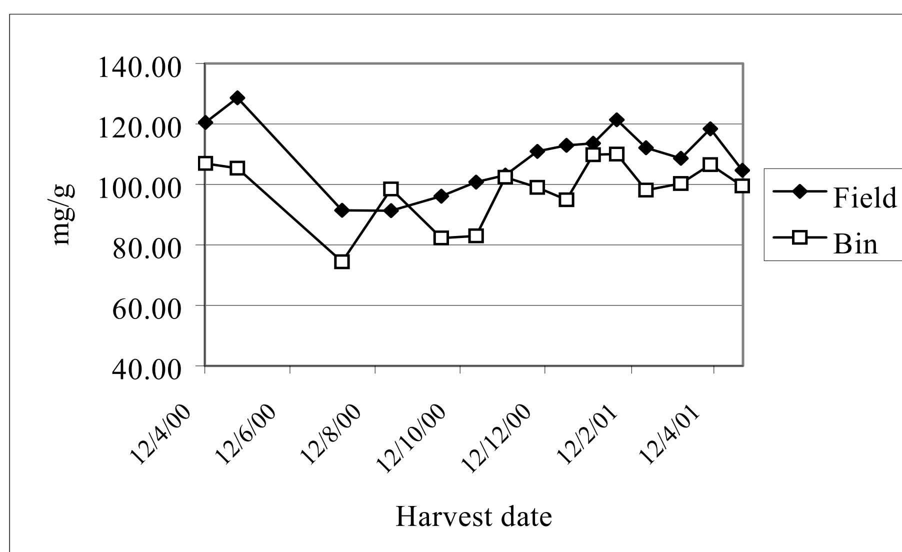


Figure 5.24 Comparison of seasonal variations of EGCG in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

Table 5.37 Monthly production of green tea leaves in different locations.

Harvest date	Production of green leaves, kg/ha[a]		
	Paddock A	Paddock B	Paddock C
04/00	1808	2003	2554
05/00	1825	1107	1337
07/00	124	217	243
08/00	557	314	1102
09/00	540	2913	2672
10/00	2391	632	845
11/00	1163	1360	3818
12/00	3483	4939	6444
01/01	1950	612	2186
02/01	3132	3333	4667
03/01	1867	2604	1881
04/01	1040	1039	3107
05/01	1646	1388	2105

[a] Supplied by Glen Allyn Tea Estates.

5.3.4.2 ECG

At all harvests except for the harvests on 23 August and 13 November 2000, the ECG levels in the mechanically harvested samples were lower than in the hand plucked samples (Tables 5.24 and 5.31). The seasonal patterns of ECG were similar to the patterns of EGCG for both the mechanically harvested and the hand plucked fresh tea leaves (Figures 5.25 and 5.24). Higher levels of ECG in the green leaves coincided with higher levels of EGCG in the same leaves. At the 23 August 2000 harvest, the peak levels of ECG and EGCG in mechanically harvested green leaves were greater than the corresponding levels in the hand plucked tea leaves.

The seasonal pattern of ECG showed a significant increase ($P < 0.05$) between December 2000 and February 2001 (Tables 5.24 and 5.31), following the fertiliser applications in November 2000 and January 2001, which was similar to that observed for the EGCG levels in the tea leaves. Therefore, fertiliser applications may be also beneficial to the synthesis of ECG in tea.

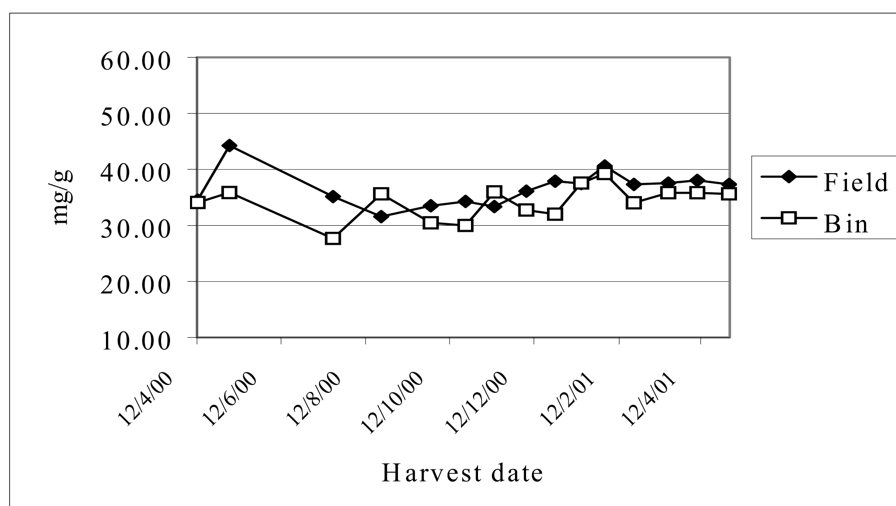


Figure 5.25 Comparison of seasonal variations of ECG in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

5.3.4.3 EGC

The content of EGC in mechanically harvested green tea leaves showed generally a similar seasonal pattern to that of hand plucked fresh tea leaves, with the peak values occuring in cooler months of 19 July to 28 September 2000 (Figure 5.26).

The applications of fertilisers in November 2000 and January 2001 did not affect the seasonal patterns of EGC since there was no obvious increases or decreases of the content of EGC after the application of fertilisers, unlike the effects observed for EGCG and ECG. Thus, EGC of tea leaves may be mainly controlled by the weather, with cold weather aiding the accumulation of EGC. It is also possible that the accumulation of EGC could result from the less active consumption of EGC for the synthesis of EGCG in the tea plant.

5.3.4.4 Total catechins

The seasonal pattern of total catechins in hand plucked fresh tea leaves is similar to that in mechanically harvested green leaves (Figure 5.27), with general increases or decreases with seasonal changes of weather. This is similar to that found for EGC, since the total catechins comprise predominately EGC. In addtion, the actual levels of total catechins in hand plucked fresh leaves is quantitatively close to the levels in mechanically harvested green leaves from the harvest dates of 23 August 2000 to 2 May 2001.

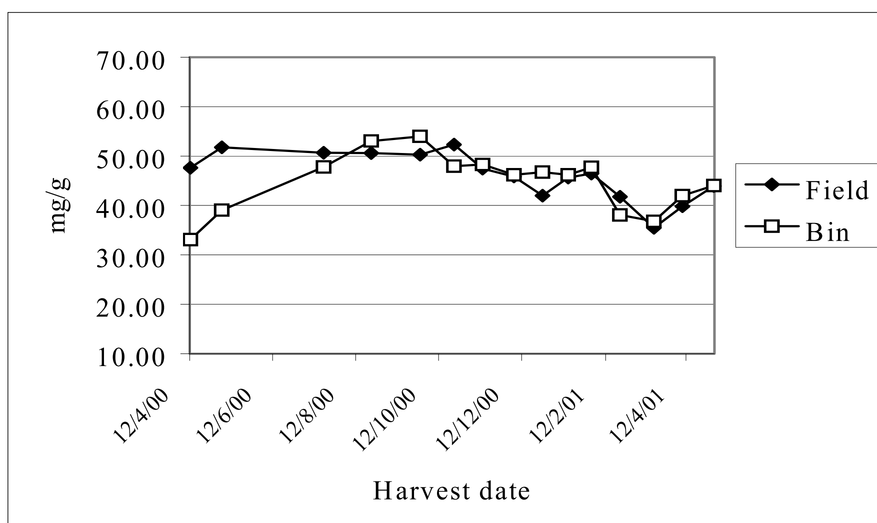


Figure 5.26 Comparison of seasonal variations of EGC in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

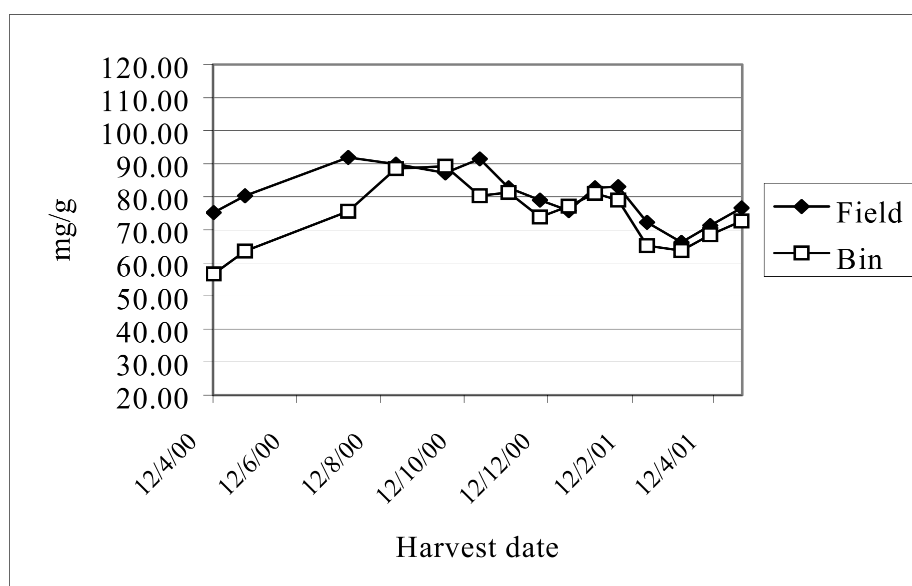


Figure 5.27 Comparison of seasonal variations of total catechins in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

However, the content of total catechins in hand plucked fresh tea leaves is much greater than that in the mechanically harvested green tea leaves from 12 April to 19 July 2000 (Figure 5.27). This result reveals that the mechanical harvests carried out during those months may have cut the tea bushes more heavily than in the harvests after August 2000, resulting in more mature tea shoots being collected in the harvests. The older shoots or the bin green leaves would be expected to contain smaller amounts of EGCG and total catechins including EGC. In addition, the gradual increase of total catechins from April to July 2000 (Figure 5.27) was generally accompanied with a gradual decrease of EGCG for the

same tea shoots during the same period (Figure 5.24), regardless of the harvest method. This result support the hypothesis that the biosynthesis of ECGC consumes the most abundant catechin, EGC.

In conclusion, the total catechins and EGC had differents seasonal patterns from that of EGCG. However, their levels during the same harvest periods were controlled by the particular harvest method used.

5.3.4.5 Total catechin gallates

The differences in the seasonal patterns of the total catechin gallates between hand plucked fresh tea leaves and mechnically harvested green tea leaves (Figure 5.28) are similar to those for EGCG and ECG (Figures 5.24 and 5.25). This is due to the total catechin gallates being comprised mainly of EGCG (about 70 %) and ECG (about 20 %) in both hand plucked and mechanically harvested tea leaves. In addition, the effect of fertiliser application on the total catechin gallates is also similar to that on EGCG, as discussed in Section 5.3.4.1.

The obvious variations between the contents of total catechin gallates in the tea leaves from 12 April to 19 July 2000 may be due to the two different harvest methods, as discussed earlier in Section 5.3.4.4, where heavy mechanical harvests may have resulted in more mature leaves with less catechin gallates.

The levels of catechin gallates in hand plucked samples are generally higher than in mechanically harvested samples. This is in contrast to the seasonal patterns of EGC and total catechins which do not differ much between the mechanically harvested and hand plucked tea leaves. Thus, catechin gallates are more variable than catechins in their seasonal distributions in young tea shoots.

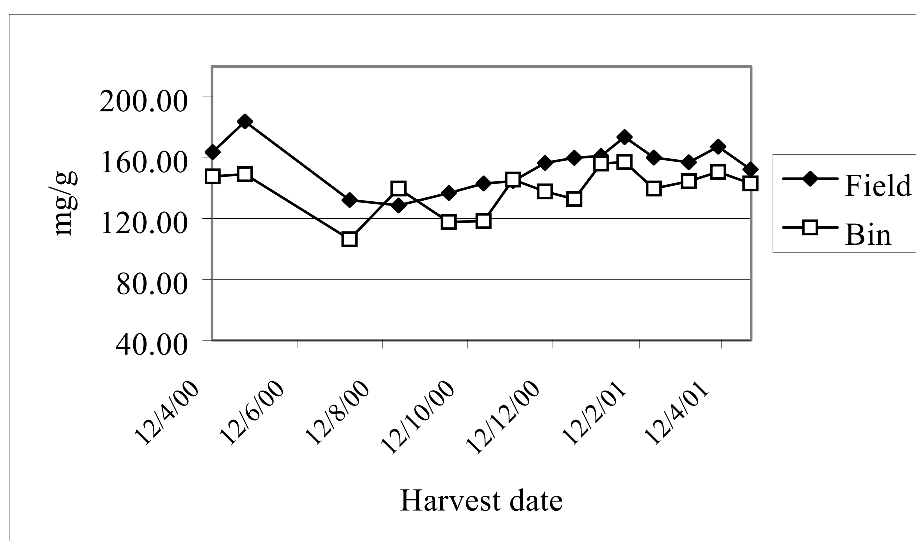


Figure 5.28 Comparison of seasonal variations of total catechin gallates in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

5.3.4.6 Combined catechins/catechin gallates

The patterns of seasonal distribution of combined catechins/catechin gallates between hand plucked and mechanically harvested tea leaves (Figure 5.29) are very similar to those of EGCG and total catechin gallates (Figures 5.24 and 5.28). However, less variation in actual levels occurs over the harvesting period from April 2000 to May 2001 for the combined catechins/catechin gallates (Figure 5.29). This result may suggest that the variations of combined catechins/catechin gallates between the two harvest methods are dominated by EGCG and total catechin gallates.

The content of combined catechins/catechin gallates in mechanically harvested tea leaves is lower than in the hand plucked fresh tea leaves for the harvests from 12 April to 19 July 2000. The possible reason for this is discussed in Section 5.3.4.4, the heavy mechanical harvests resulted in more mature leaves with less EGCG and ECG (Forrest and Bendall, 1969), and thus less combined catechins/catechin gallates.

In general, hand plucked fresh leaves comprising one apical bud with two adjoining leaves contain higher levels of combined catechins/catechin gallates than the mechanically harvested green leaves under the harvest system used by Glen Allyn Tea Estates.

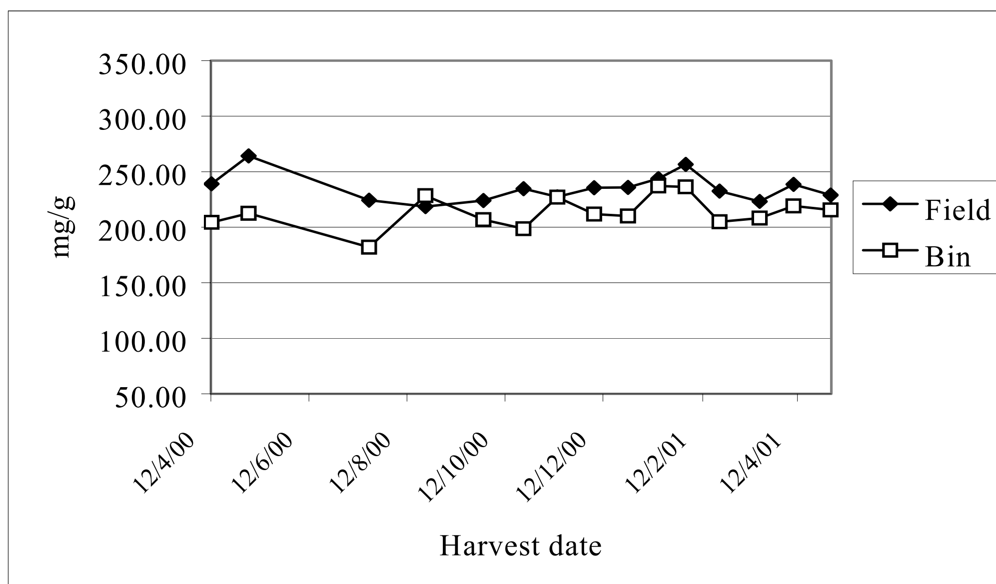


Figure 5.29 Comparison of seasonal variations of combined catechins and catechin gallates in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

5.3.4.7 Total phenolic compounds

The seasonal patterns of total phenolic compounds in tea leaves from the two different harvest methods (Figure 5.30) are similar to the patterns found for EGCG (Figure 5.24), total catechin gallates (Figure 5.28) and combined catechins/catechin gallates (Figure 5.29). This result suggests that catechins and catechin gallates play a dominant role in the seasonal distributions of total phenolic compounds in tea leaves of both harvest methods. This is an expected result since catechins and catechin gallates represent 90 % of the total phenolic compounds in both mechanically harvested and hand plucked tea leaves.

Total phenolic compounds in the mechanically harvested tea flushes range 206.98–273.11 mg/g (Table 5.36), while those compounds range 258.23–304.73 mg/g in hand plucked tea flushes (Table 5.29). Thus, hand plucked tea leaves contain more phenolic compounds than mechanically harvested tea leaves, mainly due to the variations of catechin gallates with the age of tea leaves harvested by each method.

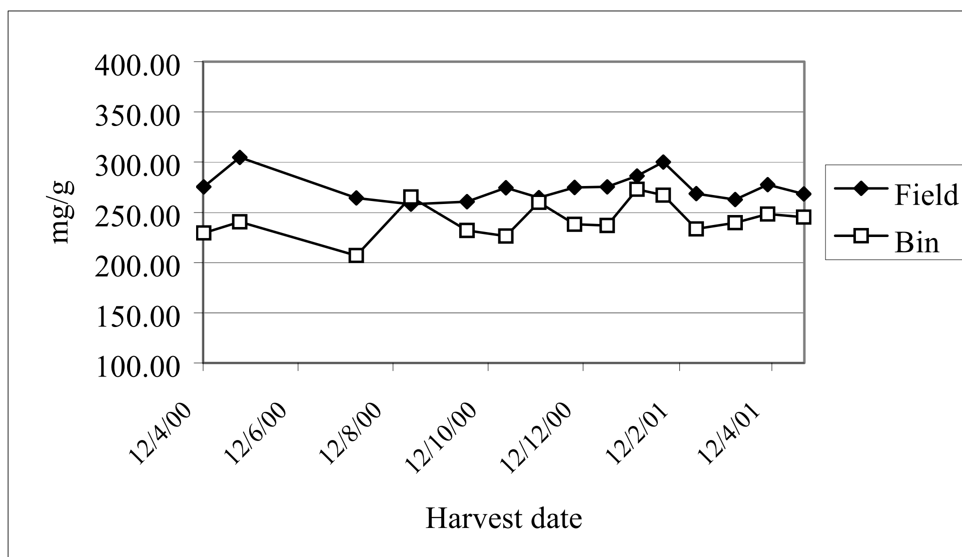


Figure 5.30 Comparison of seasonal variations of total phenolic compounds in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

5.3.4.8 Minor phenolic compounds

Minor phenolic compounds in this study have been grouped into three groups:

- Other phenolic compounds (OPS) include all the phenolic compounds measured in the tea leaves excluding catechins and catechin gallates, i.e. kaempferol glycoside, kaempferol 3-rhamnosylglucoside, quercetin glycoside, quercetin 3-glucoside and quercetin 3-rhamnosylglucoside, gallic acid, theogallin, iso-chlorogenic acid, *p*-coumaryl quinic acid, chlorogenic acid, 3-(*p*-hydroxyphenyl)-propionic acid and *p*-coumaric acid.
- Other catechins (OCS) include all the catechins measured in the tea leaves excluding EGC, i.e. C, EC, GC.
- Other catechin gallates (OGS) include all the catechin gallates measured in the tea leaves excluding EGCG and ECG, i.e. CG, GCG, ECDG, EGCDG.

Hand plucked fresh tea leaves

The other phenolic compounds (OPS) in hand plucked fresh tea leaves ranged 36.2-43.6 mg/g during the sampling seasons (Table 5.38). The minor catechin gallates (OGS) ranged 5.8-11.5 mg/g in the hand plucked fresh leaves over the seasons. The other catechins (OCS) ranged 27.6-41.3 mg/g (dry weight basis) in the hand plucked tea leaves (Table 5.38), which represents about 45 % of the total catechins (Table 5.26). Siginificant differences ($P < 0.05$) can be found from these grouped minor phenolic compounds. Generally, OPS and OGS in hand plucked leaves showed significantly higher levels ($P < 0.05$) in warmer months (January–February) than in cooler months (July-October) (Table 5.38), which mirrors the seasonal variations for the main total phenolic compounds (Section 5.3.3.7) and main total catechin gallates (Section 5.3.3.5). In contrast, OCS in hand plucked leaves generally showed significanly higher levels ($P < 0.05$) in the cooler months than in the warmer months (Table 5.38), which also mirror the seasonal variations for the main total catechins (Section 5.3.2.4). These results show that the seasonal variations of the grouped minor phenolic compounds are similar to the main grouped phenolic compounds discussed previously.

Table 5.38 Content of minor phenolic compounds in hand plucked fresh tea leaves.

Harvest date	Content of grouped minor compounds (mg/g, dry basis)*		
	OPS[1]	OCS[2]	OGS[3]
12 April 2000	36.3 a	27.6 a	8.7 cd
9 May 2000	40.6 cde	28.5 a	10.9 gh
19 July 2000	40.0 bcd	41.3 h	5.8 a
23 August 2000	39.7 abcd	39.3 gh	5.8 a
28 September 2000	36.7 ab	36.9 fg	7.3 b
23 October 2000	40.0 bcd	39.1 gh	8.1 bc
13 November 2000	37.3 abc	35.2 def	8.2 bc
6 December 2000	39.3 abcd	33.2 bcd	9.6 def
27 December 2000	39.7 abcd	33.8 cde	9.1 cde
15 January 2001	42.4 de	37.1 fg	10.0 efg
1 February 2001	43.6 e	36.6 efg	11.5 h
22 February 2001	36.2 a	30.6 ab	10.7 fgh
19 March 2001	39.6 abcd	30.7 ab	10.8 fgh
9 April 2001	38.9 abcd	31.5 bc	10.9 gh
2 May 2001	39.3 abcd	32.7 bcd	10.4 fgh
LSD	3.56	2.99	1.27

*Means (n=6) in the same column followed by a common letter are not significantly different (P > 0.05).
[1] OPS, the other phenolic compounds include all the phenolic compounds measured in the tea leaves excluding catechins and catechin gallates.
[2] OCS, the other catechins include all the catechins measured in the tea leaves excluding EGC.
[3] OGS, other catechin gallates include all the catechin gallates measured in the tea leaves excluding EGCG and ECG.

Mechanically harvested green tea leaves

Minor phenolic compounds (OPS) in mechanically harvested green tea leaves ranged 25.1-37.0 mg/g (Table 5.39). The minor catechin gallates (OGS) ranged 4.2-8.7 mg/g in the mechanically harvested leaves. The levels of minor catechins (OCS) ranged 23.5-35.5 mg/g in the mechnically harvested green tea leaves (Table 5.39). The levels of each of these groups of phenolic compounds are higher in hand plucked leaves (Table 5.38) relative to mechanically harvested leaves (Table 5.39). The level of OPS in the green leaves is significantly higher ($P < 0.05$) in the warmer months of November 2000 and January 2001 than those in the cooler months of July and September (Table 5.39), with an exceptional occurrence of higher level of total minor phenolic compounds in August. This sesonal pattern of the minor phenolic compounds mirrors the seasonal variations of main total phenolic compounds, which also shows a higher level in the August 2000 harvest (Section 5.3.3.7). In general, the seasonal variations of the grouped minor phenolic compounds, OPS, OGS and OCS in the mechanically harvested leaves fluctuated similarly to the main phenolic compounds (Sections 5.3.3.7, 5.3.3.5 and 5.3.3.4, respectively). These results suggest that these minor compounds do not affect the seasonal patterns of the main individual or grouped phenolic compounds in the tea leaves.

Table 5.39 Content of minor phenolic compounds in mechanically harvested green leaves.

Harvest date	Content of grouped minor compounds (mg/g, dry basis)*		
	OPS[1]	**OCS**[2]	**OGS**[3]
12 April 2000	25.3 a	23.5 a	6.6 bcdef
9 May 2000	28.0 ab	24.5 a	7.9 efg
19 July 2000	25.1 a	27.9 abcd	4.2 a
23 August 2000	37.0 d	35.5 f	5.7 abcd
28 September 2000	25.1 a	35.3 e	4.9 ab
23 October 2000	27.7 ab	32.3 cde	5.5 abc
13 November 2000	33.1 bcd	33.1 de	7.2 cdefg
6 December 2000	26.3 ab	27.6 abc	6.0 abcde
27 December 2000	27.0 ab	30.4 bcde	5.9 abcde
15 January 2001	35.9 cd	34.8 e	8.7 g
1 February 2001	30.9 abcd	31.3 bcde	7.8 efg
22 February 2001	28.4 ab	27.1 abc	7.7 defg
19 March 2001	31.4 abcd	26.9 ab	8.4 fg
9 April 2001	28.9 abc	26.6 ab	8.3 fg
2 May 2001	29.7 abc	28.6 abcd	7.9 efg
LSD	7.01	5.23	2.03

* Means (n=3) in the same column followed by a common letter are not significantly different (P > 0.05).

[1] OPS, the other phenolic compounds include all the phenolic compounds measured in the tea leaves excluding catechins and catechin gallates.

[2] OCS, the other catechins include all the catechins measured in the tea leaves excluding EGC.

[3] OGS, other catechin gallates include all the catechin gallates measured in the tea leaves excluding EGCG and ECG.

5.3.4.9 *Summary of seasonal variations of phenolic compounds in tea leaves with different harvest methods*

The content of various phenolic compounds shows a relatively consistent pattern in the hand plucked fresh tea leaves but a more fluctuating pattern in the mechanically harvested green tea leaves. Fertiliser application has some effects on the seasonal pattern of the phenolic components in the tea leaves, with the main catechin gallates EGCG and ECG, and the total catechin gallates being most affected.

In this study, ECG and EGCG have been found to be the principal flavanols in green tea leaves and dominate the catechin gallates and combined catechins/catechin gallates in green leaves. Earlier studies showed that ECG and EGCG are responsible for the formation of a large quantity of theaflavins (TF3G, TF3′G and TFDG) (Coxon *et al*., 1970abc; Opie, 1992). These theaflavin compounds have long been regarded as the quality indicators for black tea (Bhatia, 1963; Bhatia and Ullah, 1968; Singh *et al*., 1999; Obanda *et al*., 1997ab). These results show that tea leaves harvested in the warmer months in Australia could produce the best quality black tea, due to the dominant levels of ECG and EGCG.

In general, hand plucked fresh tea leaves contain higher levels of phenolic compounds than mechanically harvested green tea leaves because in the latter there are usually more mature tea shoots present. However, there are some occasions in which the phenolic components show higher amounts in the mechanically harvested green leaves than in the hand plucked fresh leaves. In this case, the application of fertilisers may be part of the reason for a higher than expected level of phenolic compounds in the mechanically harvested green leaves. This is because the fast growth of tea shoots may contribute more tender young shoots for the mechanical harvest.

The levels of the main and grouped phenolic compounds in samples harvested by the two different harvests show larger differences during the period from April to July 2000 than that in the rest of the experimental period. These differences could be due to heavy cutting during the mechanical harvests from April to July 2000, which resulted in more mature shoots being present in the mechanically harvested tea samples, thus reducing the levels of phenolic compounds in these samples.

5.3.5 Ratio of catechin gallates to catechins

The ratio of EGCG/ECG has been used to compare the development of tea shoots after germination (Bhatia and Ullah, 1962, 1968) and clonal selection (Bhatia and Ullah, 1968). Nakagawa and Torri (1964ab) used EC and EGC as simple flavanol, and ECG and EGCG as galloyl flavanols to compare the seasonal variations of tea shoots and found that the proportion for simple flavanols is higher in spring, while the proportion is higher in summer for galloyl flavanols. In addition, the ratio of (EGCG+ECG) × 100/EGC has been used as a quality index for Chinese green teas for forty years (Yuan, 1962; Liang *et al*., 1990). On many occasions, a number of researchers (Roberts, 1962; Hilton, 1973; Hilton and Palmer-Jones, 1973; Forrest and Bendall, 1969; Millin, 1987; Harbowy and Balentine, 1997) have suggested that the flavanol components and their proportions in tea flushes could affect the quality of resultant black tea.

There are no quantitative data available to compare the ratio of individual and/or total catechin gallates to individual and/or total catechins in young tea shoots during the production period of tea bushes or between different types of harvests. Therefore, it was thought useful to compare the ratio of total catechin gallates (galloyl flavanols) to total catechins (simple flavanols) across seasons and with the two types of harvesting methods. Furthermore, the ratio of (EGCG + ECG) / EGC is also worth considering since the former two catechin gallates represent ca 90 % of total catechin gallates, while the latter represents more than 50 % of total catechins in the green leaves of Australian grown tea.

5.3.5.1 Ratio of total catechin gallates to total catechins

The ratio of total catechin gallates to total catechins in the hand plucked fresh leaves was compared to that of mechanically harvested green leaves over the seasons (Figure 5.31). In general, they show a very similar seasonal pattern, with the ratio in mechanically harvested green leaves being more variable. This result strongly suggests that the seasonal variation of ratio of catechin gallates to the catechins is weather-dependent, with harvests in warmer months showing higher ratios and harvests in cooler months showing lower ratios.

The ratios of those two groups of flavanols in the green leaves of both harvest types are very similar throughout the experimental period (Figure 5.31). In this study, all the tea plants on the farm of Glen Allyn Tea Estates are considered to be from the same variety as described in Chapter 3. Thus, sampling of mechanically harvested leaves and determination of the ratio of total catechin gallates to total catechins can be used to monitor seasonal changes in tea leaves grown on the Australian tea farm. Further, the results (Figure 5.31) suggest mechanically harvested tea leaves mimic hand plucked tea leaves over the whole year when this ratio is considered.

However, this ratio is quite complex to determine with a large number of various flavanols to be determined. Because EGCG and ECG together represent 90 % of the total catechin gallates and EGC represents 55 % of the total catechins, then a ratio of EGCG and ECG relative to EGC may be more useful quality index for detecting seasonal variations.

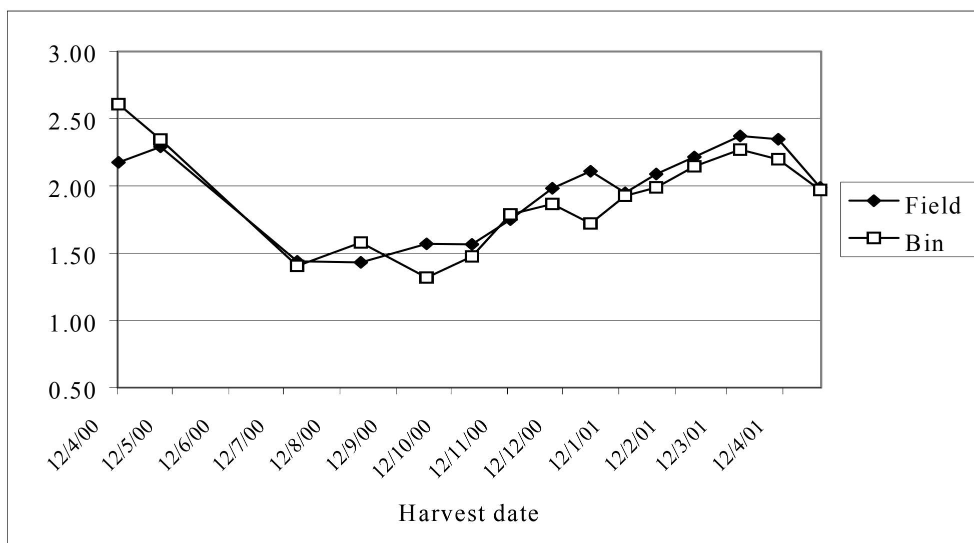


Figure 5.31 Seasonal variations of the ratio of total catechin gallates to total catechins in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

5.3.5.2 Ratio of (EGCG + ECG)/EGC

The ratio of (EGCG + ECG)/EGC in the hand plucked tea leaves also shows a similar seasonal pattern to that of mechanically harvested tea leaves (Figure 5.32). These similar seasonal distribution patterns of the ratios among the individual flavanols between the two harvest types are also close to the seasonal patterns of ratios of the two grouped flavanols (Figures 5.31 and 5.32). Therefore, it is possible, as expected, to use the ratio of (EGCG + ECG)/EGC rather than the ratio of the two grouped flavanols as a quality index of fresh green tea leaves, that is sensitive to seasonal variations.

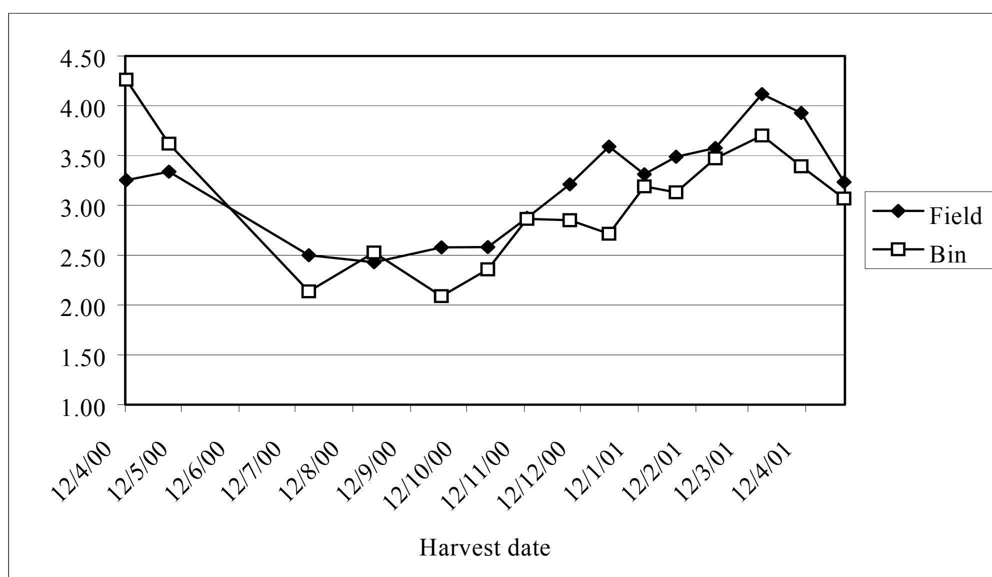


Figure 5.32 Seasonal variations of the ratio of EGCG and ECG to EGC in hand plucked (Field) and mechanically harvested (Bin) tea leaves.

As part of quality control monitoring of seasonal variations in green tea leaves using the ratio (EGCG + ECG)/EGC, the more easily obtained mechanically harvested green tea leaves can be substituted for the hand plucked tea leaves.

5.3.6 Summary and conclusions

EGCG and ECG are the main catechin gallates in tea flushes, with higher amounts produced in the warmer months of November 2000 to February 2001 (mean level: EGCG, 112.37 mg/g; ECG, 37.13 mg/g; total catechin gallates, 159.34 mg/g) and lower amounts in cooler months of July to September 2000 (mean level: EGCG, 92.94 mg/g; ECG, 33.41 mg/g; total catechin gallates, 132.61 mg/g) in Australia. In contrast, EGC, the main catechin in the tea flushes, is at higher levels in the cooler months of July to September 2000 (mean level: EGC, 50.50 mg/g; total catechins, 89.67 mg/g) and at lower levels in the warmer months of November 2000 to February 2001 (mean level: 44.86 mg/g; total catechins, 79.26 mg/g) in Australia. Since its contribution to the total phenolic compounds in tea flushes is relatively smaller than the gallates, its seasonal pattern does not affect the general patterns of phenolic compounds.

The literature is divided as to whether the catechin or catechin gallate levels in fresh tea leaves are the best indicators of black tea quality. Thus, there is potential for making good quality black tea during the months when there are higher amounts of all the phenolic compounds, including EGCG, ECG, EGC, other catechins and other catechin gallates. However, this study shows that different seasonal variation trends exist between catechins and catechin gallates. Thus, the importance of the catechin gallate levels versus the catechin levels in the freshly harvested green tea leaves needs to be resolved, if the Australian tea industry is to know which months will produce green tea leaves likely to produce higher quality black tea when processed. On a quantitative basis, the levels of EGCG and ECG together are four times the level of EGC in tea leaves. Thus, EGCG and ECG predominate over EGC for the selection of quality indicators for green leaves and for the resultant black tea.

The ratios of catechin gallates to catechins and (EGCG + ECG)/EGC are similar in both mechanically harvested and hand plucked green tea leaves. Thus, the more easily obtained mechanically harvested leaves can be analysed for the ratio, which can be used as a quality index for the processed black tea. In this study, higher ratios were found for samples harvested in the warmer months and lower ratios were found in the cooler months. Thus, these ratios may not be genetically controlled but weather dependent. The high ratios in the warmer months suggest that the biosynthesis of catechin gallates, such as EGCG and ECG, increases during the warmer months of January/February. This study also found that the biochemical synthesis of the catechin gallates (EGCG and ECG) in tea shoots, as judged by gross levels, is more active than that of catechins (EGC) over the seasons.

In conclusion, firstly, the ratio of (EGCG + ECG)/EGC can be used as an index of seasonal variations for tea grown in Malanda, North Queensland. Secondly, the results of this study suggest that during the warmer months in Australia, particularly January and February, green tea leaves will be produced with higher catechin gallate levels which, when processed, will produce a higher quality black tea, than green leaves processed at other times of the year, particularly in the cooler months of July to September. Section 5.4 details the effect of processing on these catechins and catechin gallates.

5.4 In-line analysis of flavonoids and other polyphenols during the processing of Australian black tea

5.4.1 Effect of harvest time on the phenolic compounds of in-line tea leaves during Australian black tea processing

5.4.1.1 EGCG, ECG and total catechin gallates

There was a significant effect ($P < 0.05$) of harvest time on the levels of EGCG, ECG and the total catechin gallates in the in-line tea samples throughout the black tea processing.

EGCG

EGCG levels of in-line tea samples were significantly lower ($P < 0.05$) in the first four steps of the processing of the July 2000 harvest than for these of the April 2000 and January 2001 harvests, indicating a possible effect of the cooler temperatures and/or shorter day lengths of July 2000 (Table 5.40). After these steps, the effect of harvest times on EGCG levels disappears.

EGCG content of in-line tea leaves in the first two processing steps, from bin (B) to shredder (S), showed a similar pattern within the warmer and cooler months. That is, no significant differences ($P > 0.05$) were observed in the samples harvested between the cooler months of July and October 2000 or between the warmer months of April 2000 and January 2001. The significant differences ($P < 0.05$) in EGCG levels that exist across the harvest times in these first two steps originate from the mechanically harvested green leaves because of seasonal variations, as discussed earlier in Sections 5.3.2.1 and 5.3.3.1. In the third and fourth processing steps, from rotorvane (R) to after the four CTC steps (C1), EGCG was present in significantly lower ($P < 0.05$) levels in the July 2000 harvest samples than in the samples of April 2000, October 2000 and January 2001 harvests (Table 6.1). EGCG showed irregular patterns across harvest times in the rest of the process, with no significant differences ($P > 0.05$) being observed between harvest times from fermentation 3 (F3) to the end of the process. Clearly, any differences in the EGCG levels due to harvest time are removed once oxidation of EGCG is underway in the fermentation steps (F2 onwards) and the level of EGCG begins to reduce.

Table 5.40 Comparison of mean EGCG contents of in-line tea leaves across harvest times.

Processing steps	EGCG content (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	104.25 bc	72.76 a	87.52 ab	113.86 c	20.69
S	93.88 bc	62.59 a	80.53 ab	106.88 c	20.69
R	86.21 b	56.84 a	82.77 b	95.68 b	20.69
C1	100.32 b	59.66 a	84.01 b	102.05 b	20.69
C2	62.19 ab	59.08 a	84.80 bc	97.83 c	20.69
F1	48.56 ab	34.23 a	56.36 b	66.96 b	20.69
F2	28.65 ab	9.84 a	28.40 ab	43.67 b	20.69
F3	12.52 a	4.44 a	13.99 a	23.33 a	20.69
F4	7.50 a	4.06 a	6.62 a	19.13 a	20.69
D1	4.57 a	3.79 a	4.39 a	14.10 a	20.69
D2	3.42 a	3.63 a	5.67 a	14.91 a	20.69
T	3.91 a	3.62 a	5.19 a	16.23 a	20.69

[1] Means (n=3) in rows followed by a common letter are not significantly different ($P > 0.05$)

In conclusion, the results of this study indicate that the in-line EGCG levels during the processing of black tea showed an irregular oxidation pattern on comparing the content over the four harvests (Table 5.40). This variation is due to the raw materials (fresh leaves) differences where significant differences ($P < 0.05$) in EGCG were found between the different harvests (Table 5.40). In Australia, EGCG levels in freshly harvested tea leaves may be used as a quality indicator for black tea, particularly since EGCG is known to oxidise to theaflavins, the important indicators for the black tea quality. Seasonal variation of the leaf EGCG content has been suggested to be an indicator of tea quality with a higher EGCG content in the green leaves resulting in higher residue in the final black tea (Eden, 1976). However, this was not the case in this study, where the more oxidation of EGCG that occurs as the processing of tea proceeds, the less is the observed effect of harvest times, until finally, the level of EGCG in the final black tea showed no significant differences ($P > 0.05$) due to harvest times. No published data exists on in-line EGCG oxidation and the effect of harvest times for comparison.

ECG

The July 2000 harvest (cooler month) had a significant lower ($P < 0.05$) ECG content than the January 2001 harvest (warmer month) for the first seven processing steps from bin leaves (B) to fermentation 2 (F2) (Table 5.41). The ECG level of the January 2001 harvest was irregularly significantly higher ($P < 0.05$) than that of the April and October 2000 harvests in the first seven processing steps (Table 5.41). These variations originate from the seasonal variations of freshly harvested tea leaves as discussed in Sections 5.3.2.2 and 5.3.3.2. Once the oxidation of ECG was completed after fermentation stage 3 (e.g. F3), there was no longer any significant differences ($P < 0.05$) observed between harvest times, with the ECG content, in common with the EGCG content, of the final black tea showing no significant ($P > 0.05$) harvest time effects (Table 5.41).

Table 5.41 Comparison of mean ECG contents of in-line tea leaves across harvest times.

Processing steps	**ECG content (mg/g, dry basis)[1]**				**LSD**
	April 00	**July 00**	**October 00**	**January 01**	
B	33.08 ab	23.88 a	28.83 a	40.38 b	10.07
S	29.21 a	22.31 a	27.28 a	39.84 b	10.07
R	28.40 ab	20.77 a	28.16 ab	35.40 b	10.07
C1	32.02 ab	22.51 a	28.29 a	38.59 b	10.07
C2	24.58 a	22.93 a	30.48 ab	36.69 b	10.07
F1	21.48 ab	19.47 a	22.84 ab	30.60 b	10.07
F2	14.58 a	11.90 a	17.00 ab	25.89 b	10.07
F3	8.60 a	6.67 a	11.73 a	14.42 a	10.07
F4	5.65 a	5.34 a	7.85 a	8.25 a	10.07
D1	2.52 a	4.54 a	7.51 a	6.33 a	10.07
D2	3.32 a	4.05 a	7.96 a	7.36 a	10.07
T	3.04 a	3.82 a	7.40 a	6.84 a	10.07

[1] Means (n=3) in rows followed by a common letter are not significantly different ($P > 0.05$)

Comparing the seasonal in-line levels of ECG changes to those of EGCG, the main similarity was that both compounds showed no significant changes ($P > 0.05$) between harvest times from fermentation stage 3 (F3) onwards during the black tea processing (Tables 5.40 and 5.41). Again, there are no published data available to compare the in-line ECG oxidation across harvest times under commercial processing conditions.

Total catechin gallates

The content of total catechin gallates was significantly lower ($P < 0.05$), during the first four steps of tea processing, for the July 2000 harvest than for the April 2000 and January 2001 harvests (Table 5.42). This is probably due to the significantly lower ($P < 0.05$) levels of total catechin gallates in the tea leaves harvested during the cooler month of July 2000, as discussed earlier for the hand plucked and mechanically harvested tea leaves in Sections 5.3.2.5 and 5.3.3.5.

Table 5.42 Comparison of mean contents of the total catechin gallates of in-line tea leaves across harvest times.

Processing steps	Total catechin gallates (mg/g, dry basis)[1]				LSD
	April 00	**July 00**	**October 00**	**January 01**	
B	143.72 bc	100.82 a	121.76 ab	162.04 c	30.12
S	129.29 bc	88.66 a	112.76 ab	155.51 c	30.12
R	121.37 b	81.41 a	115.69 b	138.42 b	30.12
C1	139.99 bc	86.71 a	117.08 b	150.26 c	30.12
C2	95.76 ab	86.70 a	121.56 bc	144.01 c	30.12
F1	79.05 ab	59.06 a	87.20 ab	107.54 b	30.12
F2	55.52 ab	27.78 a	53.63 ab	80.30 b	30.12
F3	34.81 ab	17.59 a	34.80 ab	49.14 b	30.12
F4	26.62 a	16.10 a	23.38 a	38.70 a	30.12
D1	18.22 a	15.06 a	20.45 a	30.58 a	30.12
D2	17.32 a	13.69 a	23.34 a	32.68 a	30.12
T	17.40 a	13.65 a	20.81 a	33.44 a	30.12

[1] Means (n=3) in rows followed by a common letter are not significantly different ($P > 0.05$)

Total catechin gallates of in-line tea samples in the first two processing steps, from bin (B) to shredder (S), showed a similar pattern between the harvest times, with no significant differences ($P > 0.05$) in total catechin gallates levels being observed in the samples harvested between the cooler months of July and October 2000 or between the warmer months of April 2000 and January 2001. In the third and fourth processing steps, from rotorvane (R) to after the four CTC's (C1), the levels of total catechin gallates were significantly lower ($P < 0.05$) in the coldest month of July 2000 harvest than in the April 2000, October 2000 and January 2001 harvests (Table 5.42). Thereafter, in the tea processing, levels of total catechin gallates showed irregular patterns across the harvest times. Again, oxidation is well underway in the fermentation stages of the black tea processing, with the levels of total catechin gallates reducing rapidly until there was no significant effects ($P > 0.05$) due to harvest time in the in-line tea samples from F4 onwards until the final black tea product.

This seasonal pattern of total catechin gallates appears to be similar to that of EGCG in the in-line samples, because of the major contribution of EGCG levels to the levels of total catechin gallates. The main difference between EGCG and total catechin gallates is that there was no significant difference ($P > 0.05$) in the levels of EGCG between harvest times for the in-line samples from F3 onwards, while for the levels of total catechin gallates it was F4 onwards (Tables 5.40 and 5.42). There are no published data available to compare the in-line oxidation of total catechin gallates across harvest times under commercial processing conditions.

5.4.1.2 EGC and total catechins

There were significant ($P < 0.05$) harvest time effects on the levels of EGC and total catechins in the in-line tea samples throughout the black tea processing.

EGC

Significant differences (P < 0.05) in EGC content across harvests were found for the in-line samples from the bin leaves (B) to the fermentation stage 2 (F2) (Table 6.4). No significant differences (P > 0.05) in the EGC levels were found for the in-line samples from F3 to the final tea (T) across harvest times. The higher levels of EGC found during the cooler months of July to September 2000 for hand plucked and mechanically harvested tea leaves was not observed in this study for the in-line samples from the bin (B) to the Rotorvane (R). However, the EGC level was significantly higher (P < 0.05) for the October 2000 harvest than for the April 2000, July 2000 and January 2001 harvests, for the in-line samples from B to F1 (Table 5.43). Only hand plucked tea leaves at the October 2000 harvest showed higher EGC level (52.33 mg/g) than the July 2000 harvest (50.65 mg/g) of the hand plucked leaves (Table 5.11), which is in common with the difference between these two corresponding harvests of the in-line samples. Clearly, the seasonal differences in EGC between cooler and warmer months observed in Section 5.3 are not being duplicated in this study, particularly for the mechanically harvested leaves. The reason why is not clear.

Table 5.43 Comparison of mean EGC contents of in-line tea leaves across harvest times.

Processing steps	EGC content (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	34.72 a	47.76 b	56.93 c	46.70 b	7.66
S	30.88 a	42.78 b	55.73 c	42.49 b	7.66
R	31.68 a	42.44 b	56.07 c	37.54 ab	7.66
C1	28.80 a	38.37 b	56.76 c	39.63 b	7.66
C2	25.98 a	39.04 b	56.82 c	38.43 b	7.66
F1	23.86 a	20.62 a	42.13 b	25.09 a	7.66
F2	17.99 ab	12.27 a	20.14 b	18.09 ab	7.66
F3	14.85 a	9.70 a	16.95 a	12.01 a	7.66
F4	14.23 a	9.29 a	9.24 a	11.22 a	7.66
D1	11.74 a	10.90 a	7.75 a	9.33 a	7.66
D2	10.90 a	9.21 a	7.52 a	7.87 a	7.66
T	10.87 a	8.32 a	6.58 a	8.00 a	7.66

[1] Means (n=3) in rows followed by a common letter are not significantly different (P > 0.05)

EGC of the in-line samples showed a different seasonal pattern from those of EGCG, ECG and total catechin gallates and was also less variable than those components during the first several steps of black tea processing. There are no published data available to compare the in-line oxidation of EGC across harvest times directly under the commercial processing conditions. Again, EGC differences in the bin leaves due to harvest times are not carried through into the final black tea (Table 5.43).

Total catechins

Significant differences (P < 0.05) across harvest times were found in the contents of total catechins in the in-line samples during the processing of black tea, except for the steps of fermentation 4 (F4) and dry 1 (D1) (Table 5.44). Levels of total catechins were significantly lower (P < 0.05) for the April 2000 harvest than for the July 2000, October 2000 and January 2001 harvests, for the in-line samples from bin (B) to CTC 2 (C2), except for the in-line samples at the rotorvane (R) step where the levels of total catechins for the April 2000 and January 2001 harvests were not significantly different (P > 0.05) (Table 5.44). These differences in the bin leaves may originate from the seasonal variations of tea shoots similar to that discussed earlier for the hand plucked and mechanically harvested tea leaves in Sections 5.3.2.4 and 5.3.3.4.

No significant differences ($P > 0.05$) in the levels of total catechins across harvest times were found for the in-line samples from F4 to the final tea, except for the January 2001 harvest in-line samples from dry 2 (D2) to final tea (Table 5.44). The final black tea product from the January 2001 harvest had a significantly higher ($P < 0.05$) residue of total catechins than the final black tea produced from the April and July 2000 harvests. This difference was not observed for the EGC levels in the in-line samples (Table 5.43).

Table 5.44 Comparison of mean content of the total catechins of in-line tea leaves across harvest times.

Processing steps	Total catechins (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	58.29 a	78.45 b	89.81 b	81.41 b	13.51
S	52.33 a	72.72 b	89.03 c	73.58 b	13.51
R	53.06 a	70.22 b	89.74 c	65.97 ab	13.51
C1	54.56 a	72.63 b	91.32 c	76.37 b	13.51
C2	53.34 a	73.97 b	100.18 c	70.56 b	13.51
F1	53.75 a	54.93 a	76.98 b	65.04 ab	13.51
F2	46.00 ab	42.10 a	56.14 bc	65.38 c	13.51
F3	40.40 a	35.28 a	47.70 ab	55.22 b	13.51
F4	37.37 a	34.92 a	36.39 a	47.42 a	13.51
D1	31.62 a	30.95 a	35.99 a	43.78 a	13.51
D2	28.14 a	26.62 a	30.32 a	46.77 b	13.51
T	27.71 a	26.10 a	34.13 ab	43.96 b	13.51

[1] Means (n=3) in rows followed by a common letter are not significantly different ($P > 0.05$)

The rate of oxidation of EGC and total catechins across harvest times was different, particularly during the early stages of fermentation (in-line samples F1 and F2). Differences between harvests took longer to disappear for the total catechins (F4) than for EGC (F3). While there were some differences in total catechin levels between black tea samples produced by different harvests, it is not totally consistent with the differences in the total catechin levels in the fresh tea leaves from different harvests. It appears that the catechins other than EGC may be oxidising at a different rate to EGC. Thus, total catechins in the fresh tea leaves may not be suitable as a quality indicator for the black tea in the Australian black tea processing factory.

5.4.1.3 TF, TF3G, TF3'G, TFDG and total theaflavins

There were significant ($P < 0.05$) harvest time effects on the levels of theaflavin (TF), theaflavin 3-gallate (TF3G), theaflavin 3′-gallate (TF3′G), theaflavin 3,3′-digallate (TFDG), and total theaflavins in the in-line tea samples throughout the Australian black tea process examined in this study.

Theaflavin (TF)

No significant differences ($P > 0.05$) in the TF levels were found between harvests for the in-line samples from the bin leaves (B) to the rotorvane stage (R), from C2 to F1, for F4 and for the final tea (T) (Table 5.45). Thus, the TF content was less variable across harvest times than the contents of catechins and catechin gallates discussed previously. Since no significant harvest time effects ($P > 0.05$) were observed in the TF levels in the produced black tea, the TF level in the in-line tea samples can not be used as an indicator of seasonal variations during black tea processing.

Theaflavin 3-gallate (TF3G)

No significant differences (P > 0.05) in TF3G contents were found across harvest times for the in-line tea samples from the first four processing steps, from stage B to C1 (Table 5.46). In-line tea samples from stage C2 for the April 2000 harvest had significantly higher (P < 0.05) TF3G levels than those for the July and October 2000 harvests. Thereafter, no significant differences (P > 0.05) between harvest times were found for the in-line tea samples from the early fermentation stages of F1 to F2. Finally, stages F3 to final tea (T) of the in-line tea samples from the January 2001 harvest had significantly higher (P < 0.05) TF3G levels than those corresponding samples from the April and July 2000 harvests (Table 5.46). This correlates with significantly higher (P < 0.05) levels of EGCG for the in-line tea samples from the January 2001 harvest (Table 5.40), which, along with EC, are known to oxidise to produce TF3G (Bryce *et al*., 1970; Coxon *et al*., 1970a).

The results reveal that the formation of TF3G during the processing of black tea was more variable than that of TF. Significant differences (P < 0.05) in the TF3G content can be found in the later periods of fermentation (F3, F4) across harvest times, and these differences continue into the drying stages (D1, D2) and final tea (T). This result may indicate that the formation and the further oxidation of TF3G and TF occur at different rates, with TF3G more sensitive to the seasonal variations of catechin and catechin gallate levels in the green tea leaves. Thus, while TF3G levels increased (Tables 5.46 and 5.59) due to oxidation of catechins and catechin gallates, particularly during the fermentation stages, the differences in black tea due to harvest times are not consistent enough for any quality considerations relating to seasonal variations to be made for the TF3G levels.

Table 5.45 Comparison of mean TF contents of in-line tea leaves across harvest times.

Processing steps	TF content (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	0.91 a	1.20 a	1.02 a	0.77 a	2.55
S	1.56 a	2.32 a	3.10 a	2.14 a	2.55
R	1.73 a	2.85 a	1.89 a	1.80 a	2.55
C1	1.13 a	3.87 b	1.88 ab	2.86 ab	2.55
C2	4.75 a	5.52 a	3.49 a	3.09 a	2.55
F1	6.05 a	6.98 a	6.66 a	5.26 a	2.55
F2	7.64 a	10.46 b	9.49 ab	7.16 a	2.55
F3	8.35 a	9.25 ab	11.51 b	9.22 ab	2.55
F4	9.17 a	9.39 a	11.19 a	9.03 a	2.55
D1	9.22 ab	9.09 ab	10.79 b	7.86 a	2.55
D2	9.17 ab	8.07 a	10.69 b	8.04 a	2.55
T	8.87 a	8.13 a	10.41 a	8.02 a	2.55

[1] Means (n=3) in rows followed by a common letter are not significantly different (P > 0.05)

Table 5.46 Comparison of mean TF3G contents of in-line tea leaves across harvest times.

Processing steps	TF3G content (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	0.27 a	0.39 a	0.35 a	0.38 a	3.04
S	0.34 a	0.41 a	0.54 a	0.65 a	3.04
R	0.48 a	0.48 a	0.43 a	0.98 a	3.04
C1	1.24 a	0.56 a	0.43 a	1.76 a	3.04
C2	4.64 b	0.60 a	0.96 a	2.26 ab	3.04
F1	4.62 a	2.94 a	4.03 a	4.48 a	3.04
F2	7.64 a	8.20 a	6.54 a	7.92 a	3.04
F3	8.35 a	8.38 a	10.06 a	13.35 b	3.04
F4	9.17 a	8.66 a	11.16 ab	14.19 b	3.04
D1	9.22 a	8.58 a	10.83 ab	13.04 b	3.04
D2	8.08 ab	7.60 a	10.81 bc	12.76 c	3.04
T	7.99 a	7.79 a	10.39 ab	13.09 b	3.04

[1] Means (n=3) in rows followed by a common letter are not significantly different (P > 0.05)

Theaflavin 3'-gallate (TF3'G)

There were no significant differences (P > 0.05) between harvest times for the levels of TF3′G in the in-line samples from the first five processing stages, B to C2 (Table 5.47). The in-line samples harvested in October 2000 and January 2001 showed significantly higher (P < 0.05) TF3′G levels than those harvested in April and July 2000 from in-line stage F1 to the final tea, except for those from F1 and F4 for the October 2000 harvest, which were not significantly different (P> 0.05) to those from the April 2000 harvest (Table 5.47).

There is a possible correlation of the significantly higher (P < 0.05) TF3′G levels (Table 5.47) with the significantly higher (P < 0.05) EGCG and ECG levels (Tables 5.40 and 5.41) for the in-line samples, D2 and final tea (T) from the harvest in the warmer months of January 2001 relative to those from the harvest in the cooler month of July 2000. These results are similar to those noted earlier for TF3G, which support the findings of others that TF3′G is formed by the oxidation of ECG and EGC (Bryce *et al*., 1970; Coxon *et al*., 1970a).

Table 5.47 Comparison of mean TF3′G contents of in-line tea leaves across harvest times.

Processing steps	TF3′G content (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	0.32 a	0.32 a	0.38 a	0.15 a	1.85
S	0.84 a	0.523 a	0.89 a	0.88 a	1.85
R	1.13 a	0.76 a	0.46 a	1.09 a	1.85
C1	0.80 a	1.49 a	0.43 a	1.92 a	1.85
C2	2.84 a	1.34 a	1.34 a	2.33 a	1.85
F1	3.50 a	2.85 a	4.33 ab	5.46 b	1.85
F2	4.72 a	4.12 a	6.57 bc	7.60 c	1.85
F3	5.29 b	3.25 a	7.59 c	8.97 c	1.85
F4	5.02 ab	3.22 a	7.08 bc	8.40 c	1.85
D1	5.20 a	3.45 a	7.10 b	7.14 b	1.85
D2	4.82 a	3.01 a	7.15 b	7.05 b	1.85
T	4.61 a	3.10 a	6.81 b	7.00 b	1.85

[1] Means (n=3) in rows followed by a common letter are not significantly different (P > 0.05)

The main difference between TF3G and TF3′G data for the final teas is that TF3G is present in higher amounts than TF3′G. In addition, no significant differences ($P > 0.05$) in TF3G of the final tea occurred between the April, July and October 2000 harvests, while a significant higher ($P < 0.05$) TF3′G level occurred for the October 2000 harvest relative to the April and July 2000 harvests. In conclusion, these results may indicate that the formation of TF3′G follows a seasonal trend possibly related to the seasonal levels of the catechin gallates in fresh leaves. In conclusion, TF3′G shows potential for possible use as a quality indicator for black tea due to harvest time variations being evident in the final black tea produced from this Australian processing.

Theaflavin 3,3′-digallate (TFDG)

No significant differences ($P > 0.05$) in the levels of TFDG were found in the first five processing stages from B to C2 across harvest times (Table 5.48). The in-line samples from F1 to the final tea (T) for the April 2000 and January 2001 harvests had significantly higher ($P < 0.05$) TFDG levels than those from the July 2000 harvest (Table 5.48). Furthermore, TFDG levels were significantly higher ($P < 0.05$) for in-line samples F3 to the final tea (T) from the January 2001 harvest than from the other three harvests, again suggesting a correlation with the significantly higher ($P < 0.05$) levels of EGCG, ECG and total catechin gallates in green leaves from the warmer January 2001 harvest. This can be explained by the fact that January is one of the warmest months on the tea farm and higher levels of EGCG, ECG and total catechin gallates have been shown earlier to form in fresh tea leaves during January 2001. The opposite was observed for the cooler month of July 2000.

Table 5.48 Comparison of TFDG content of in-line tea leaves across harvest times.

Processing steps	TFDG content (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	0.35 a	0.31 a	0.28 a	0.18 a	3.37
S	0.40 a	0.78 a	1.28 a	1.00 a	3.37
R	0.44 a	1.10 a	0.84 a	1.33 a	3.37
C1	0.93 a	1.73 a	0.75 a	2.33 a	3.37
C2	3.33 a	1.98 a	1.67 a	2.76 a	3.37
F1	6.13 b	2.05 a	4.24 ab	6.70 b	3.37
F2	10.17 bc	6.14 a	6.90 ab	12.19 c	3.37
F3	12.14 b	6.18 a	9.98 b	18.55 c	3.37
F4	12.79 b	6.87 a	10.23 ab	18.73 c	3.37
D1	13.08 b	7.03 a	10.22 ab	17.54 c	3.37
D2	11.66 b	6.30 a	10.24 b	17.82 c	3.37
T	11.64 b	6.62 a	9.98 ab	18.03 c	3.37

[1] Means (n=3) in rows followed by a common letter are not significantly different ($P > 0.05$)

According to Cloughley (1979), Owuor *et al*. (1984) and Owuor and Reeves (1986), the formation of theaflavins occurs mainly during the fermentation period of black tea processing. This is supported by the results of this study where the main increase in TFDG levels occurs at the fermentation stage (Table 5.48).

The variability between harvests for the TFDG levels in the final black tea suggests TFDG levels in black tea may be useful as a quality indicator for seasonal variation of black tea produced by this Australian processing. In addition, EGCG, ECG and total catechin gallate levels in freshly harvested green tea leaves is also a quality indicator of black tea produced from these leaves in Australia.

Total theaflavins
No significant differences (P > 0.05) were found in the levels of total theaflavins across harvest times for the in-line samples from B to F2 (Table 5.49). The July 2000 harvest showed significantly lower (P < 0.05) levels of total theaflavins than the October 2000 and January 2001 harvests for the in-line from F3 to the final product.

The total theaflavins included four individual compounds, TF, TF3G, TF3′G and TFDG in this study, thus the seasonal pattern of total theaflavins during the processing of black tea represents a combination of the patterns for all four individual theaflavins. The results of the seasonal pattern of total theaflavins (Table 5.49) showed a similar distribution to those of TF3G (Table 5.46), TF3′G (Table 5.47) and TFDG (Table 5.48) rather than that of TF (Table 5.45), indicating that those gallated theaflavins dominate the distribution of total theaflavins across harvest times.

The levels of total theaflavins in the final black tea were low for the July 2000 harvest. This is because July is one of the cooler months for 2000, and low levels of catechin gallates are present in fresh tea leaves used to make the black tea at that time of the year.

Table 5.49 Comparison of mean contents of the total theaflavins of in-line tea leaves across harvest times.

Processing steps	Total theaflavins (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	1.85 a	2.22 a	2.04 a	1.48 a	10.47
S	3.13 a	4.03 a	5.81 a	4.67 a	10.47
R	3.78 a	5.20 a	3.62 a	5.19 a	10.47
C1	4.10 a	7.02 a	3.49 a	8.87 a	10.47
C2	15.56 a	10.07 a	7.45 a	10.44 a	10.47
F1	20.29 a	14.82 a	19.26 a	21.89 a	10.47
F2	30.18 a	28.92 a	29.50 a	34.87 a	10.47
F3	34.12 ab	27.05 a	39.14 b	50.09 c	10.47
F4	36.15 ab	28.15 a	39.66 b	50.34 c	10.47
D1	36.71 ab	28.16 a	38.95 b	45.57 b	10.47
D2	33.73 ab	24.99 a	38.89 b	45.67 b	10.47
T	33.11 ab	25.64 a	37.60 b	46.15 b	10.47

[1] Means (n=3) in rows followed by a common letter are not significantly different (P > 0.05)

5.4.1.4 Combined catechins/catechin gallates and total phenolic compounds

There were significant harvest time effects (P < 0.05) on the levels of combined catechins/catechin gallates and the total phenolic compounds in the in-line samples throughout the black tea processing.

Combined catechins/catechin gallates
The results of combined catechins/catechin gallates, which are comprised of four catechins and six catechin gallates, showed that there were significantly higher (P < 0.05) contents for the January 2001 harvest than for the July 2000 harvest in the in-line samples from B to F3 step (Table 5.50).

From the later stage of fermentation (F4) to the final black tea, the in-line samples showed no significant effect (P > 0.05) of harvest time for the combined catechins/catechin gallates (Table 5.50). This is similar to the distribution pattern of total catechin gallates (Table 5.42), indicating that catechin gallates dominate the distribution of combined catechins/catechin gallates of the in-line samples since they are the main contributors to this group of compounds.

Hilton *et al*. (1973) reported that the concentration of total flavanols (including EGC, GC, EGCG, EC, C, and ECG) measured using paper chromatography in fresh apical tea shoots was highest during the cold season in central Africa (Malawi). In the North Hemisphere, three flavanols (EGCG, ECG and EGC) are reported to represent 90 % of total flavanols (Wood *et al*., 1964a; Bhatia and Ullah, 1968). Measurement by paper chromatography of these three flavanols has found that the total flavanol concentration is the greatest during the height of the growing season (warmer months) and least at the end of the season (cooler months) (Wood *et al*., 1964a; Bhatia and Ullah, 1968). The result of this study supports the findings in the North Hemisphere, but not the finding by Hilton *et al*. (1973).

Total phenolic compounds

The in-line samples for July 2000 harvest showed significantly lower ($P < 0.05$) levels of total phenolic compounds than those for the January 2001 harvest during the all stages of the black tea processing (Table 5.51).

The results of this study showed a different seasonal pattern for the total phenolic compounds from that of combined catechins/catechin gallates, suggesting that the oxidation products of the catechins and catechin gallates, the theaflavins are influencing the seasonal variations. Thus, the seasonal distribution patterns of total phenolic compounds are complicated.

Table 5.50 Mean contents of the combined catechins and catechin gallates of the in-line tea leaves across harvest times.

Processing steps	Total catechins and catechin gallates (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	202.01 a	179.26 a	211.57 ab	243.45 b	41.37
S	181.62 a	161.39 a	201.79 ab	229.09 b	41.37
R	174.43 ab	151.63 a	205.43 b	204.39 b	41.37
C1	194.55 ab	159.34 a	208.41 b	226.63 b	41.37
C2	149.10 a	160.68 a	221.74 b	214.57 b	41.37
F1	132.81 ab	113.99 a	164.18 b	172.57 b	41.37
F2	101.52 a	69.88 a	109.77 ab	145.68 b	41.37
F3	75.21 ab	52.88 a	82.49 ab	104.35 b	41.37
F4	63.99 a	51.02 a	59.78 a	86.12 a	41.37
D1	49.84 a	46.01 a	56.44 a	74.37 a	41.37
D2	45.46 a	41.48 a	53.66 a	79.46 a	41.37
T	45.10 a	38.64 a	54.93 a	77.40 a	41.37

[1] Means ($n = 3$) in rows followed by a common letter are not significantly different ($P > 0.05$)

Table 5.51 Mean contents of total phenolic compounds of the in-line tea leaves across harvest times.

Processing steps	Total phenolic compounds (mg/g, dry basis)[1]				LSD
	April 00	July 00	October 00	January 01	
B	228.15 a	206.95 a	240.05 ab	282.75 b	48.60
S	208.32 a	188.10 a	232.74 ab	269.65 b	48.60
R	204.68 ab	180.81 a	234.09 b	242.88 b	48.60
C1	229.05 a	199.96 a	238.01 ab	278.13 b	48.60
C2	201.55 a	200.68 a	262.22 b	266.80 b	48.60
F1	188.98 ab	163.33 a	225.01 bc	240.67 c	48.60
F2	169.13 a	134.17 a	181.09 a	233.67 b	48.60
F3	145.44 ab	110.63 a	172.43 bc	207.96 c	48.60
F4	134.12 a	110.13 a	144.86 ab	185.43 b	48.60
D1	117.53 ab	106.60 a	133.87 ab	163.82 b	48.60
D2	102.94 a	93.53 a	133.37 ab	172.31 b	48.60
T	103.01 a	94.15 a	132.38 ab	170.19 b	48.60

[1] Means (n = 3) in rows followed by a common letter are not significantly different ($P > 0.05$)

The total phenolic compounds in the final tea showed significant differences ($P < 0.05$) across harvest times, with a trend of higher levels in the warmer months (January 2001) and lower levels in the cooler months (July 2000) (Table 5.51); this is the same pattern for the phenolic compounds in the fresh green leaves (B). Therefore, the fresh tea leaves harvested in warmer months (e.g. January 2001) have higher levels of phenolic compounds (Section 5.3), resulting from the higher accumulation of catechin gallates (Table 5.42) in the tea shoots, which then oxidise during the processing of black tea to yield higher levels of theaflavins in the final black tea (Table 5.49).

5.4.1.5 Ratio of the total catechin gallates to the total catechins (TCG/TC)

There were significant ($P < 0.05$) harvest time effects on the ratio of total catechin gallates (TCG) to total catechins (TC) in tea leaves throughout black tea processing in this study. The ratio of TCG/TC in the samples from the April 2000 and January 2001 harvests showed significantly higher ($P < 0.05$) levels than those from the July and October 2000 harvests for the in-line samples from step B to the F1 step (Table 5.52). As observed earlier for total catechin gallates and total catechins, no significant differences ($P > 0.05$) in the TCG/TC ratio were found between harvest times for in-line samples from the dry 1 step (D1) to the final tea (T) in all harvests. Thus, this ratio for the final black tea is not a useful index of seasonal variation, but, the ratio for freshly harvested tea leaves can be used as an index of seasonal variation of these flavonoids.

5.4.1.6 Summary

ECG, EGC, EGCG, total catechin gallates, and the combined catechins/catechin gallates showed significant differences ($P < 0.05$) across harvest times in the first few stages during black tea processing, but no significant differences ($P > 0.05$) from dry 1 (D1) stage to the final tea (T). TF3G, TF3′G, TFDG and total theaflavins showed the opposite distribution patterns to the above catechins and the gallates. Only the compound TF did not vary between harvest times during the black tea processing.

Table 5.52 Comparison of the mean TCG/TC ratios of in-line tea leaves across harvest times.

Processing steps	TCG/TC ratio[1]				LSD
	April 00	July 00	October 00	January 01	
B	2.48 c	1.28 a	1.35 a	1.99 b	0.34
S	2.47 b	1.22 a	1.27 a	2.13 b	0.34
R	2.28 b	1.16 a	1.29 a	2.09 b	0.34
C1	2.58 c	1.19 a	1.28 a	1.96 b	0.34
C2	1.78 b	1.17 a	1.21 a	2.03 b	0.34
F1	1.47 b	1.07 a	1.10 a	1.64 b	0.34
F2	1.13 b	0.65 a	0.89 ab	1.20 b	0.34
F3	0.84 b	0.50 a	0.70 ab	0.87 b	0.34
F4	0.71 ab	0.46 a	0.64 ab	0.81 b	0.34
D1	0.58 a	0.49 a	0.56 a	0.70 a	0.34
D2	0.62 a	0.52 a	0.75 a	0.71 a	0.34
T	0.63 a	0.52 a	0.60 a	0.76 a	0.34

[1] Means (n = 3) in rows followed by a common letter are not significantly different ($P > 0.05$)

There are no published data available to compare the in-line oxidation of catechins and the gallates, and the formation of theaflavins and theaflavin gallates across harvest times under commercial black tea processing conditions. The results of this study show that the seasonal distributions of catechin gallates are generally reflected, after oxidation during fermentation, in the theaflavin levels of black tea. Therefore, the seasonal variations of their gallates in fresh tea leaves could be used to predict their corresponding oxidation products, theaflavins and their gallates in black tea under similar processing systems.

5.4.2 Effect of processing stage (sampling point) on the phenolic compounds of in-line tea leaves during Australian black tea processing

5.4.2.1 EGCG, ECG and total catechin gallates

There were significant effects ($P < 0.05$) of the in-line sampling point (processing stage) on the levels of EGCG, ECG and total catechin gallates during the black tea processing.

EGCG

The results of the oxidation of EGCG during the processing of black tea showed that generally no significant changes ($P > 0.05$) in the levels of EGCG occurred from the B to C2 stages during the processing, regardless of the harvest times (Table 5.53). However, significant differences ($P < 0.05$) were found in the levels of EGCG between the B and R in-line samples in all harvests except for the October 2000 harvest. This reduction in EGCG levels is not consistent enough, according to the differences in EGCG levels found between bin samples (B) and those after the four CTC stages (C1) which were not significant ($P > 0.05$). This dip in EGCG levels at R step is probably due to the difficulties encountered with the samples from the rotorvane (R) stage where freezing of these samples was more difficult than for the other in-line samples due to the nature of R samples.

Table 5.53 Mean contents of EGCG during the processing of black tea.

Processing steps	EGCG content (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	104.25 e	72.76 d	87.52 d	113.86 e
S	93.88 de	62.59 cd	80.53 d	106.88 de
R	86.21 d	56.84 c	82.77 d	95.68 d
C1	100.32 de	59.66 cd	84.01 d	102.05 de
C2	62.19 c	59.08 cd	84.80 d	97.83 d
F1	48.56 c	34.23 b	56.36 c	66.96 c
F2	28.65 b	9.84 a	28.40 b	43.67 b
F3	12.52 a	4.44 a	13.99 a	23.33 a
F4	7.50 a	4.06 a	6.62 a	19.13 a
D1	4.57 a	3.79 a	4.39 a	14.10 a
D2	3.42 a	3.63 a	5.67 a	14.91 a
T	3.91 a	3.62 a	5.19 a	16.23 a
LSD	14.39	14.39	14.39	14.39

[1] Means (n=3) in columns followed by a common letter are not significantly different (P > 0.05)

The trend during the early stages of fermentation (F1-F3) in the black tea processing is a significant decrease (P < 0.05) in EGCG levels for all harvests (Table 5.53, Figure 5.33). In fact, rapid reduction in EGCG levels occurs during these fermentation stages as oxidation of EGCG to the theaflavins occurs (Tables 5.58 - 5.62). However, by the last two steps of the fermentation (F3 and F4), oxidation of EGCG had now ceased with no significant changes (P > 0.05) occurring in the EGCG levels for the in-line samples from F3 to the final black tea.

These findings are supported by the work of Roberts (1957a and 1962) and Bhatia (1964) who reported that EGCG was oxidised rapidly during fermentation. In black tea processing of this study, the fermentation or oxidation of polyphenolic compounds occurs immediately after the CTC stages where all the enzymes and substrates are brought together and a complex biochemical reaction proceeds.

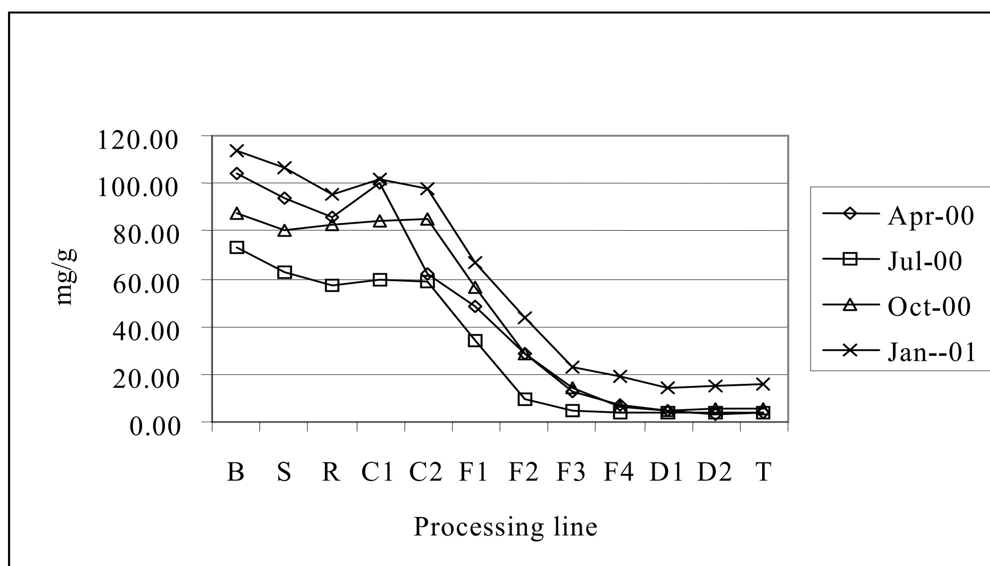


Figure 5.33 Mean contents of EGCG during the processing of black tea.

According to Roberts (1957a, 1958ab, 1962), the oxidation products in black tea are mainly derived from EGC and EGCG. The oxidation of EGC and EGCG leads to the formation of TF. Takino *et al.*

(1964, 1965), Coxon *et al*. (1970abc) and Collier *et al*. (1973) all reported that the oxidation of EGCG with EC produced TF3G, while oxidation of EGCG with ECG produced TFDG. Yao and Nursten (1997) reported that the chemical oxidation of EGCG alone yielded an HPLC polyphenolic profile similar to that of Malawi tea. Thus, EGCG has been recognised as the most important compound for black tea processing, based on its dominating proportion in the polyphenolic constituents of the green leaves and its various quality-oriented oxidation products in the final tea. In conclusion, EGCG is an appropriate compound to use to follow the oxidation process during black tea processing in the Australian factory, particularly if changes to the processing (e.g.to fermentation) are to be considered.

ECG

No significant changes ($P > 0.05$) in the ECG levels were found in the in-line samples from bin (B) to after the CTC's (C1) for all the harvests (Table 5.54, Figure 5.34). Significant ($P < 0.05$) decrease or oxidation of ECG occurred after the C1 stage as described below.

The start of oxidation of ECG (and thus reduction in levels) was variable across the harvests: for the April 2000 harvest, oxidation began at stage C2; for the July 2000 harvest, oxidation started during the second fermentation stage (F2); and for the October 2000 and January 2001 harvests, oxidation began during the first fermentation stage (F1). The reason for such variation between harvests for the start of oxidation is not clear. Thereafter, oxidation is complete by fermentation stage 3 (F3) for the July and October 2000 harvests, and by fermentation stage 4 (F4) for the April 2000 and January 2001 harvests. The oxidation of ECG and EGCG was very fast during the early fermentation periods (Figures 5.33, 5.34). The rapid reduction of ECG and EGCG levels during fermentation correlates with a rapid increase in theaflavin levels (Tables 5.58 - 5.62).

The main difference between ECG and EGCG is that no significant differences ($P > 0.05$) were found in the in-line EGCG levels after the F3 step but for ECG it was after the F4 step for all the harvests. This result indicates that ECG may be oxidised more slowly than EGCG during fermentation (rate of decrease of EGCG > rate of decrease of ECG in Figures 5.33 and 5.34). Roberts (1957a, 1962) has suggested that ECG does not undergo appreciable oxidation during the fermentation, while Bhatia (1964) showed that ECG does undergo oxidation during this period. The results of this Australian study support the findings of Bhatia (1964) rather than of Roberts (1957a, 1962). Further, Bhatia (1964) suggested that ECG is oxidised at a slower rate than EGC and EGCG, which is also in agreement with the results of this study.

Table 5.54 Mean contents of ECG during the processing of black tea.

Processing steps	**ECG content (mg/g, dry basis)[1]**			
	April 00	**July 00**	**October 00**	**January 01**
B	33.08 f	23.88 c	28.83 d	40.38 e
S	29.21 ef	22.31 c	27.28 cd	39.84 e
R	28.40 ef	20.77 c	28.16 d	35.40 de
C1	32.02 f	22.51 c	28.29 d	38.59 e
C2	24.58 de	22.93 c	30.48 d	36.69 e
F1	21.48 d	19.47 c	22.84 c	30.60 cd
F2	14.58 c	11.90 b	17.00 b	25.89 c
F3	8.60 b	6.67 a	11.73 a	14.42 b
F4	5.65 ab	5.34 a	7.85 a	8.25 a
D1	2.52 a	4.54 a	7.51 a	6.33 a
D2	3.32 a	4.05 a	7.96 a	7.36 a
T	3.04 a	3.82 a	7.40 a	6.84 a
LSD	5.18	5.18	5.18	5.18

[1] Means (n = 3) in columns followed by a common letter are not significantly different ($P > 0.05$)

According to Coxon *et al*. (1970 abc) and Collier *et al*. (1973) the biochemical oxidation of ECG with EGC formed TF3′G, with EGCG formed TFDG, and with gallic acid formed epitheaflavic acid gallate. Berkowitz *et al*. (1971) found that ECG could be oxidised with gallic acid during a very short reaction period to form epitheaflavic acid gallate, which then condenses to thearubigins. These theaflavins and related compounds are contributors to good quality tea (Ellis and Cloughley, 1981; Malec and Vigo, 1987). Later discussion in Section 5.4.2.3 explores the formation of these theaflavins during fermentation.

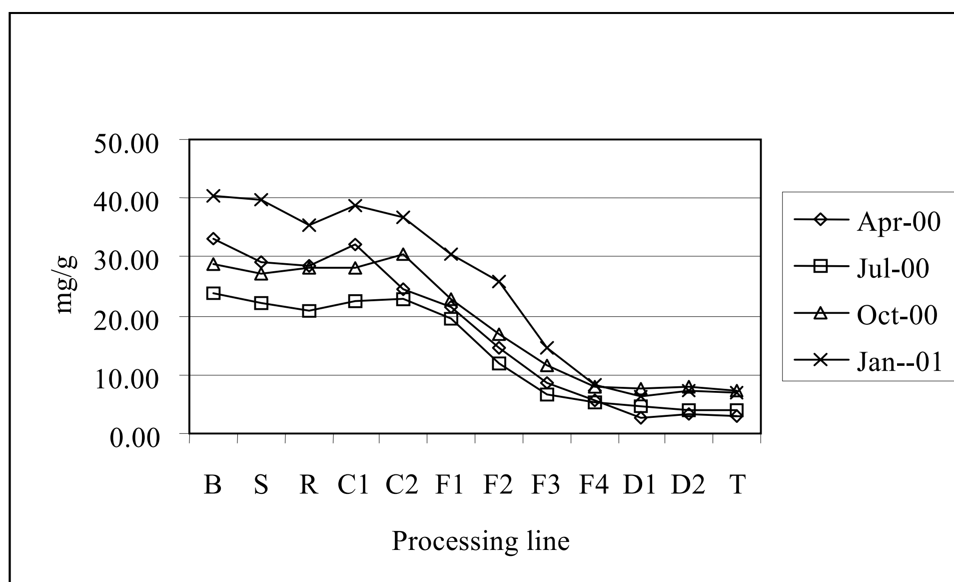


Figure 5.34 Mean content of ECG during the processing of black tea.

Total catechin gallates

Total catechin gallates showed a similar oxidation pattern as for EGCG during the processing of black tea (Tables 5.53 and 5.55; Figures 5.33 and 5.35) as discussed earlier.

Table 5.55 Mean contents of the total catechin gallates during the processing of black tea.

Processing steps	Total catechin gallates (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	143.72 e	100.82 d	121.76 d	162.04 f
S	129.29 de	88.66 cd	112.76 d	155.51 ef
R	121.37 d	81.41 c	115.69 d	138.42 e
C1	139.99 de	86.71 cd	117.08 d	150.26 ef
C2	95.76 c	86.70 cd	121.56 d	144.01 ef
F1	79.05 c	59.06 b	87.20 c	107.54 d
F2	55.52 b	27.78 a	53.63 b	80.30 c
F3	34.81 a	17.59 a	34.80 a	49.14 b
F4	26.62 a	16.10 a	23.38 a	38.70 ab
D1	18.22 a	15.06 a	20.45 a	30.58 a
D2	17.32 a	13.69 a	23.34 a	32.68 ab
T	17.40 a	13.65 a	20.81 a	33.44 ab
LSD	18.18	18.18	18.18	18.18

[1] Means (n = 3) in columns followed by a common letter are not significantly different ($P > 0.05$)

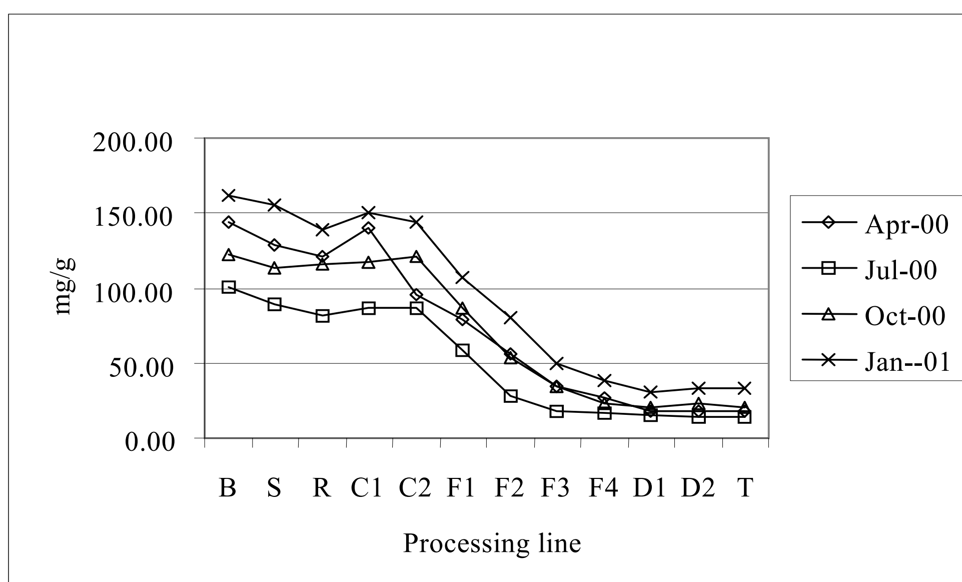


Figure 5.35 Mean contents of the total catechin gallates during the processing of black tea.

The result of this study suggests that all the main catechin gallates, particularly EGCG and ECG were similarly oxidised by the polyphenolic oxidases with most oxidation occurring during fermentation. The minor catechin gallates such as GCG and CG seem to play little role in affecting the oxidation pattern of total catechin gallates, regardless of their occurrence and oxidation rate. Thus, oxidation of EGCG during the tea processing in the factory could be used as an indicator of the oxidation of total catechin gallates.

5.4.2.2 EGC and total catechins

There were significant effects ($P < 0.05$) of the in-line sampling point (processing stage) on the levels of EGC and total catechins during the black tea processing.

EGC

Oxidation of EGC occurred at the fastest rate for the in-line samples from July and October 2000 harvests during the fermentation steps F1 to F4 with oxidation complete by F2 for July 2000 harvest and by F4 for October 2000 harvest (Table 5.56, Figure 5.36). Oxidation of EGC occurred at a slower rate in tea leaves from the January 2001 harvest samples during the fermentation stages F1 to F3 but was complete after F4. Finally, the tea leaves from the April 2000 harvest showed the slowest EGC oxidation rate during the fermentation stages of the black tea processing (Figure 5.36).

Table 5.56 Mean contents of EGC during the processing of black tea.

Processing steps	EGC content (mg/g, dry basis)[1]			
	April 00	**July 00**	**October 00**	**January 01**
B	34.72 f	47.76 d	56.93 d	46.70 e
S	30.88 ef	42.78 cd	55.73 d	42.49 de
R	31.68 ef	42.44 cd	56.07 d	37.54 d
C1	28.80 def	38.37 c	56.76 d	39.63 d
C2	25.98 de	39.04 c	56.82 d	38.43 d
F1	23.86 cd	20.62 b	42.13 c	25.09 c
F2	17.99 bc	12.27 a	20.14 b	18.09 b
F3	14.85 ab	9.70 a	16.95 b	12.01 ab

F4	14.23 ab	9.29 a	9.24 a	11.22 ab
D1	11.74 ab	10.90 a	7.75 a	9.33 a
D2	10.90 a	9.21 a	7.52 a	7.87 a
T	10.87 a	8.32 a	6.58 a	8.00 a
LSD	6.90	6.90	6.90	6.90

[1] Means (n = 3) in columns followed by a common letter are not significantly different (P > 0.05).

EGC has been found to show a similar oxidative pattern to those of EGCG, ECG and the total catechin gallates (Figures 5.33, 5.34, 5.35 and 5.36). The similarity among these components during the processing of black tea is that most oxidation occurred from stage C2 to stage F3. Roberts (1957a, 1962) and Roberts and Smith (1961) suggested that only EGCG and EGC underwent appreciable oxidation during the processing of black tea, while Bhatia (1964) reported that EGC and its gallate EGCG were consumed faster than ECG during fermentation. The result of this study showed that EGC was not consumed faster than EGCG and ECG.

Total catechins
Oxidation of total catechins does not follow the same pattern as for EGC. When the actual levels of EGC and the total catechins are compared (Tables 5.56 and 5.57), it appears that the change in the levels of the total catechins through the factory is mostly due to oxidation of EGC, and not to oxidation of other catechins, i.e. EC. It is interesting to note that for the Jan uary 2001 tea samples, only the EGC component of the total catechins is being oxidised. The other months show this to a slightly less extent. Therefore, the other catechins appear to be relatively unaffected by fermentation. Overall, about 30-50 % of the total catechins were not oxidised (Table 5.57).

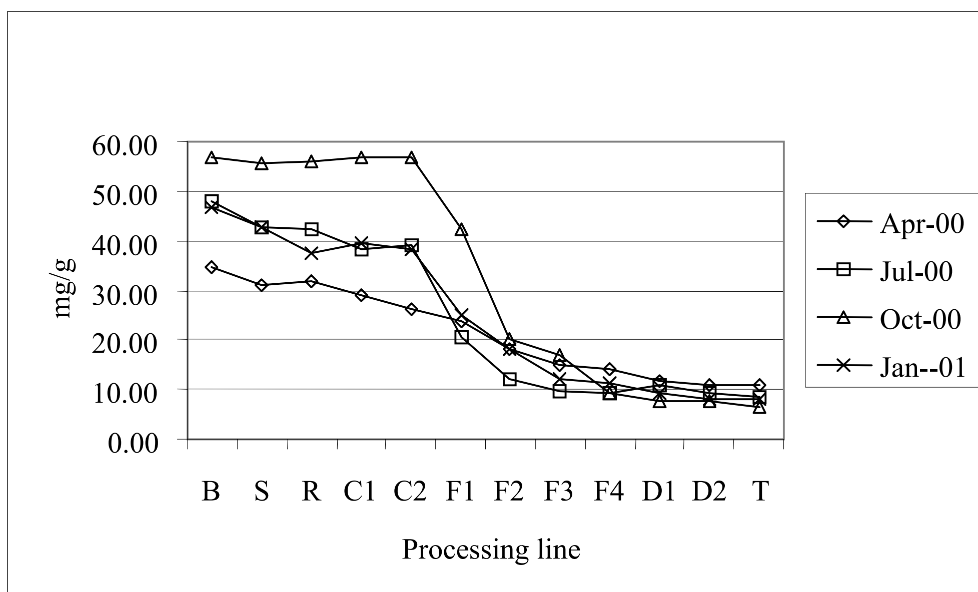


Figure 5.36 Mean contents of EGC during the processing of black tea.

As for EGC, most oxidation in the factory process occurred from stage C2 (after the CTC's but before the fermentation) through to the fourth fermentation stage (F4). There was little change in catechin levels after fermentation (Figure 5.37).

Table 5.57 Mean contents of the total catechins during the processing of black tea.

Processing steps	Total catechins (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	58.29 f	78.45 d	89.81 d	81.41 e
S	52.33 ef	72.72 d	89.03 d	73.58 cde
R	53.06 ef	70.22 d	89.74 d	65.97 c
C1	54.56 ef	72.63 d	91.32 de	76.37 de
C2	53.34 ef	73.97 d	100.18 e	70.56 cd
F1	53.75 ef	54.93 c	76.98 c	65.04 c
F2	46.00 de	42.10 b	56.14 b	65.38 c
F3	40.40 cd	35.28 ab	47.70 b	55.22 b
F4	37.37 bcd	34.92 ab	36.39 a	47.42 ab
D1	31.62 abc	30.95 a	35.99 a	43.78 a
D2	28.14 ab	26.62 a	30.32 a	46.77 ab
T	27.71 a	26.10 a	34.13 a	43.96 a
LSD	9.39	9.39	9.39	9.39

[1] Means (n = 3) in columns followed by a common letter are not significantly different (P > 0.05)

Apart from EGC, other catechins in tea include C, EC and GC. According to Takino *et al.* (1964, 1965) and Coxon *et al.* (1970abc), oxidation of paired catechins and/or catechin gallates produces corresponding theaflavins during the fermentation of black tea. As such, oxidation of EC and EGC forms theaflavin; oxidation of EC and GC forms isotheaflavin; oxidation of C and GC forms neotheaflavin; oxidation of EC and EGCG forms TF3G; oxidation of C and gallic acid forms theaflavic acid; and oxidation of EC and gallic acid forms epitheaflavic acid. These theaflavins and related compounds formed by the oxidation of the tea polyphenols contribute to the astringency and other quality properties of the black tea liquor (Opie 1992, Harbowy and Balentine 1997). Therefore, the oxidation process of catechins, as that of catechin gallates, is an important process for producing quality black tea.

5.4.2.3 TF, TF3G, TF3′G, TFDG and total theaflavins

There were significant effects (P < 0.05) of the in-line sampling point (processing stage) on the levels of TF, TF3G, TF3′G, TFDG and total theaflavins during black tea processing.

Theaflavin (TF)

No significant differences (P > 0.05) in TF content were found for the in-line samples taken at stages B to C1 of the black tea process from all harvests, except for the July 2000 harvest, which showed a significant increase (P < 0.05) in TF levels from in-line samples B to C1 (Table 5.58). Significant increases (P < 0.05) in TF levels after the C1 stage are described as follows (Table 5.58, Figure 5.38).

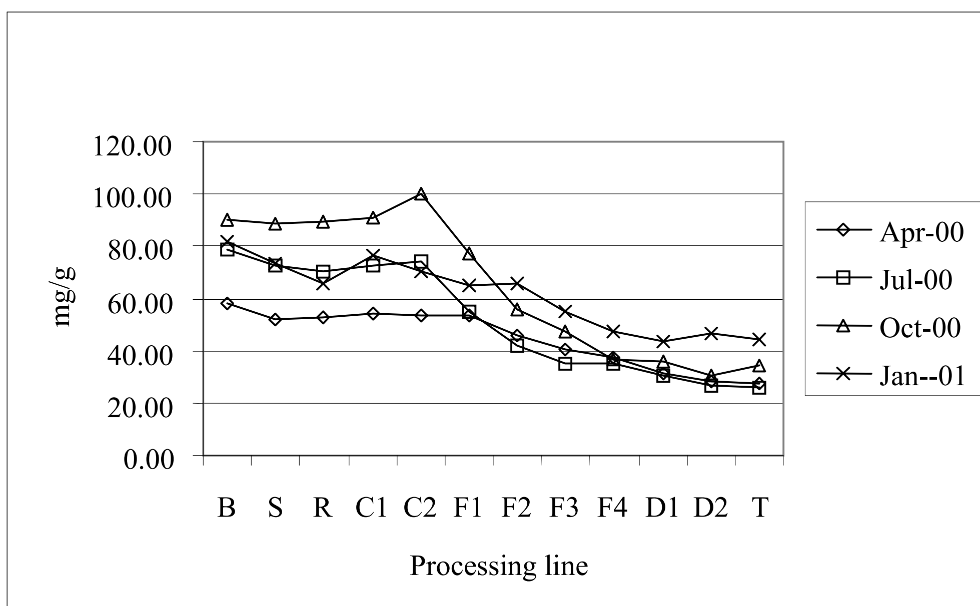


Figure 5.37 Mean contents of the total catechins during the processing of black tea.

The major formation of TF occurred in the in-line samples from stage C2 to the second fermentation step (F2), when oxidation of catechins and catechin gallates was most significant (Tables 5.53-5.57, Figure 5.33-5.37). Formation of TF is quite rapid during these early stages of fermentation suggesting the later fermentation stages F3-F4 were not really required to produce maximum levels of TF, i.e. the black tea is being over-fermented in this Australian tea processing factory, when the TF levels are considered.

The results of this study showed that TF levels peaked in the second stage of the fermentation (F2) and thereafter remain unchanged in the process (Table 5.58 and Figure 5.38). This correlated well with reducing EGC levels which for most harvests reached a minimum at the in-line stage F3 of the black tea processing. The compound, TF is the enzymatic oxidative product of EC and EGC (Roberts 1958ab; Brown *et al*., 1966; Coxon *et al*., 1970abc, Collier *et al*. 1973; Takeo and Oosawa, 1976). Further, it appears that TF formed during the Australian black tea process does not undergo further oxidation to form more polymeric compounds as the processing progresses, as has been reported previously (Roberts, 1962; Obanda and Owuor, 1993).

Table 5.58 Mean contents of TF during the processing of black tea.

Processing steps	TF content (mg/g, dry basis)[1]			
	April 00	**July 00**	**October 00**	**January 01**
B	0.91 a	1.20 a	1.02 a	0.77 a
S	1.56 a	2.32 ab	3.10 ab	2.14 ab
R	1.73 a	2.85 ab	1.89 ab	1.80 ab
C1	1.13 a	3.87 bc	1.88 ab	2.86 ab
C2	4.75 b	5.52 cd	3.49 b	3.09 bc
F1	6.05 bc	6.98 de	6.66 c	5.26 cd
F2	7.64 cd	10.46 g	9.49 d	7.16 de
F3	8.35 d	9.25 fg	11.51 d	9.22 e
F4	9.17 d	9.39 fg	11.19 d	9.03 e
D1	9.22 d	9.09 efg	10.79 d	7.86 e
D2	9.17 d	8.07 ef	10.69 d	8.04 e
T	8.87 d	8.13 ef	10.41 d	8.02 e
LSD	2.21	2.21	2.21	2.21

[1] Means (n = 3) in columns followed by a common letter are not significantly different (P > 0.05)

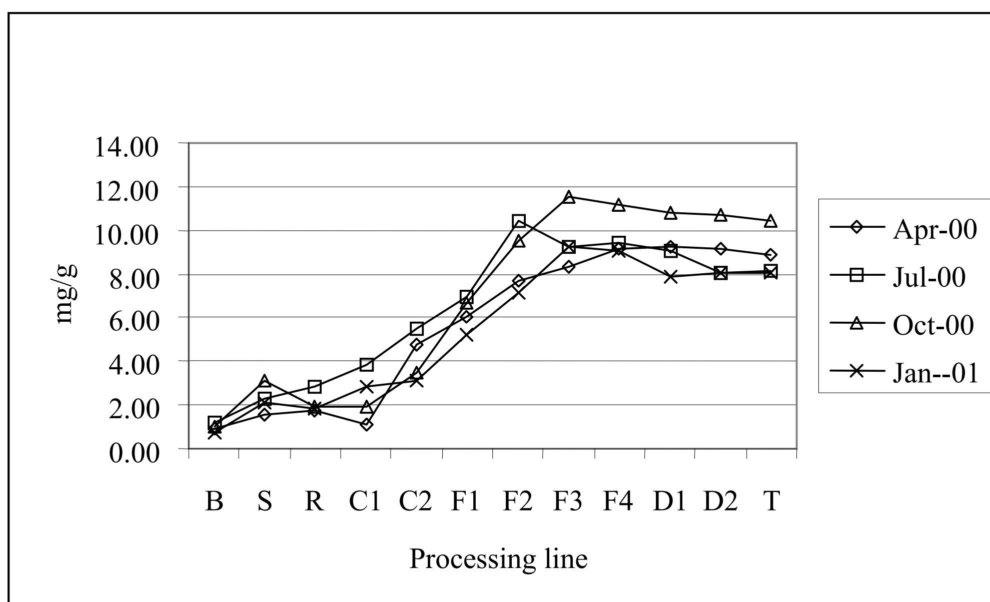


Figure 5.38 Mean contents of TF during the processing of black tea.

In most literature TF is referred to as theaflavins or whole theaflavin fractions, with determinations being based on their spectral characteristics, such as their absorbance at particular wavelengths (Biswas and Biswas, 1971; Biswas *et al*., 1971; Biswas, 1973; Cloughley, 1980a; Millin, 1987). In the broadest sense, theaflavins are generally correlated to the quality of black tea (Roberts and Smith, 1961, 1963; Deb and Ullah, 1968; Hilton and Ellis, 1972). Thus, it is recommended that the processing of black tea should maximise the content of theaflavins in the end products (Cloughley and Ellis, 1980; Roberts and Chandradasa, 1982). However, there are only a few reports on the levels of individual theaflavins in commercial black tea. One is a survey of the individual and total theaflavins of black tea from Kenya, India, Sri Lanka and other countries in the German market (Steinhaus and Engelhardt, 1989), where the levels of the compound TF range 1.35-5.27 mg/g, with an average of 2.48 mg/g. Another survey on commercial Kenyan black tea showed that TF ranged 6.26-9.70 mg/g, with an average 7.68 mg/g (Owuor and Obanda, 1995a). The results of this study show that the levels of TF in Australia black tea (8.02-10.41 mg/g; average 8.86 mg/g) are generally higher than the levels reported in other countries (Table 5.58); thus the quality of Australian black tea should be higher than the teas produced in other countries.

Theaflavin 3-gallate (TF3G)
For all the harvests, no significant differences ($P > 0.05$) in TF3G content were found between the in-line samples taken at the first four processing stages from B to C1 (Table 5.59). Rapid increases in TF3G levels occurred for all harvest samples from in-line stage C2 to the third fermentation stage (F3), at which all harvest samples had peaked with maximum TF3G levels. Thereafter, there was no significant change ($P > 0.05$) in the TF3G levels for all harvests (Table 5.59, Figure 5.39). As for TF, the TF3G levels suggest that this Australian black tea processing is over-fermenting the tea leaves, with only three fermentation steps required to maximise the TF3G levels.

Table 5.59 Mean contents of TF3G during the processing of black tea.

Processing steps	TF3G content (mg/g, dry basis)[1]			
	April 00	**July 00**	**October 00**	**January 01**
B	0.27 a	0.39 a	0.35 a	0.38 a
S	0.34 a	0.41 a	0.54 a	0.65 a
R	0.48 a	0.48 a	0.43 a	0.98 a
C1	1.24 a	0.56 a	0.43 a	1.76 ab
C2	4.64 b	0.60 a	0.96 a	2.26 ab
F1	4.62 b	2.94 a	4.03 b	4.48 b
F2	7.64 c	8.20 b	6.54 b	7.92 c
F3	8.35 c	8.38 b	10.06 c	13.35 d
F4	9.17 c	8.66 b	11.16 c	14.19 d
D1	9.22 c	8.58 b	10.83 c	13.04 d
D2	8.08 c	7.60 b	10.81 c	12.76 d
T	7.99 c	7.79 b	10.39 c	13.09 d
LSD	2.77	2.77	2.77	2.77

[1] Means (n = 3) in columns followed by a common letter are not significantly different (P > 0.05)

TF3G is the oxidative product of EGCG and EC (Roberts 1958ab, Coxon *et al*., 1970abc, Collier *et al*. 1973). The results of this study for in-line samples from stage C2 onwards showed that a significant decrease (P < 0.05) in EGCG levels corresponds with a significant increase (P < 0.05) in TF3G levels during the processing of all the harvest samples (Tables 5.53 and 5.59, Figures 5.33 and 5.39). Thus, the results support the possibility that oxidation of EGCG produces TF3G.

Sanderson *et al*. (1976), and Owuor and Obanda (1995) reported that TF3G has higher astringency than TF. Astringency of tea is due to the interactions of polyphenols with proteins, polysaccharides and caffeine at the molecular level (Haslam and Lilley, 1988) in the black tea quality complex. Thus, TF3G could be used as an important indicator of black tea quality, together with TF since both are formed during black tea fermentation.

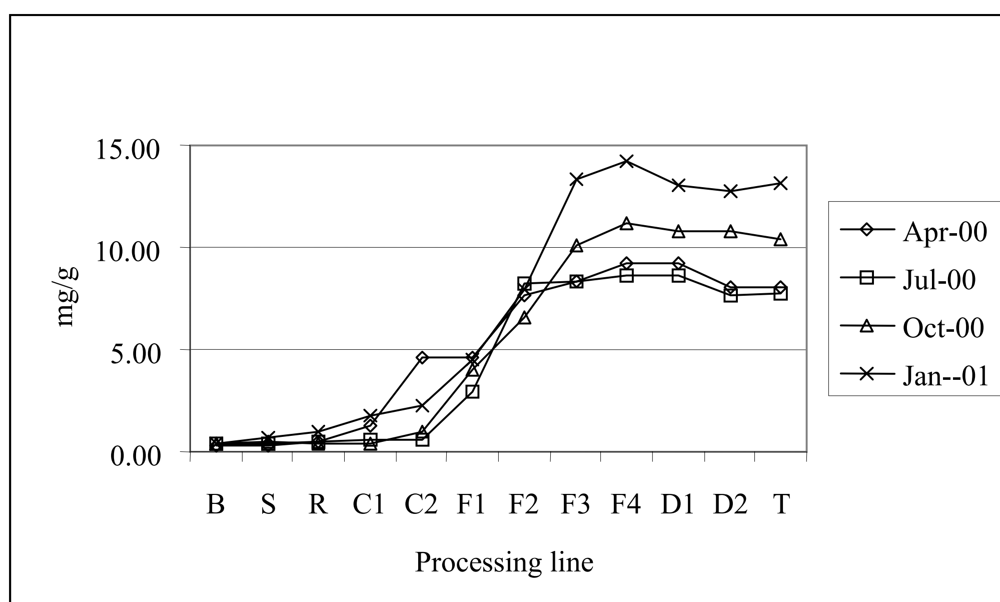


Figure 5.39 Mean contents of TF3G during the processing of black tea

The levels of TF3G range 1.10-4.13 mg/g with an average of 2.44 mg/g in commercial black teas produced in Kenya, India, Sri Lanka and other countries, and sourced in German market (Steinhaus and Engelhardt, 1989). In the commercial Kenyan black tea, the levels of TF3G range 5.48-6.57 mg/g, with an average 6.19 mg/g (Owuor and Obanda, 1995a). The results of this study show that the levels of TF3G in Australia black tea (7.79-13.09 mg/g; average 9.82 mg/g) are much higher than the levels reported in other countries (Table 5.59). Thus, the quality of Australian processed black tea should be higher than the teas produced in other countries because of the higher levels of TF3G and TF.

Theaflavin 3′-gallate (TF3′G)

The levels of TF3′G significantly increased ($P < 0.05$) from in-line stage C2 to the second fermentation stage (F2) (Table 5.60, Figure 5.40). This significant increase ($P < 0.05$) in the TF3′G levels corresponds with significant decrease ($P < 0.05$) in the levels of ECG (Table 5.54, Figure 5.34) and EGC (Table 5.56, Figure 5.36), two flavonoids that Coxon *et al*. (1970 abc) and Collier *et al*. (1973) suggested were oxidised to TF3′G. Again, as for TF and TF3G, maximum levels of TF3′G were reached early in the fermentation (F2) suggesting the tea leaves are being over-fermented in the Australian tea processing factory.

Furthermore, it was found that the levels of TF3′G were lower than the levels of TF3G (Tables 5.59 and 5.60). This could be due to the quantitative differences of their precursors, EGC, ECG and EGCG, particularly the correspondence of TF3′G to EGC, and TF3G to EGCG (Roberts 1958ab, Coxon *et al*., 1970abc, Collier *et al*. 1973).

Similar to TF3G, TF3′G also has a higher astringency than TF (Sanderson *et al*., 1976; Owuor and Obanda, 1995a). In most cases, TF3′G and TF3G are generally referred to as theaflavin monogallates by some researchers (Takeo and Oosawa, 1976; Opie *et al*., 1990; Chen *et al*. 1999). Coxon *et al*. (1970a) reported that TF3G and TF3′G make up 50 % of the total theaflavin fraction, while Takeo and Ossawa (1976) estimated them at 46 % of the total theaflavins. This study found that the combined TF3G and TF3′G levels ranged from 42 % to 46 % of the total theaflavins in black tea, indicating that these two theaflavins could play the major role in determining the quality of black tea.

Table 5.60 Mean contents of TF3′G during the processing of black tea.

Processing steps	TF3′G content (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	0.32 a	0.32 a	0.38 a	0.15 a
S	0.84 a	0.52 a	0.89 a	0.88 ab
R	1.13 a	0.77 a	0.46 a	1.09 ab
C1	0.80 a	1.49 abc	0.43 a	1.92 b
C2	2.84 b	1.34 ab	1.34 a	2.33 b
F1	3.50 bc	2.85 bcd	4.33 b	5.46 c
F2	4.72 cd	4.12 d	6.57 c	7.60 de
F3	5.29 d	3.25 d	7.59 c	8.97 e
F4	5.02 cd	3.22 d	7.08 c	8.40 de
D1	5.20 d	3.45 d	7.10 c	7.14 d
D2	4.82 cd	3.01 cd	7.15 c	7.05 d
T	4.61 cd	3.10 d	6.81 c	7.00 cd
LSD	1.58	1.58	1.58	1.58

[1] Means (n = 3) in columns followed by a common letter are not significantly different ($P > 0.05$)

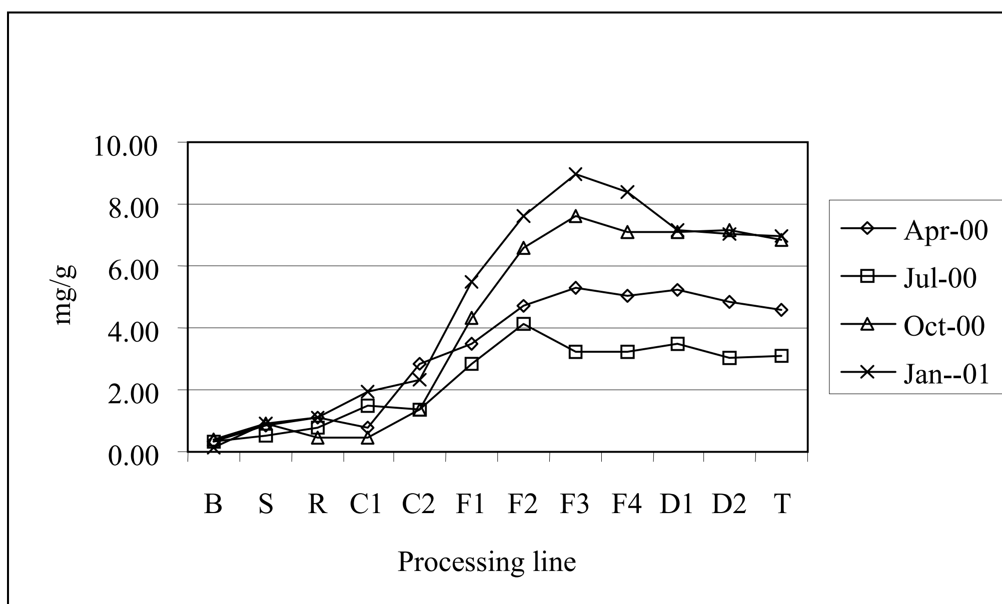


Figure 5.40 Mean contents of TF3′G during the processing of black tea.

In black teas (German market) produced in Kenya, India, Sri Lanka and other countries, the levels of TF3′G range 0.92-2.60 mg/g with an average of 1.64 mg/g (Steinhaus and Engelhardt, 1989). In commercial Kenyan black tea, the levels of TF3′G range 2.48-3.45 mg/g, with an average 2.89 mg/g (Owuor and Obanda, 1995a). These results show that the TF3′G levels in black tea are much lower than the TF and TF3G levels, which has also been found in this study of the TF3′G levels in Australian black tea. Again, black tea processed in this Australian factory has higher levels of TF3′G (3.10-7.00 mg/g; average 5.38 mg/g) than those found in other countries (Table 5.60). Thus, the quality of Australian processed black tea should be higher than the teas produced in other countries because of the higher levels of TF3′G, and the higher levels of TF3G and TF.

Theaflavin 3,3′-digallate (TFDG)
There were no significant changes ($P > 0.05$) in the TFDG levels in the in-line samples from the bin (B) to C2 stage of black tea processing for all the harvests (Table 5.61, Figure 5.41). For all harvests, the formation of TFDG occurred at a fast rate during the first three fermentation steps (F1-F3), and thereafter did not significantly change ($P > 0.05$) (Table 5.61, Figure 5.41). The level of TFDG peaked at either the F2 stage (April and July 2000 harvests) or at the F3 stage (October 2000 and January 2001) in the Australian black tea process. This was similar to the results for TF3G and TF3′G.

TFDG has been reported to be the oxidative product of ECG and EGCG (Takino *et al*., 1964; Bryce *et al*., 1970; Coxon *et al*., 1970b). The results of this Australian study showed that significant decreases ($P < 0.05$) in ECG and EGCG (Tables 5.53 and 5.54, Figures 5.33 and 5.34) occurred during the early fermentation stages (F1-F3) when significant increases ($P < 0.05$) in the TFDG levels occurred (Table 5.61, Figure 5.41).

Table 5.61 Mean contents of TFDG during the processing of black tea.

Processing steps	TFDG content (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	0.35 a	0.31 a	0.28 a	0.18 a
S	0.40 a	0.78 a	1.28 ab	1.00 a
R	0.44 a	1.10 a	0.84 a	1.33 a
C1	0.93 a	1.73 a	0.75 a	2.33 a
C2	3.33 ab	1.98 a	1.67 ab	2.76 a
F1	6.13 b	2.05 a	4.24 bc	6.70 b
F2	10.17 c	6.14 b	6.90 cd	12.19 c
F3	12.14 c	6.18 b	9.98 de	18.55 d
F4	12.79 c	6.87 b	10.23 e	18.73 d
D1	13.09 c	7.03 b	10.22 e	17.54 d
D2	11.66 c	6.30 b	10.24 e	17.82 d
T	11.64 c	6.62 b	9.98 de	18.03 d
LSD	3.12	3.12	3.12	3.12

[1] Means (n = 3) in columns followed by a common letter are not significantly different (P > 0.05)

The TFDG levels peaked at least by third stage of the fermentation (F3) in this study. Moreover, it has been estimated that TFDG is about 40 % of the theaflavin fractions in black tea (Coxon *et al*. 1970a), while Takeo and Ossawa (1976) measured TFDG at only 19 % of the theaflavins. This study of Australian black tea found that the proportion of TFDG in the total theaflavin fractions of the final black tea ranged 25-27 % during the cooler months (July and October 2000) and 35-39 % during the warmer months (April 2000 and January 2001). Since EGCG levels were generally higher in the warmer months (Section 5.3), the result of this study suggests that the level of EGCG in the green leaf could primarily affect the formation of TFDG, since TFDG is the oxidative product of ECG and EGCG (Takino *et al*., 1964; Bryce *et al*., 1970; Coxon *et al*., 1970b).

The levels of TFDG in black teas (German market) produced in Kenya, India, Sri Lanka and other countries range 0.94-4.96 mg/g, with an average of 2.55 mg/g (Steinhaus and Engelhardt, 1989). These levels of TFDG in Kenyan black tea range 3.77-6.18 mg/g, with an average of 4.61 mg/g (Owuor and Obanda, 1995a). These results show that the TFDG levels in black tea are more variable than the levels of TF, TF3G and TF3′G; this was also found in this study of the TFDG levels in the Australian black tea. Again, black tea processed in this Australian factory has higher levels of TFDG (6.62-18.03 mg/g; average 11.57 mg/g) than those found in other countries (Table 6.22). Thus, the Australian processed black tea should possess higher quality than the teas produced in other countries.

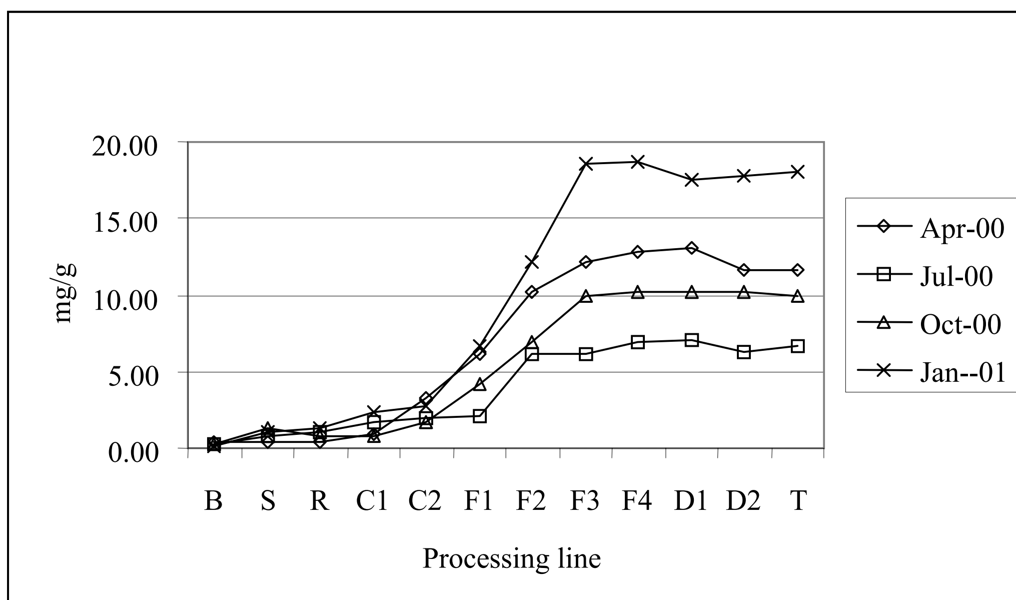


Figure 5.41 Mean contents of TFDG during the processing of black tea.

Sanderson *et al*. (1976) found that TFDG has an astringency about three times higher than that of the theaflavin monogallates (TF3G and TF3′G) and six times higher than that of TF. Owuor and Obanda (1995) also reported a similar finding and suggested that TFDG or TFDG equivalent of total theaflavins could be used as better quality indicator than total theaflavins. Therefore, the results of this study on the formation of TFDG during tea processing could be related to the quality of black tea.

Total theaflavins

No significant differences ($P > 0.05$) were found in the total theaflavins from stages B (green leaves) to C1 of the tea processing for all the harvests (Table 5.62 and Figure 5.42). There were significant increases ($P < 0.05$) in the total theaflavin levels during the in-line stages from C1 to the third fermentation stage (F3) depending on the harvest time.

- April 2000: significant increases ($P < 0.05$) from in-line stages C1 to C2/F1, and then from C2/F1 to F2
- July 2000: significant increase ($P < 0.05$) from in-line stages F1 to F2.
- October 2000 and January 2001: significant increases ($P < 0.05$) from in-line stages C2 to F1, F1 to F2, and finally F2 to F3.

The components of theaflavins are synthesised by the oxidation of catechins and catechin gallates during the fermentation stage of the Australian black tea processing. However, theaflavins are not the end products of tea fermentation. These compounds tend to undergo further oxidation to form a complex mixture called thearubigins (Roberts and Smith, 1961; Roberts, 1962; Deb and Ullah, 1968).

Earlier studies suggested that the oxidation of theaflavins is a polyphenolic oxidase driven oxidation (Robert, 1962), while recent studies have indicated that the four main theaflavins (examined in this Australian study) are not substrates for tea polyphenol oxidase (Opie *et al*., 1993). Instead, the theaflavins may be degraded by a coupled oxidation with EC-quinone, which is synthesised through the oxidation of EC catalysed by polyphenolic oxidase (Opie *et al*., 1993, 1995). Nevertheless, the oxidation of theaflavins and the formation of the thearubigin-like substances have not yet been fully elucidated.

Table 5.62 Mean contents of the total theaflavins during the processing of black tea.

Processing steps	Total theaflavins (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	1.85 a	2.22 a	2.04 a	1.48 a
S	3.13 a	4.03 a	5.81 a	4.67 a
R	3.78 a	5.20 a	3.62 a	5.19 a
C1	4.10 a	7.02 ab	3.49 a	8.87 a
C2	15.56 b	10.07 ab	7.45 a	10.44 a
F1	20.29 b	14.82 b	19.26 b	21.89 b
F2	30.18 c	28.92 c	29.50 c	34.87 c
F3	34.12 c	27.05 c	39.14 d	50.09 d
F4	36.15 c	28.15 c	39.66 d	50.34 d
D1	36.71 c	28.16 c	38.95 d	45.57 d
D2	33.73 c	24.99 c	38.89 d	45.67 d
T	33.11 c	25.64 c	37.60 cd	46.15 d
LSD	9.32	9.32	9.32	9.32

[1] Means (n = 3) in columns followed by a common letter are not significantly different (P > 0.05)

It is known that the oxidation of TF3G and TF3′G is more rapid than the oxidation of TFDG (Bajaj *et al*., 1987), and the possible oxidation products of theaflavins may include the proanthocyanidin polymers or a theafulvin fraction (Bailey *et al*., 1991, 1994; Powell *et al*., 1995). However, in this study, the levels of all four theaflavins did not significantly change (P > 0.05) in the in-line samples from the third fermentation stage (F3) to the final black tea product. It appears that oxidation of theaflavins in this black tea process may not be occurring although it can not be determined quantitatively from the data. Roberts and Smith (1961) found that the levels of total theaflavins decreased as the fermentation progressed, from 16.1 mg/g for 1 h fermentation to 12.7 mg/g for 4 h fermentation. Thus, longer hours of fermentation are detrimental to the formation of theaflavins.

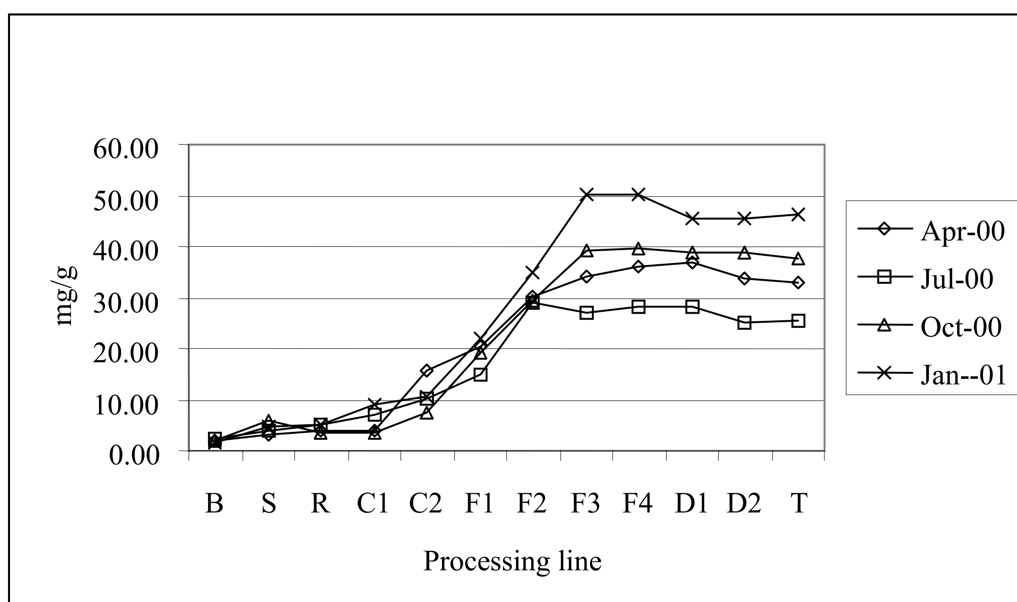


Figure 5.42 Mean contents of the total theaflavins during the processing of black tea.

As discussed previously, the content of theaflavins is extremely important in determining the quality of black tea and almost all the best quality characteristics of black tea are associated with the levels of theaflavins (Roberts and Smith, 1961, 1963; Roberts, 1962; Deb and Ullah, 1968; Hilton and Ellis, 1972; Cloughley and Ellis, 1980; Roberts and Chandradasa, 1982). In their early observation on the effects of theaflavins on black tea quality, Roberts and Smith (1963) found that commercial black teas with the highest levels of theaflavins (16.3 mg/g) had the highest sensory quality, with tasters' remarks including "very bright liquor, rich colour." Owuor *et al*. (1986a) found that the levels of total theaflavins in black tea varied from 3.06 mg/g for orthodox tea to 16.40 mg/g for CTC tea. The highest levels of total theaflavins were found in clonal black teas, ranging 16.30-22.88 mg/g with an average of 19.44 mg/g (Owuor *et al*., 1987). The variable reports of theaflavin levels in the literature are due to the methods used for the measurement. In the past, the most extensively used method for theaflavin determination was a spectrophotometric method (Roberts and Smith, 1961 and 1963), measuring the absorption of black tea liquor at 380 nm. This method cannot attain the true levels of theaflavins in black tea, as discussed earlier. This study of Australian black tea using HPLC analysis has found theaflavin levels (25.64-46.15 mg/g; average 35.63 mg/g) that are nearly twice as much as those found using the spectrophotometric method. While most of this difference may be due to the different analysis methods, black tea produced using the Australian process (CTC tea process as that used in Kenya) clearly has high levels of theaflavins.

Steinhaus and Engelhardt (1989) and Owuor and Obanda (1995) measured the individual and total theaflavins using a HPLC method similar to that used in this study. Steinhaus and Engelhardt (1989) extracted theaflavins using 2 g tea in 200 mL boiling water for 10 min extraction. These researchers found that the levels of the total theaflavins in black teas (German market) produced in Kenya, India, Sri Lanka and other countries range 4.49-14.48 mg/g with an average of 9.21 mg/g. This is a low level of total theaflavins for black tea. The possible reason for the low levels of total theaflavins found by these researchers may be that their extraction is incomplete. The second reason may be that when the commercial black tea samples were collected in the German market, these commodities might have various stages of storage since they were produced in Kenya, India, Sri Lanka and other countries. It has been found that theaflavins decrease during the storage of black tea; the longer the storage, the larger amounts of the loss for the theaflavins in black tea (Cloughley, 1981bc; Ellis and Cloughley, 1981; Obanda and Owuor, 1995). The third possibility may be that these samples may include some black teas produced using orthodox process (not mentioned in the original paper), which usually produces black tea with low levels of total theaflavins (Hilton, 1973; Hara et al, 1995; Sud and Baru, 2000).

In Kenyan black tea, the levels of total theaflavins range 18.94-22.71 mg/g, with an average of 21.37 mg/g (Owuor and Obanda, 1995a). On comparing these results to those found in Australian black tea, it has been found that the level of total theaflavins in the tea produced in July 2000 (25.64 mg/g) is very close to the level found in the Kenyan black tea sourced from the Kenyan market (21.37 mg/g; Owuor and Obanda, 1995a). However, the level of total theaflavins in the Australian black tea produced in January 2001 is 46.15 mg/g, being twice as much as the level found in the Kenyan black tea. These results demonstrate that black tea produced in Australia has much higher levels of total theaflavins than the teas from other countries (Table 5.62). Therefore, it can be concluded that the very high levels of individual and total theaflavins in black tea produced in the Australian factory should indicate a better quality tea being produced in Australia than in other countries when the theaflavin levels are considered.

Compared to the volatile flavour compounds, the astringency of theaflavins has been recognised as a better estimator than the aroma of the volatile flavour compounds in assessing the quality of black tea (Owuor, 1996). Results of this study showed that the formation of theaflavins varies with different harvests (Table 5.49), and the formation of each individual theaflavins occurs at a different rate within a harvest. These results are in accordance with an earlier report by Owuor and McDowell (1994a), who found that the rate of formation of total theaflavins varied with clones, within a clone, and that the individual theaflavins formed at different rates as fermentation progressed.

It has been found that the contributions of the four main theaflavins (TF, TF3G, TF3′G and TFDG) to the astringency and the quality of black tea are different (Sanderson *et al*., 1976; Owuor and Obanda, 1995a; Owuor, 1996). Therefore, use of the total theaflavins along with the individual theaflavins to predict the total astringency and, hence, the quality of black tea is suggested.

5.4.2.4 Combined catechin/catechin gallates and total phenolic compounds

There were significant effects ($P < 0.05$) of the in-line sampling point (processing stage) on the levels of combined catechins/catechin gallates and the levels of the total phenolic compounds during the black tea processing.

Combined catechins/catechin gallates
Generally, the levels of combined catechins/catechin gallates in the in-line samples showed no significant differences ($P > 0.05$) from the bin leaves (B) to the C1 stage for all harvests (Table 5.63 and Figure 5.43). Significant decreases ($P < 0.05$) in the levels of combined catechins/catechin gallates were found between the B and R stages in all the harvests except for the October 2000 harvest. Significant decreases ($P < 0.05$) in the levels of combined catechins/catechin gallates occurred in in-line samples after the C1 stage during the processing of black tea are described as follows (Table 5.63):

Table 5.63 Mean contents of the combined catechins/catechin gallates during the processing of black tea .

Processing steps	Total catechins and catechin gallates (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	202.01 f	179.26 e	211.57 e	243.45 g
S	181.62 ef	161.39 de	201.79 e	229.09 fg
R	174.43 e	151.63 d	205.43 e	204.39 e
C1	194.55 ef	159.34 de	208.41 e	226.63 efg
C2	149.10 d	160.68 de	221.74 e	214.57 ef
F1	132.81 d	113.99 c	164.18 d	172.57 d
F2	101.52 c	69.88 b	109.77 c	145.68 c
F3	75.21 b	52.88 ab	82.49 b	104.35 b
F4	63.99 ab	51.02 ab	59.78 ab	86.12 ab
D1	49.84 a	46.01 ab	56.44 a	74.37 a
D2	45.46 a	41.48 a	53.66 a	79.46 a
T	45.10 a	38.64 a	54.93 a	77.40 a
LSD	24.68	24.68	24.68	24.68

[1] Means (n = 3) in columns followed by a common letter are not significantly different ($P > 0.05$)

- In the April 2000 harvest, significant decreases ($P < 0.05$) in the combined catechins/catechin gallates occurred from stage C1 to C2/F1, from stage C2/F1 to F2, from stage F2 to F3, and finally from stage F3 to D1, after which there were no significant changes ($P > 0.05$) to the final black tea;
- In the July 2000 harvest, significant decreases ($P < 0.05$) in the combined catechins/catechin gallates occurred from stage C2 to F1, from stage F1 to F2, and finally from stage F2 to D2, after which there were no significant changes ($P > 0.05$) to the final black tea;
- In both the October 2000 and January 2001 harvests, significant decreases ($P < 0.05$) in the combined catechins/catechin gallates occurred from stage C2 to F1, from stage F1 to F2, from stage F2 to F3, and finally from stage F3 to D1, after which there were no significant changes ($P > 0.05$) to the final black tea.

Oxidation of all catechins and catechin gallates had slowed down after the third fermentation stage (F3), suggesting that the oxidation of the catechins and catechin gallates occurred mainly during the early stages of the fermentation period (e.g. F1 and F2). The oxidation of EGCG and the other catechin gallates plays an important role in determining the oxidative trend of the combined catechins and their gallates. That is, the oxidation of catechin gallates could dominate the oxidation process during the processing of black tea, due to the levels of these flavonoids constituting approximately 50 % of the levels of the combined catechins/catechin gallates in freshly harvested tea leaves.

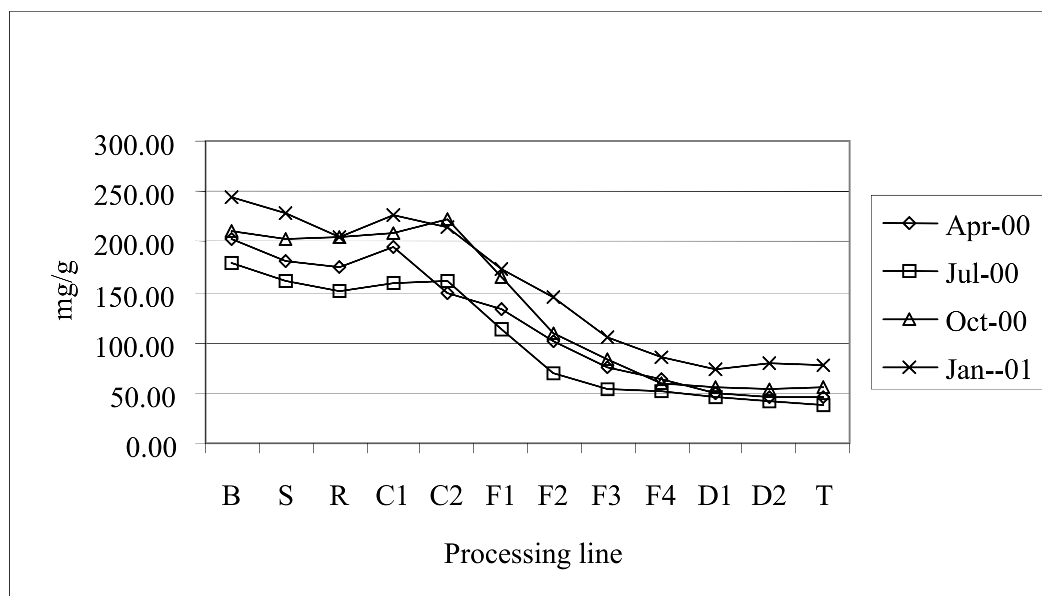


Figure 5.43 Mean contents of the combined catechins/catechin gallates during the processing of black tea.

Hara *et al* (1995) and Harbowy and Balentine (1997) suggested that the residues of catechins and their gallates in black tea are important black tea polyphenols in determining the liquor characteristics of the teas. In this study, it was found that the levels of combined catechins/catechin gallates in the final black tea (Table 5.42) showed no significant differences ($P > 0.05$) among the four harvests. Thus, the levels of these flavonoids in black tea, although important for black tea quality, are not useful indicators of the seasonal variation effect on tea liquor characteristics.

Total phenolic compounds

Significant differences ($P < 0.05$) in the levels of total phenolic compounds were found in the in-line samples for all the harvests (Table 5.64 and Figure 5.44).

- April 2000: significant decreases ($P < 0.05$) in the level of total phenolic compounds occurred from stage C1 to C2;
- July 2000: significant decreases ($P < 0.05$) in the levels of total phenolic compounds occurred from stage C2 to F1 and from stage F1 to F2;
- October 2000: significant decreases ($P < 0.05$) in the levels of total phenolic compounds occurred from stage C2 to F1, from stage F1 to F2, and finally from stage F3 to F4;
- January 2001: significant decreases ($P < 0.05$) in the total phenolic compounds occurred from stage C2 to F1, and then from stage F2 to F3.

Table 5.64 Mean contents of the total phenolic compounds during the black tea processing.

Processing steps	Total phenolic compounds (mg/g, dry basis)[1]			
	April 00	July 00	October 00	January 01
B	228.15 g	206.95 e	240.05 cd	282.75 e
S	208.32 fg	188.10 cde	232.74 c	269.65 e
R	204.68 fg	180.81 cd	234.09 c	242.88 cd
C1	229.05 g	199.96 de	238.01 cd	278.13 e
C2	201.55 f	200.68 de	262.22 d	266.80 de
F1	188.98 ef	163.33 c	225.01 c	240.67 c
F2	169.13 de	134.17 b	181.09 b	233.67 c
F3	145.44 cd	110.63 ab	187.86 b	207.96 b
F4	134.12 bc	110.13 ab	152.38 a	185.43 ab
D1	117.53 ab	106.60 a	133.87 a	163.82 a
D2	102.94 a	93.53 a	133.37 a	172.31 a
T	103.01 a	94.15 a	132.38 a	170.19 a
LSD	24.79	24.79	24.79	24.79

[1] Means (n = 3) in columns followed by a common letter are not significantly different ($P > 0.05$)

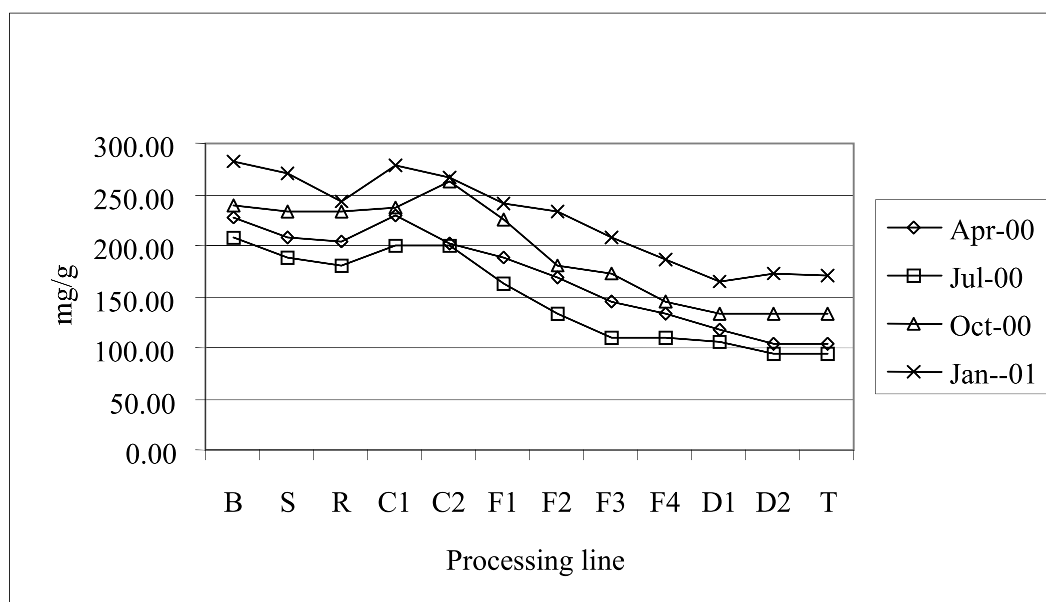


Figure 5.44 Reduction of total phenolic compounds during the processing of black tea.

The clear trend in this data is that catechins and catechin gallates that constitute 86-88 % of the total phenolic compounds in freshly harvested leaves decreased significantly ($P < 0.05$) during the fermentation stages F1 to F4 for all harvests, while the theaflavins significantly increased ($P < 0.05$). However, overall there is a significant decrease ($P < 0.05$) in the levels of total phenolic compounds measured in this study from the harvested fresh leaves to the final black tea. Total phenolics in black tea are residues and the oxidation products of the phenolics of green tea leaves. The decrease in the level of total phenolics during black tea processing results from the oxidation of the catechins and their gallates (Sections 5.4.2.1 and 5.4.2.2) to form relatively lower levels of theaflavins (Section 5.4.2.3) and so-called thearubigins, complexes that were not quantified in this study. In the final black tea, theaflavins constitute 27-32 %, and residue catechins and catechin gallates constitute 41-45 % of total phenolic compounds (Tables 5.62-5.64). Thus, the other phenolic compounds (the five flavonol

glycosides and seven simple phenolic compounds), which constitute 11-14 % of total phenolic compounds in the fresh tea leaves, appear to increase in levels during the processing of black tea and to finally constitute 23-32 % of the final black tea phenolic compounds analysed.

5.4.2.5 Ratio of total catechin gallates to total catechins (TCG/TC)

Ninomiya *et al*. (1997) suggested that the ratio of catechins and catechin gallates greatly affect the flavour and taste of green tea infusion. From this point of view, the ratios of total catechin gallates to total catechins (TCG/TC) in fresh green tea leaves could be used as one of the parameters for the quality of black tea. There was a significant effect ($P < 0.05$) of the in-line sampling point (processing stage) on the ratio of total catechin gallates to the total catechins (TCG/TC) during the black tea processing. The ratio of TCG/TC showed no significant differences ($P > 0.05$) from the first three processing stages from B to R for all the harvests (Table 5.65 and Figure 5.45). Significant decreases ($P < 0.05$) in TCG/TC ratio during the processing are described as follows (Table 5.65):

- In the April 2000 harvest, significant decreases ($P < 0.05$) in TCG/TC ratio were found from stage C1 to C2, from stage C2 to F1, from stage F1 to F2, from stage F2 to F3 and from stage F3 to D1.
- In the July 2000 harvest, the only significant decrease ($P < 0.05$) in TCG/TC ratio was found from stage F1 to F2.
- In the January 2001 harvest, significant decreases ($P < 0.05$) in TCG/TC ratio were found from stage C2 to F1, from stage F1 to F2, and from stage F2 to F3.
- In the October 2000 harvest, significant decreases ($P < 0.05$) in TCG/TC ratio were found from stage C2 to F2 and from stage F2 to F4.

Table 5.65 Mean ratios of TCG/TC during the processing of black tea.

Processing steps	TCG/TC ratio[1]			
	April 00	**July 00**	**October 00**	**January 01**
B	2.48 fg	1.28 b	1.35 e	1.99 d
S	2.47 fg	1.22 b	1.27 de	2.13 d
R	2.28 f	1.16 b	1.29 de	2.09 d
C1	2.58 g	1.19 b	1.28 de	1.96 d
C2	1.78 e	1.17 b	1.21 de	2.03 d
F1	1.47 d	1.07 b	1.10 cd	1.64 c
F2	1.13 c	0.65 a	0.89 bc	1.20 b
F3	0.84 b	0.50 a	0.70 ab	0.87 a
F4	0.71 ab	0.46 a	0.64 a	0.81 a
D1	0.58 a	0.49 a	0.56 a	0.70 a
D2	0.62 ab	0.52 a	0.75 ab	0.71 a
T	0.63 ab	0.52 a	0.60 a	0.76 a
LSD	0.24	0.24	0.24	0.24

[1] Means (n = 3) in columns followed by a common letter are not significantly different ($P > 0.05$)

In this study, the sharp reduction in the TCG/TC ratios during the fermentation stage (F1-F4) of black tea processing suggests that the catechin gallates undergo oxidation more rapidly than the catechins (Figure 5.45). Thereafter, there were no significant changes ($P > 0.05$) in the ratio TCG/TC (Table 5.65) and the differences among harvest times were not significant ($P > 0.05$) (Table 5.52). The ratios of TCG/TC became closer and closer between different harvest times as the tea process progresses (Table 5.65). This result indicates that oxidation of both catechins and their gallates would progress towards a fixed ratio and this ratio was the same for all the harvests during the last stages during the processing of black tea (Table 5.52).

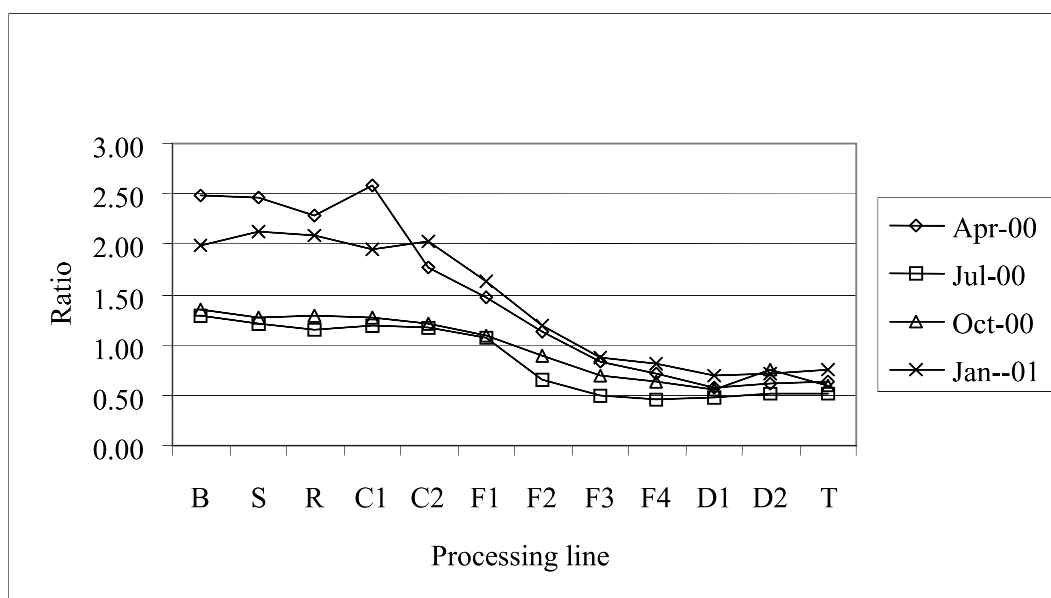


Figure 5.45 Mean ratios of TCG/TC during the processing of black tea.

In an early study, the oxidations of catechins and catechin gallates were also found to occur at different rates and to different extents during the fermentation of tea leaves (Bhatia and Ullah, 1962). Correspondingly, the formation of theaflavins occurred at varied rates among clones and within a clone (Owuor and McDowell, 1994a). Thus, the ratio of TCG/TC may be used to reflect the oxidation rate of the catechins and catechin gallates and, therefore, to monitor the formation and/or oxidation of the known quality indicators for black tea during processing.

5.4.2.6 Summary

No significant oxidation or decrease ($P > 0.05$) of the total and individual catechins and catechin gallates, together with no significant change ($P > 0.05$) in the total phenolic compounds and the ratio of TCG/TC were found in the early stages of black tea processing from the in-line stages of the bin (B, fresh green tea leaves) to stage C 1 after the CTC's for all harvests. EGCG, ECG, total catechin gallates and total catechins showed similar oxidation or decrease patterns. Most of the significant oxidations ($P < 0.05$) of the catechins and their gallates were detected during the early fermentation periods. Thus, the oxidation of polyphenols could be initiated by the polyphenolic enzymes and accelerated during the early fermentation stages. Thereafter, the oxidation of catechins and catechin gallates slowed down because of the availability of these substrates and the activity of the enzymes. This oxidation seemed to be stopped by the third or fourth stages of the fermentation in the black tea processing.

Oxidation of the catechins and their gallates leads to the formation of theaflavins. No significant change ($P > 0.05$) in the levels of theaflavins was found in the early processing stages from bin (B, fresh leaves) to after the CTC's (C1) for all harvests. The formation of theaflavins was generally significant ($P < 0.05$) during the early fermentation stages (F1-F3) in the processing of black tea. Thus, the early stages of fermentation in this Australian factory appears to be very critical in the formation of quality important theaflavins. As for the decrease in catechins and catechin gallates in the in-line samples, the levels decreased to a significantly ($P < 0.05$) low point from the third stage of the fermentation (F3) onward, while the increase in theaflavins peaked also by F3. Thus, the oxidation of flavanols and the formation of theaflavins corresponded and was completed by fermentation stage (F3) suggesting that the processing of Australian black tea is over-fermenting the tea leaves. The

results of this study suggest that the oxidation pattern of individual or grouped catechins and/or catechin gallates, along with the formation of individual or total theaflavins through the factory, could be used for on-line monitoring of black tea quality.

5.4.3 Summary and conclusions

The results for black tea processing in the Australian tea factory indicate that the processing or harvest times, the initial flavonoid levels (in tea green leaves), and the types of tea catechins and catechin gallates influence the levels and types of the resultant oxidation products, the theaflavins that accumulated in the black tea. The oxidation pattern of the catechins and their gallates varied accordingly to their initial levels in fresh green tea leaves and according to the harvest times. Correspondingly, the formation of the individual theaflavins was also dependent on these parameters. Therefore, the leaf composition and the environmental factors must be taken into considerations during the processing of black tea to maximise the quality of the end product.

The seasonal variation of the theaflavin levels in Kenyan black tea showed a highest level in September (18.98 mg/g) and lowest in April (12.63 mg/g) (Owuor, 1992). The highest and lowest levels did not occur correspondingly to the warmest month (January, 16.42 mg/g; February 18.13 mg/g) and the coldest month (June, 16.36 mg/g; July, 17.25 mg/g). Thus, the levels of theaflavins in the Kenyan black tea appear to be an irregular pattern across seasons. In Australian black tea, the levels of total theaflavins are higher in the January 2001 harvest than the other harvests, and all harvests show higher levels of total theaflavins than the corresponding harvests in Kenyan black tea (Table 5.66). Therefore, Australian black tea should have a quality as good as that of Kenyan CTC black tea when the theaflavin levels are taken into account.

In Britain, Rice-Evans's group compared the level of total theaflavins in orthodox and CTC black teas and found the former contained 21.2 mg/g theaflavins, while the latter reached 40.1 mg/g theaflavins on dry weight basis (Unilever, 1996). Compared to Australian black tea produced in this factory, the average level of total theaflavins (35.63 mg/g, Table 5.66) is close to this report. Again, Australian black tea should have a good quality on the basis of its total theaflavins.

Table 5.66 Comparison of the mean contents of total theaflavins in black tea produced in Kenya and Australia across harvest times.

Country	Total theaflavins (mg/g, dry basis)				
	April	July	October	January	Overall mean
Kenya[1]	12.63	17.25	17.64	16.42	16.30
Australia[2]	33.11	25.64	37.60	46.15	35.63

[1] Adapted from Owuor, 1992; measured by spectrophotometric method.
[2]. Data from Table 6.23; measured by HPLC.

A comparison of mean levels of individual and total theaflavins in Kenyan and Australian black teas produced using the CTC process is shown in Table 5.67. In this Kenyan study, a similar HPLC analytical method to this Australian study was used and a number of commercial Kenyan black teas were analysed. From this comparison, it has been noted that the HPLC analytical method used for individual and total theaflavins usually provides higher levels of these compounds relative to the spectrophotometric method used in the past. The levels of individual and total theaflavins in Australian black tea analysed in this study are generally higher than the levels of those found in the Kenyan black tea (Table 5.67). Therefore, the quality of Australian black tea is high compared with the black teas in the world on the basis of its theaflavin levels.

Table 5.67 Comparison of mean contents of individual and total theaflavins in Kenyan and Australian CTC black teas.

Theaflavins	Kenya (mg/g, dry basis)*			Australia (mg/g, dry basis)		
	Min	**Max**	**Average**	**Min**	**Max**	**Average**
TF	6.26	9.70	7.68	8.02	10.41	8.86
TF3G	5.48	6.57	6.19	7.79	13.09	9.82
TF3'G	2.48	3.45	2.89	3.10	7.00	5.38
TFDG	3.77	6.18	4.61	6.62	18.03	11.57
Total theaflavins	18.94	22.71	21.37	25.64	46.15	35.63

* Adapted from Owuor and Obanda, 1995a.

It should be noted that all of the above-mentioned levels of individual and/or total theaflavins in the black teas compared to the Australian black teas were the levels present in commercial black teas or in teas analysed by different methods. Thus, until all the sampling and analytical conditions are the same, it is too early to draw a final conclusion about the comparison. However, the trends in the levels of individual and total theaflavins found in this study clearly show a potential for high quality of Australian black tea.

For the levels of the individual catechins and catechin gallates, seasonal variations were observed in the initial stages of the tea processing, but not in the final black tea products. Higher contents of the catechins or their gallates in the freshly harvested green tea leaves from particular harvests did not result in higher residues of these compounds in the end products. However, it was found that for the January 2001 harvest, the levels of catechin gallates were generally higher in freshly harvested green tea leaves, while theaflavin levels were also generally higher in the final black tea when compared to the other harvests. Thus, it can be concluded that the levels of catechin gallates in fresh green tea leaves may be useful index of the level of theaflavins that will be produced in the black tea by the Australian processing. Therefore, it is recommended that further work should be conducted on improving the processing conditions, particularly the fermentation stages, with changes in the individual and grouped polyphenolic compounds for in-line teas being used to monitor the success or failure of such changes of the process.

The oxidation of catechins and catechin gallates, and the formation of the individual theaflavins occur most during the early stages of the fermentation in the Australian black tea process, suggesting that that this process is over-fermenting the tea leaves. The processing of black tea in the Australian factory could be modified by shortening the time that the tea leaves are fermented, with a possible improvement in quality. However, it is known that Australian tea drinkers have a preference for over-fermented black tea, so any changes in the fermentation step would need to be carefully considered by the Nerada Tea Pty. Ltd. with their customer's preferences in mind. Correlation with sensory data would then be of prime importance. However, the overseas markets would demand a less-fermented black tea.

5.5 Development of an on-line method for assessing optimum fermentation of black tea

The emphasis of this part of the study was on testing out the methods for suitability for use in a factory situation. As mentioned in Section 4.5 tea samples collected from the processing line in the factory at Glen Allyn Tea Estates (see Section 4.4) were analysed for some quality parameters, namely theaflavins, thearubigins, soluble solids, and total colour. The tea samples used were from the fermentation stages. There were 3 replicate sets of samples taken during the study at three monthly intervals in April, July and October 2000, and January 2001 (Section 4.4). The samples from January and July were measured here.

5.5.1 Changes in total theaflavins, thearubigins, soluble solids, and colour during fermentation

Some of the results for the different methods and parameters are shown in Tables 5.68-5. All values are in mg/g and are on a dried weight basis.

The results from one of the three January 2001 replicates is shown in Table 5.68 and Table 5.69 shows the results for the means of the three January replicates of fermentation stages 2-4 for a number of the measured parameters. Only 1 replicate had samples for fermentation stage 5.

Table 5.68 Comparison of different spectophotmetric methods for measuring theaflavins during fermentation of tea harvested in January 2001 (1 replicate).

Ferm Stage	TF HPLC	W TR %	W TF	W&T TR % 440nm	W&T TF 460nm	W&T TF 440nm	TF Flavognost	TF Method 6	TF Ullah	TF $AlCl_3$ (525nm)	Total Colour	%TSS	% Brightness
2	23.66						4.80	5.13	10.08	4.27	6.18	47.10	45.30
3	56.01	13.10	10.90	10.80	10.80	9.90	11.18	13.32	12.60	9.03	4.85	44.59	72.17
4	63.29	24.23	17.35	13.83	14.30	13.90	15.00	15.70	13.50	11.10	7.30	42.56	51.37
5	66.17	22.60	15.00	15.30	15.70	15.20	12.94	12.30	12.05	11.08	8.03	42.44	41.70

Table 5.69 Changes to quality parameters during fermentation of tea harvested in January 2001.

Ferm Stage	TF HPLC	TF $AlCl_3$ (525nm)	TF Flavognost	%TSS	% Brightness	Total Colour
2	34.87	6.29	7.65	44.77	49.05	6.08
3	50.10	9.47	11.99	44.04	49.64	7.42
4	50.34	9.57	12.49	42.97	38.58	9.18

The results (Table 5.69) show TF plateauing at fermentation stage 4. The values for total soluble solids (TSS) and % brightness appear to follow a similar pattern while total colour continually rises. There does appear to be agreement with the strong correlation between the concentration of thearubigins and the level of total colour found by Whitehead and Muhime (1989) (Figure 5.46)

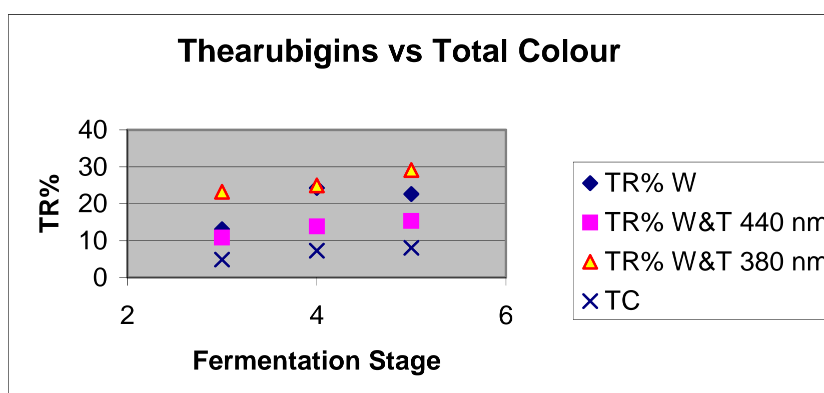


Figure 5.46 Relationship between thearubigens and total colour during fermentation of tea harvested in January 2001(1 replicate).

The results from one of the three July 2000 replicates is shown in Table 5.70 and Table 5.71 shows the results for the means of the three July replicates for fermentation stages 2-5 for a number of the measured parameters.

Table 5.70 Comparison of different spectophotmetric methods for measuring theaflavins during fermentation of tea harvested in July 2000 (1 replicate).

Ferm Stage	TF HPLC	TF Flavognost	TF Method 6	TF Ullah	TF $AlCl_3$ (525nm)	TF $AlCl_3$ (520nm)	Total Colour	% TSS	% Brightness
2	31.36	4.70	5.90	5.445	3.75	5.56	5.56	42.56	27.19
3	30.44	8.30	5.74	7.335	6.01	9.10	6.20	41.30	27.53
4	35.06	7.97	5.36	10.20	6.15	-	7.40	40.05	38.29
5	30.78	6.73	-	-	5.11	-	6.90	39.05	18.30

Table 5.71 Changes to quality parameters during fermentation of tea harvested in July 2000.

Ferm Stage	TF HPLC	TF $AlCl_3$ (525nm)	TF Flavognost	%TSS	% Brightness	Total Colour
2	28.92	4.03	5.09	41.57	32.61	6.23
3	27.05	4.66	6.26	40.06	33.79	6.55
4	28.15	4.53	5.82	39.56	31.10	7.28
5	24.00	4.18	5.46	39.65	19.56	7.93

The results (Table 5.71) show TF peaking at fermentation stage 3. The values for total soluble solids (TSS) and % brightness appear to follow a similar pattern while total colour continues to rise

Overall the results were consistent with the finding (Section 5.4) that the January 2001 samples had higher TF values and that the July 2000 samples had lower TF values.

Total colour also tended to be higher for January 2001 but the most important observation was the way colour varied with stage of fermentation.

There are considerable variations in the data obtained using the different methods with all values using the spectrophotometric methods being lower than the values obtained by HPLC. However as already explained the deterioration of the samples with time cannot be eliminated as the cause.

Considering just the spectrophotometric methods, the results seemed to indicate that the Sep-Pak methods of Whitehead (1991) and Whitehead and Temple (1992) seemed to be in general agreement with the other methods. However the Aluminium Chloride method measured at 525 nm consistently gave lower results. Some of the measurements taken at 520 nm were higher than those at 525 nm and gave results comparable to the other methods. The results obtained using the Aluminium Chloride method would be expected to parallel the results obtained using the Flavognost method due in part to the fact that the same solvent extract is used for both procedures, as Aluminium Chloride solution is simply used in place of the Flavognost reagent.

5.5.2 Assessment of methods

The Sep-Pak methods offer a rapid method for analysing both TF and TR. There appeared to be good agreement between the results obtained using the Sep-Pak methods and those obtained using the Flavognost method. The Whitehead and Temple Sep-Pak method has an advantage over the Flavognost method, as it also measures TR, and is less taxing in terms of time and solvent. The

Whitehead and Temple method uses water, acetic acid and methanol, which are less toxic than the IMBK required in the Flavognost and Aluminium Chloride methods. The Whitehead and Temple (1992) method does require some technical knowledge and ability as does the Flavognost and Aluminium Chloride methods.

Measurement of total colour was relatively simple but clearly measures TR rather than TF.

Considerable effort went in to assessing the accuracy of the TF measurements by the various methods by comparing them with published values in a number of publications. This is not reported here as in the end none of the methods were simple enough to fit the criteria for adaptation to the factory situation at Malanda. Whilst the measurement of TF itself is simple enough, the need to extract the theaflavins and separate them from thearubigens make the tests more complex requiring space, equipment and skilled technical support.

5.5.3 Relationships between theaflavins, thearubigen fractions and colour

Takeo (1974) undertook a photometric evaluation of infused teas. A range of blended teas were assessed for TF and the role of thearubigins and highly polymerised substance was discussed. Theaflavin, thearubigin and especially theaflavin plus thearubigin showed significant positive correlations with quality, while highly polymerised substance showed a negative correlation. In Section 4.1 the thearubigin fraction was separated into butanol-soluble thearubigin and butanol-insoluble theabrownin (TB). Highly polymerised substance referred to by Takeo (1974) is most probably theabrownin.

Malec and Vigo (1988) monitored seasonal variations in TF, TR and caffeine in Argentinian black teas. These authors also stated that there was a positive correlation between total colour and TF, but not TR. In contrast, as already mentioned, Whitehead and Muhine (1989) stated that TR is correlated with total colour.

Obanda *et al.* (2003) called the thearubigen butanol-insoluble fraction, TRSII (which is probably equivalent to TB or highly polymerised substance) and the butanol-soluble fraction, TRSI. They stated that the substances that determine total colour are TRSII and TF. They also stated that TF positively influenced liquor brightness, while total TR, and especially TRSII (not so much TRSI) negatively influences liquor brightness. Different fermentation times were studied. Interestingly, in contrast to the findings of Whitehead and Muhime (1989), when TF peaked, so did total TR and total colour, although TRSI and TRSII continued to rise.

Roberts and Chandradas (1982) presented a method for measuring colour to monitor fermentation. They discussed a method utilising a simple comparitor, the Lovibond instrument and claimed that the colour development in tea is primarily associated with the formation of TF, TR, and TRP (thearubigin polymers). The data presented show TR and TF peaking at 2.5 hrs with TRP and colour continuing to rise throughout the process. The end of the fermentation is when the colour reaches approximately 19 on the (EBC) colour scale.

Takeo and Oosawa (1976) used the Hunterlab colour meter and a spectrometer to investigate coloured components in black tea. They reported that the L, a and b values determined on the infusions showed high correlation to the level of thearubigin and lower correlations with theaflavin.

Liang *et al.* (2003) studied chemical composition (including TF) relating to both sensory evaluation as well as colour. This information was then used for developing a model to estimate black tea quality. Seventeen samples from the major tea estates across China were analysed. HPLC was utilised to quantify TF. A colour difference meter was used to measure the colour components, i.e Δa, Δb, ΔL, and ΔE. The components Δa, Δb, and ΔE were found to be positively correlated to total TF, TF, TF3'G and TF3,3'DG while ΔL was found to be negatively correlated. ΔL might in fact positively correlate to thearubigin polymers.

The finding that that total colour is related to total TR content is clearly not that simple. Thearubigens themselves are a complex mixture. It would appear that some components of colour are in fact positively correlated to TF and perhaps even the equivalent of the TRS1 fraction of Obanda *et al.* (2003), whereas the higher molecular weight polymers of TR are positively correlated to different colour components. Thus there would appear to be a potential opportunity to investigate colour components Δa, Δb, ΔL, and ΔE and compare them not only to total TF, TF, TF3'G and TF3,3'DG as Liang *et al*. (2003) did, but also TR including TRSI and TRSII. The Minolta colorimeter is a hand-held device requiring minimal training to use. If TF and TR components throughout fermentation could be monitored and correlated with certain colour components as measured with the Minolta colorimeter and standardised in the factory situation this would represented a simple and easy to use method for monitoring optimum fermentation.

More information is first required on the fractions of TR. A useful starting point is the investigation of the variety of phenolics in various tea samples by gel-permeation chromatography. Millin et al. (1969) subjected whole tea solids and various tea extracts to gel-permeation chromatography. The resulting chromatograms were illustrative of the particular components. Millin et al. (1969) stated that non-dialysable pigments may be equated with Roberts' SII thearubigins which in turn are equivalent to the TRS11 fractions above. This method is able not only to separate out the TR higher molecular weight polymers but also to separate the TF's into 3 peaks.

Some preliminary analytical gel-permeation chromatography experiments were undertaken on an analytical scale in order to compare tea samples that previous analysis pointed to observable differences in the resulting gel-permeation chromatography chromatograms. Standardised infusions of these samples were prepared and freeze-dried. TSK-40 was utilised as the absorbent, which has a capacity to work with organic solvents. An acetone:water mixture was used with this phase as reported by Ozawa (1982). Initially 40% acetone was used. There appear to be solubility problems with using such a high proportion of acetone. When the freeze-dried tea was dissolved in the solvent, there was varying amounts of insoluble dark material. The resulting filtered solutions were not darkly coloured indication. The insoluble material may correspond to the theabrownins, which are water soluble. A more aqueous solvent system would allow these components to be analysed by this gel-permeation chromatography system. This procedure needs to be optimised so that the TR and TF fractions can be properly separated.

5.5.4 Summary

Theaflavins were analysed by several methods (6 in all) in order to assess their ease of use and adaptability for on-line use given the constraints at the tea factory. They were also assessed for their ability to distinguish between levels of theaflavins.

None of the methods were simple enough to fit the criteria for adaptation to the factory situation at Malanda. Whilst the measurement itself is simple enough, the need to extract the theaflavins make the tests more complex requiring space, equipment and skilled technical support.

Next the feasibility of using colour as measure of theaflavin content was investigated. The drawback found here was that as the theaflavin content peaked during fermentation, total colour and thearubigens continued to rise. So it was not possible to separate the contribution of theaflavins to colour using these methods. This was supported by reported work in the literature.

However some recent work suggested that the colour contribution of theaflavins could be separated out using a colour meter such as the Hunter Lab or Minolta colour meter. These instruments measure 4 different components of colour and it appears that theaflavins can be positively correlated with some of these. Also the Minolta colour meter is a hand held instrument making it ideal for on-line use.

More information is first required on the fractions of TR and their contribution to the colour of tea. Some preliminary analytical gel-permeation chromatography work has been undertaken.

6 Summary, conclusions and recommendations

An extensive study was undertaken to investigate the flavonoids and other polyphenols in Australian grown and made tea. Five major experiments were involved:

1. The quality profiles of teas on Australian market were measured and a comparison of Australian grown teas was made with other teas.
2. A method was developed and optimised and could efficiently and repeatedly extract flavonoids and other polyphenols from fresh green leaves.
3. The profiles and contents of flavonoids and other phenolic compounds in the green leaves of Australian grown tea were analysed. The experiments were conducted for fifteen commercial harvests from April 2000 to May 2001 in order to determine seasonal variations in the flavonoids and other polyphenols.
4. The compositional changes of the flavonoids and other phenolic compounds during the processing of Australian black tea were determined. These in-line analyses were conducted for four harvests at three-month intervals to cover seasonal influences under commercial production conditions.
5. Methods were investigated for developing an online method for monitoring and optimising fermentation of Australian grown and made black tea.

6.1 Summary

6.1.1 Phenolic compounds in teas from Australian supermarkets

Samples of both green and black leaf tea and teabags were randomly collected from supermarkets, including Australian teas. Moisture, water extraction, total polyphenols (PPs), theaflavin, thearubigen and theabrownin were measured in the samples. This observational study was an initial investigation into the quality of teas available in Australian supermarkets. The aim was to provide an overall profile of the quality of teas available in Australia.

6.1.2 Flavonoids and other polyphenols in Australian grown and made tea

One bud with first two adjourning leaves was hand plucked and extracted with boiling water and methanol and then concentrated to dryness. The dry solid was re-dissolved with chloroform, ethyl acetate, methanol and water for HPLC analysis. Methanol was selected for a further extraction trial due to its efficiency in extracting tea polyphenols. Thereafter, blending time and enzymatic activity were examined, and the final method was optimised. The method involved extracting polyphenolic compounds by blending green tea leaves with methanol for 5 min and then immediately analysing the methanol extract by HPLC.

Next, hand-plucked fresh tea leaves were analysed across seasons for their contents of individual flavonoids including EGCG, ECG, EGC and grouped flavonoids including total catechins, total catechin gallates, the combined catechins/catechin gallates, and total phenolic compounds. The results from the hand-plucked green tea leaves were compared with those from the mechanically harvested green tea leaves. This study was done to determine the chemical changes that occur for these phenolic compounds, so as to determine the best time to harvest tea in Australia to maximise the quality of the processed black tea.

The chemical analyses of the in-line tea leaves (from green leaves to final black tea) were extended to include TF, TF3G, TF3′G and TFDG, in addition to those individual and grouped flavonoids analysed for the hand-plucked green tea leaves. These in-line analyses permitted monitoring of the formation of compounds important for quality (i. e. the theaflavins) during the processing of black tea.

6.1.3 Development of an on-line method for assessing optimum fermentation of black tea

One of the major findings from the above study was the need to optimise the fermentation conditions of the Australian black tea process. It was decided to investigate the development of a process to monitor and optimise the tea fermentation using theaflavin levels, the major quality indicators of black tea.

Theaflavins were directly analysed by several methods in order to assess their ease of use and adaptability for on-line use given the space and technical constraints of the tea factory. They were also assessed for their ability to distinguish between levels of theaflavins. The feasibility of using colour as measure of theaflavin content was also investigated.

6.2 Main findings and conclusions

6.2.1 Phenolic compounds in teas from Australian supermarkets

- Total polyphenols in black tea leaf was between 14 % and 20 %, with an average of 17 %. This was slightly lower than the total polyphenols detected in black teabags, which ranged from 13 % to 27 % with an average of 18 %. Two of the Australian grown and made black leaf teas had the lowest total polyphenol content of 14 %. This may suggest that Australian tea processors prefer teas with a slight over-fermentation that allows more polyphenols to oxidise than with moderate fermentation during the black tea processing.
- The total polyphenol content of green teabags was between 21 % and 33 %, with an average of 25 %. This was much higher than the polyphenols content of black teabags with an average of 18 %.
- More polyphenols were extracted from green tea bags in comparison to green leaf tea than from black tea bags in comparison to black leaf. The percentage of the polyphenols dissolved from the teabags could be a useful index of the quality of the filter paper used for teabags.
- The average TF content of 30 kinds of black teabags was 0.81 % and that of 17 black leaf tea samples was 0.75 %. The contents of TR and TB in teabags were about 1 % higher than in leaf teas. The TF content of the teabags had a range from 0.29% to 1.25%, which showed higher variation than leaf teas that had a range from 0.32% to 1.10%.
- The solubility of TR and TB from teabags was between 82 % to 86 % and between 83 % to 92 %, respectively indicating that the permeability of teabags was variable.
- The price of black teas from markets was not related to the content of TF, TR, or TF/TR though it was assumed to be for the tea producers. Similarly, the price of green teas from the markets showed the same trend.
- Black teas from Australian markets had lower contents of TF and TR as well as TF/TR ratio than those from overseas markets. This implies that education of Australian consumers is necessary to encourage the consumption of quality black teas with higher TF content and TF/TR ratio.

6.2.2 Flavonoids and other polyphenols in Australian grown and made tea

- Significant differences ($P < 0.05$) existed in the levels of EGCG, ECG and total catechin gallates in both hand-plucked and mechanically harvested green tea leaves from different months during the year, with the warmer months producing tea leaves with higher levels.
- Green tea leaves harvested in the warmer months of November to February in Australia should produce higher quality black tea based on the findings of this study: with the high levels of catechin gallates, particularly, EGCG and ECG, in fresh leaves harvested in these months being a quality indicator for the black tea produced from these leaves. These results are supported by previous findings (Bokuchava and Skobeleva, 1969; Biswas *et al*., 1973; Graham, 1992; Obanda *et al*., 1997)
- Significant differences ($P < 0.05$) were found in the levels of EGC and total catechins in both hand-plucked and mechanically harvested green tea leaves from different months during the year,

with the cooler months producing leaves with higher levels. This is opposite to that found for the catechin gallates.

- It is concluded that levels of catechins are not as good an indicator of black tea quality as are the levels of catechin gallates, based on the gross levels of catechins and catechins gallates in green leaves.
- Significant differences ($P < 0.05$) existed in the levels of all the individual and grouped flavanols and total phenolic compounds at the early stages of black tea processing (prior to fermentation steps) across different harvest months. These differences between harvests originate from the differences in the levels of these compounds in the mechanically harvested green tea leaves.
- During the period of black tea processing, significant decreases ($P < 0.05$) occurred in the levels of the individual catechins and catechin gallates, and in the grouped flavanols, including total phenolic compounds, during the early stages of fermentation due to oxidation. However, the residues of all the individual and grouped flavanols in the final black tea showed no significant differences ($P > 0.05$) due to harvest date.
- Analysis of the reduction in EGCG levels could be used to optimise the fermentation stage of this Australian tea processing factory as it is used in India (Bhatia, 1960; Owuor, 1984).
- A significant increase ($P < 0.05$) in individual and total theaflavins during the processing of black tea occurred mainly in the first half of the fermentation period. Except for TF, the levels of all individual and total theaflavins showed significant ($P< 0.05$) harvest date differences in the final black tea, with the warmer month of January 2001 producing processed tea that had the highest theaflavin levels.
- Analysis of the increase in the theaflavin levels could be used to optimise the fermentation stage of this Australian tea processing factory.
- EGCG and ECG levels in freshly harvested green leaves could be positively related to the quality of black tea as individual compounds occur at appreciable levels in Australian grown green tea leaves. However, their roles as black tea quality indicators in the green tea leaves are usually translated into the potential for the formation of the theaflavins during the black tea processing. Thus, these compounds could be the indirect indicators for black tea quality, particularly when harvest variations in EGCG and ECG were translated into harvest variations in the theaflavins in the final black tea.
- A ratio of the catechin gallates to catechins could be used to show seasonal variations in green leaves, with a lower ratio occurring in the cooler months and a higher ratio in the warmer months.
- EGCG levels of fresh leaves in Australian grown tea (91.31-128.63 mg/g, by season) are high compared to the levels reported from other countries: Japan, 88.0-122.0 mg/g by season (Chu and Juneja, 1997); Kenya, 60.6-117.5 mg/g by clone (Obanda *et al*., 1997a).
- Levels of total flavanols (combined catechins/catechin gallates) in the fresh tea leaves (218.49-264.11 mg/g, by season) are generally higher than the levels found in Kenyan tea (128.1-226.0 mg/g, by clone) (Obanda *et al*., 1997a).
- Individual and total theaflavins showed significant differences ($P < 0.05$) in the black tea produced from different harvests throughout the year, except for TF, with black teas produced in the warmer months showing significantly higher ($P < 0.05$) levels of TF3G, TF3′G, TFDG and total theaflavins. This was consistent with the higher catechin gallate levels in fresh green leaves in the warmer months. Thus, it can be concluded that the black teas produced in Australia using leaves harvested in the warmer months may possess better quality than those produced using leaves harvested in the cooler months. However, further studies are required to test this conclusion.
- The levels of total theaflavins found in the final black tea in this Australian factory (25.60-46.15 mg/g, by season) are generally higher than the levels found in commercial teas from the other countries: e.g. orthodox black tea, 21.2 mg/g and CTC black tea, 40.1 mg/g (Unilever, 1996); black teas (German market) produced in Sri Lanka, India, Kenya and other countries, 4.49-14.48 mg/g (Steinhaus and Engelhardt, 1989); black tea from the Kenyan market, 17.99-25.90 mg/g (Owuor and Obanda, 1995a).
- Levels of theaflavins in the final black tea could be used to optimise the fermentation stage of this Australia tea processing factory, as it is used in Kenya and Malawi (Cloughley, 1980a; Owuor, 1984).

6.2.3 Development of an on-line method for assessing optimum fermentation of black tea

- None of the spectrophotmetric methods used for measuring theaflavins fitted the criteria for adaption for on-line monitoring of fermentation in the factory situation where space and technical support is limited.
- Using colour to monitor theaflavins levels could be more promising than at first thought if the colour contribution of theaflavins could be separated from that of the thearubigens using detailed colour analysis with a colour meter such as the Hunter Lab or Minolta colour meter.
- More information is first required on the fractions of thearubigens and their contribution to the colour of tea.

6.3 Recommendations for further work

- Based on the results and conclusions from the studies on flavonoids and other polyphenols in Australian tea, the following recommendations are suggested:
- The ratio of (ECG+EGCG)/EGC be used to measure seasonal variation in the levels of flavonoids in green tea leaves in the field and thus be used for monitoring the best time of the year to harvest tea leaves to produce quality black tea. This ratio can be used as a quality index for the processed black tea.
- The fermentation conditions of the Australian black tea process could be optimised through monitoring:
  (1) the reduction in the in-line levels of EGCG;
  (2) the increase in the in-line levels of theaflavins (individual and total);
  (3) the sensory qualities of the final black tea;
  (4) the theaflavin content of the final black tea;
  (5) the volatile content of the final black tea.
  Thus, a further major study of the Australian black tea manufacturing process that focuses on the fermentation stage is recommended. It should consist of the analysis of the theaflavin and volatile contents of the final black tea, and sensory evaluation of the final black tea, as each change is made to the fermentation stage of the process.
- Sensory data on black tea throughout a full year should be correlated with theaflavin levels in this black tea and with the (ECG + EGCG)/EGC ratio of the green leaves prior to processing.
- A study on the thearubigins in Australian black tea should be conducted qualitatively and quantitatively, and the data from the study of thearubigins should be investigated as a means of monitoring the manufacturing process of Australian black tea.
- The development of an on-line method for measuring theaflavins using colour measurement should be further investigated. Such a method has the potential to greatly assist the tea industry to optimise fermentation.

7 Implications and intellectual property

Prior to this study, no published research has been conducted on the flavonoids and other polyphenols of Australian tea. The results have shown that Australian tea has the polyphenol chemical profile to produce high quality tea especially during the warmer months of the year. Black tea showed polyphenol levels equivalent or better than high quality overseas teas. Nonetheless Australian tea is generally regarded as of moderate quality. Fermentation was identified as an area where changes could be made to maximise the quality potential. Progress was made on the development of an on-line method for monitoring fermentation by measuring theaflavins using colour analysis. Not only does such a method have the potential to greatly assist the Australian tea industry to optimise fermentation but would be applicable worldwide and as such as would have commercial significance.

8 Publications from project

This study has been published or presented in part in the following papers:

Yao, L.H., Caffin, N., D'Arcy, B., Xu, Y., Wansri, R. and Liu, X. 2000. *Phenolic compounds in tea from Australian markets*. Proceedings of 33rd Annual Australian Institute of Food Science and Technology Convention and Exhibition, Brisbane, Australia, 20-23 August. pp41. (Oral paper)

Yao, L.H., D'Arcy, B., Caffin, N., Liu, X., Wansri, R. and Xu, Y. 2000. *Quality aspects of teas from Australian markets*. Proceedings of 33rd Annual Australian Institute of Food Science and Technology Convention and Exhibition, Brisbane, Australia, 20-23 August. pp60. (Poster)

Yao, L.H., Caffin, N. and D'Arcy, B., 2002. Flavonoids in Australian tea. Proceedings of the 26th Annual Scientific Meeting of the Nutrition Society of Australia, Wollongong, NSW, 1-4 December. Published in the Asia Pacific Journal of Clinical Nutrition. 11(supp): S252.

9 Appendix

Appendix 5.1 Moisture content of the hand plucked and mechanically harvested fresh tea leaves

The moisture content in the hand plucked (Field) and mechanically harvested (Bin) fresh tea leaves is presented as dry mass of the tea leaves (Table appendix 5.1 and Figure appendix 5.1). That is, if the value in the following table is low, the moisture content in the tea leaves from the corresponded harvest is high. Thus, the mean value of the real moisture content of the tea leaves equals to the value of that 100 % minus the corresponding value in Table appendix 5.1. For examples, the moisture content for hand plucked fresh tea leaves harvested on 11 April 2000 is: 100 % - 17.15 % = 82.85 %; for the mechanically harvested tea leaves is: 100 % - 19.97 % = 80.03 %.

Table Appendix 5.1 Mean content of dry mass in hand plucked (Field) and mechanically harvested (Bin) fresh tea leaves.

9.1 Harvest date	**Mean content of the dry mass in tea leaves (%)**	
	Field leaves	**Bin leaves**
11 April 2000	17.25	19.97
9 May 2000	16.88	18.86
19 July 2000	25.72	36.75
23 August 2000	22.68	24.04
27 September 2000	23.75	26.75
24 October 2000	19.05	26.40
13 November 2000	22.31	21.08
6 December 2000	19.62	20.54
27 December 2000	22.53	24.44
15 January 2001	21.04	21.00
1 February 2001	21.03	19.25
22 February 2001	22.10	22.68
19 March 2001	22.81	21.84
9 April 2001	19.00	20.54
2 May 2001	22.19	21.03

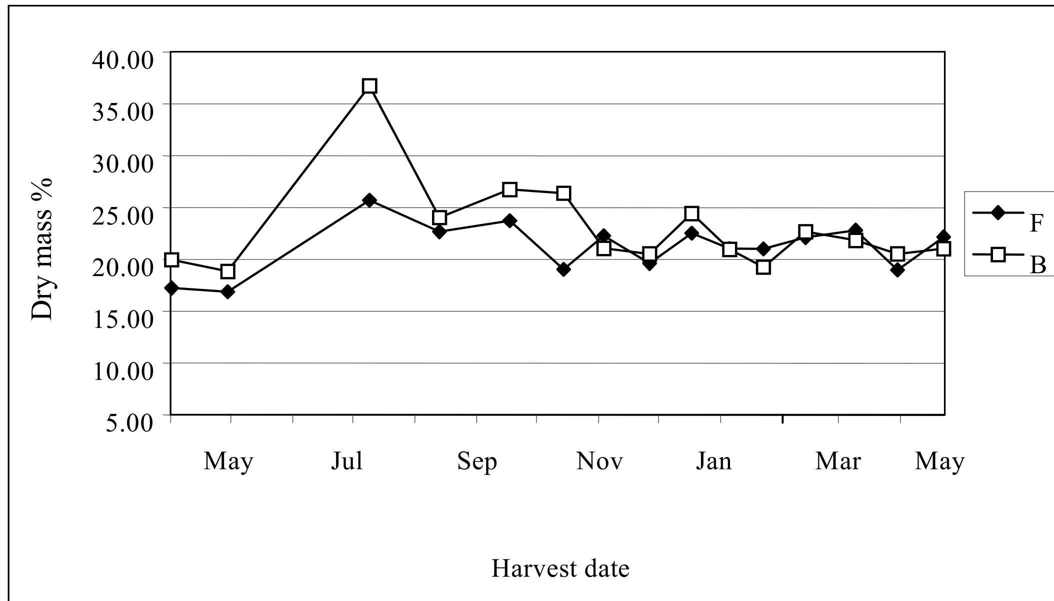


Figure Appendix 5.1 Mean content of dry mass in hand plucked (Field) and mechanically harvested (Bin) fresh tea leaves.

Appendix 5.2 Abbreviations for stages in the black tea processing line

B	green leaves from the Bin
S	green leaves that passed the Shredder
R	green leaves that passed the Rotorvane
C1	green leaves that passed the four CTC machines
C2	after C1 before fermentation
F1-F4	after each fermentation stage from 1-4
D1	after preliminary dry stage
D2	after full dry stage
T	final black tea

Appendix 5. 3 Moisture content of the in-line tea samples taken from the black tea processing line.

The moisture content in the in-line samples taken from the tea factory is present as dry mass of the tea leaves (Table appendix 5.2 and Figure appendix 5.2). Use of the value is the same as indicated in Appendix 5.1 earlier.

Table Appendix 5.2 Mean content of dry mass in the in-line tea samples (%).

Processing steps	Mean content of the dry mass in the in-line tea samples (%)			
	April 00	**July 00**	**October 00**	**January 01**
9.2 B	19.88	32.97	23.63	20.49
S	20.54	34.49	25.52	22.33
R	20.96	29.43	24.83	24.75
C1	21.05	30.98	25.38	25.62
C2	24.40	32.04	26.74	26.36
F1	25.02	33.11	28.74	27.58
F2	25.91	33.52	29.11	26.30
F3	25.71	33.77	28.43	26.28
F4	25.53	33.30	28.09	26.20
D1	55.45	64.45	55.56	58.04
D2	97.44	97.16	97.08	98.44
T	96.83	97.13	97.16	98.49

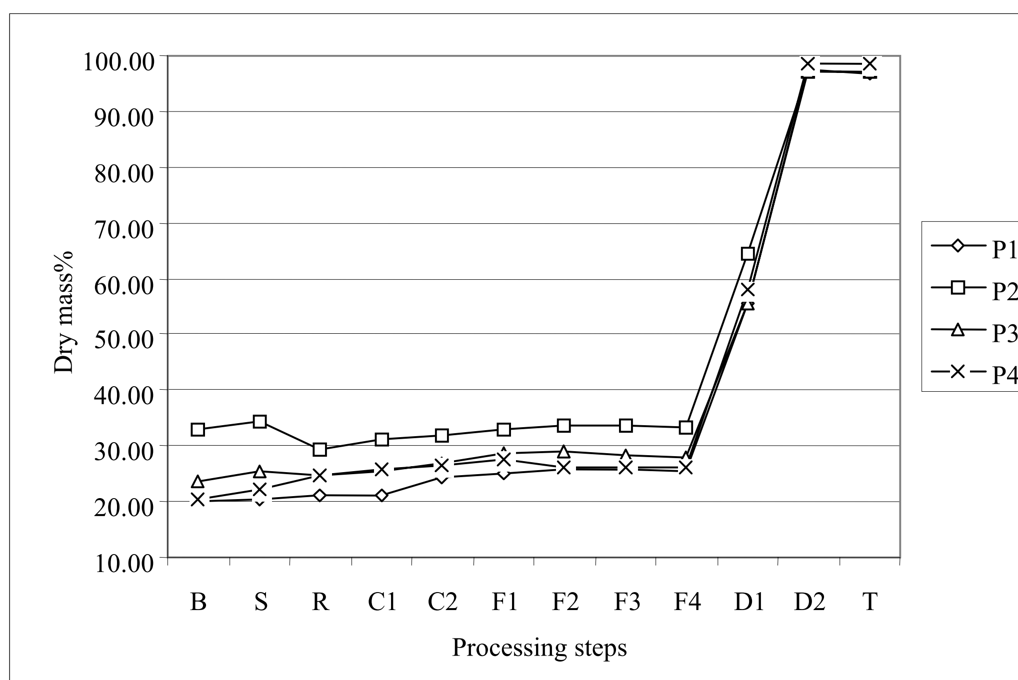


Figure Appendix 5.2 Mean content of the dry mass in the in-line tea samples (%).

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