The Characteristics of New Zealand Oolong Tea

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Abstract

Tea is a beverage made from steeping dried leaves in hot water. Worldwide, more than 10,000 different teas are made from different varietals of Camellia sinensis. Tea is a relatively new tea crop to New Zealand and they do grow well in New Zealand conditions. Research to date in New Zealand indicated is present some unique features compared to tea will wide. The main unique problem with New Zealand Oolong tea is the character of special colour and flavour and different process of manufacture. No systematic trials of the EGCG content had been performed on New Zealand Oolong tea and therefore this research investigated the characteristics of New Zealand Oolong tea processing and use HPLC to determine the content of EGCG in fresh tea leaves of New Zealand Oolong tea.

This study used HPLC to determine EGCG the content of leaves used to make New Zealand Oolong tea. In the fresh tea leaves that have not been through the indoor wilting stage, the content of EGCG is 124mg/g. In finished tea the content of EGCG is 112mg/g. That is significantly higher than other teas such as Pu-erh, Fujian Oolong tea, DaHongPao Black tea.

The results indicated that the early stages of the current tea manufacturing process are critical for controlling the EGCG content and extend of EGCG oxidation in the finished product.

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List of Nomenclature

R_i	The rates of evaporation
M	The average mass of the sample
C_s	Concentration of EGCG
V_s	Dried tea samples solution
M_s	The mess of EGCG in Original dried tea samples solution
M_0	Dried tea sample's mass
V_f	Fresh tea samples solution
M_f	The mess of EGCG in Original fresh tea samples solution
M_{f0}	Fresh tea sample's dry mass
R	The rate of the EGCG oxidisation

List of Abbreviations

EGCG	Epigallocatechingallate
EC	Epicatechin
ECG	Epicatechingallate
EGC	Epigallocatechin
C	Catechin
GCG	Gallocatechingallate
DM	Dry Matter
QS	Quality Score
TS	Taste Score

Chapter 1: Introduction

1.1 Tea

Tea is the most popular beverage after water throughout the world. Tea was first used in China for its medicinal properties 5000 years ago, [1] but it is only in the last couple of decades that the potential health benefits of this ancient beverage have been documented on a scientific basis. [2]

Today tea bushes and trees are grown commercially in about 40 countries around the globe, predominantly in areas close to the equator where the tropical conditions suit the plant best. The main tea-producing countries, world-wide, are China, India, Japan, Sri Lanka, Turkey, Kenya, and Indonesia. [3-4] New Zealand is one of the new tea growing countries. Worldwide, demand exceeds supply and therefore there is much potential for the New Zealand Oolong Tea industry to fill the gap because the New Zealand Oolong Tea is free of chemical sprays or fertilizers, with fast growth rates, anti-season product and high yields. [4-5]

1.2 The types of tea

The four types of tea most commonly found on the market are black tea, oolong tea, green tea and white tea, all of which can be made from the same bushes, processed differently. White tea and green tea are non-fermented. Oolong tea is the semi-fermented. Black tea is fully-fermented. [3]

1.3 Oolong tea

Oolong is a semi-fermented tea which retains all of the nutrients and natural healing factors contained in unfermented green tea, but without the "raw" grassy

taste and harsh impact on the stomach that make green tea disagreeable to many people. [6] The very brief fermentation process eliminates harsh irritants from the raw tea and creates the subtle fragrances and flavours which distinguish this tea from all other varieties, without producing the tannins and other toxic compounds found in fully fermented black tea. [7] The cultivation and appreciation of Oolong is similar to wine, with each plantation and each mountain producing its own unique flavours, and each year's harvest yielding its own special character. [8]

1.4 Oolong Tea in New Zealand and New Zealand Oolong Tea Industry

In 1996, 1500 tea plants were imported from Taiwan, however only 130 made it past a thorough quarantine process by New Zealand customs officials. After 13 years of development, a New Zealand company (Zealong) operates a 50 hectare tea plantation on the outskirts of Hamilton which produces New Zealand's first Oolong tea for the international market. New Zealand Oolong Tea is the world's first commercial Oolong Tea Plantation outside of Asia. Keeping with the clean New Zealand environment, New Zealand Oolong Tea believes in producing pure oolong tea without the use of chemical sprays, fertilisers and additives and they operate to world leading ISO 22000 HACCP food safety standards. [8]

1.5 History of New Zealand Oolong Tea Research

To date no research has been performed on New Zealand Oolong tea. There are some special characteristics of New Zealand Oolong Tea. It is easy to reduce the acerbity, get the high aroma that different from tradition manufacture processing. And it is better to control the body weight than the other tea. Therefore I want to use HPLC to identify and measure key aromatics in the New Zealand Oolong Tea.

Chapter2: Literature Review

2.1 Tea Plant

The tea plant (Thea sinensis), classified by the famous Swedish botanist Linnaeus in 1753, is an evergreen plant of the Camellia genus and is also known as Camellia sinensis. [9]This is further sub–divided into two main subspecies. Camellia sinensis, the variety originally found in China and Camellia sinensis assamica, the plant growing as a native in India's north-eastern province of Assam. A third, subspecies Camellia sinensis lasio-calyx, the Cambodian variety, is not generally used for commercial production [9-11].The plant is an evergreen shrub or tree with sharply serrated thick and shiny leaves and produces delicate little flowers with five or seven white or creamy-white petals and bright sunshine-yellow stamens. The bitter-tasting fruit is the size of a hazelnut and contains one to three seeds [12].

2.2 Tea plants growth situation and process

The tea plant is cultivated in tropical and subtropical regions of varying climatic conditions: with a temperature range of 13–29°C altitude 2460 m above sea level, and acidic soil rich in iron and manganese with a pH range of 3.3–6.0, preferably 4.5–5.5 [13].

The plant's shallow root system lays only a metre or so below the surface so it is vulnerable to droughts and floods [14]. Soil that is too dry or drains too swiftly will cause the bush to quickly wilt and stop producing new leaf buds. Too much rain will drown the roots. Therefore, good drainage and suitable acid soil (clay, peat and sand are all good) are essential to successful cultivation. [13-14]

Today, rather than growing new bushes from seed; planters produce new stock by vegetative propagation from cuttings and cloned leaves taken from suitable ‘mother’ plants. Parent bushes are carefully selected for their ability to grow vigorously, have high yields, able to withstand the pests and diseases, resistant to drought or flooding and producing high-quality leaf. Tea Research Centres all over the tea-growing world have isolated higher-yielding plants that allow farmers to develop plantations more efficiently and to significantly raise profit margins. [13]

The young plants are carefully tended in a nursery for 6 to 20 months, depending on the climate, before being planted into the tea garden at a spacing of 1-1.5m^2. Planting follows the natural contours of the landscape and the land is sometimes specially terraced to help eliminate soil erosion. The new plants are left unplucked and unpruned for two years until they are roughly 1.5-2m high. [15-16]The plants are then regularly pruned to keep them just under a metre and are encouraged to spread sideways and fill across to neighbouring bushes. The surface created is called the ‘plucking table’ and new leaves produced after the plants have been growing for three years (at lower altitudes) or five years (high elevations) are ready for processing into tea. In regions where very hot, direct sunshine can affect the health and growth of plants, shade trees are planted. In regions where weather conditions are constant throughout the year (such as Kenya), the plant grows without rest but in regions with seasons (such as China and north-eastern India) there is a dormant period when bushes do not grow. [16]

Plucking occurs as the bushes ‘flush’ and pushes out new leaf shoots. In hot, steady climates, plants flush regularly while in cooler, seasonal regions picking occurs from early spring to late autumn. These teas develop slowly in the cooler spring sunshine and have a concentrated essence after the winter dormancy. The first flush in seasonal areas is considered to give the best, most fragrant leaves and therefore best teas (for example in Darjeeling). In other regions such as Assam,

the second flush wins prizes and fetches higher prices. [14-16]

2.3 Tea Processing

2.3.1 Introduction

Across the globe, more than 10,000 different teas are made from different varietals of Camellia sinensis. As with production of wine, the character, colour and flavour of each tea when it is brewed and served are determined by a long list of variable factors such as -location of the plantation, altitude, climate, seasonal changes, the soil, the minerals it contains, the way it drains, cultivation methods, plucking methods, how the leaf is processed, what happens to the leaf at the end of the manufacturing process and the way the tea is eventually brewed. [17]

Teas are classified by the process used to make them and, although the name of the different categories (white, green, oolong, and black) often tells us about the colour and appearance of the dry leaf, the manufacturing method that decides the category. Levels of caffeine (in the past also known as theine) vary in different teas and are thought to depend on the varietal of the bush, the age of the leaf when it is picked, its location on the stem, the length of oxidation time, the size of the tea leaves brewed, the quantity of leaf used to make the brew, and the length of the brewing time. New buds and young leaves have been found to contain higher levels of both caffeine and catechins. [15, 18]

White teas are originally named after the tiny white or silver hairs that cover the bud as it develops at the tip of each tea shoot. Originally it was only made in China from two varieties of the tea plant. Once the new buds and baby leaves have been carefully gathered, they are dried in the sun or in a warm, drying room. When brewed they give a very pale, champagne-coloured liquor that has a very light, soft, sweet, velvety flavour. [8, 19]

Green teas are generally described as ‘unoxidized’ teas and no chemical change occurs during manufacture. Processing differs from country to country but the basic manufacture sometimes involves a short period of withering to allow some of the water content in the leaf to evaporate, then steaming or pan-firing, to de-enzyme the leaf. [13, 18]

Oolong teas, known as partially or semi-oxidized (or partially or semi-fermented) and sometimes referred to as ‘blue’ or ‘blue-green’ teas, are traditionally manufactured in China, but other countries are now also producing them. [13]

Black teas are defined as ‘red teas’ because of the coppery-red because of the coppery-red colour of the liquor that they yield. For black tea, methods of manufacture and the varieties produced vary enormously from country to country and from region to region, but the process always involves four basic stages – withering, rolling, oxidation (also misleadingly referred to within the tea industry as ‘fermenting’) and firing (drying). [13]

2.3.2 New Zealand Oolong tea processing

2.3.2.1 Leaf picking

The raw materials for New Zealand Oolong tea should be the young tender leaves with soft texture, thick, and light green in color. The leaf should be banjhi leaves. Younger leaves produce brew liquor with stronger bitter and astringent taste and dull green color, whereas older leaf produces brew liquor with light taste and its leaf is yellowish green.

With over application of nitrogen fertilizer, the fresh leaf is dark green and has high moisture content. The final product has a darker color with lighter aroma.

Fresh leaf from different sources (such as different cultivars, morning-plucked leaf, afternoon-plucked leaf, or evening-plucked leaf) should be separated and processed separately in order to control quality.

When temperature in the environment is high, suitable quantity control of intake fresh leaf is critical in order to avoid reddening of fresh leaf due to high temperature and muggy environment, resulting in ‘dead’ leaf. [5]

2.3.2.2 Solar Wilting

The purpose of solar withering is to reduce the moisture content in cells of tea leaf by evapotranspiration of the water in the cells as accelerated by the solar heat. At the same time of reduced water activity, the cell membrane loses its semipermeability capability. The different components in the cells (particularly the catechins) are oxidized by its enzymes and proceed with its fermentation. [20]

The procedure for solar withering is to spread out the fresh leaf on cloth trays to conduct withering on the floor in the workplace, with loading density of 0.6–1.0 kgm^{-2}. Temperature on the tea leaf should be maintained at 30–40°C. When the solar temperature is above 40°C, the tea leaf should be protected with shading networks to avoid sunburn of the leaf, causing ‘dead’ leaf. During the withering process, depending on the rate of moisture evaporation from the fresh leaf, the leaf should be turned over 2–3 times to provide even withering. Withering time usually is 30-40 minutes. It can be extended to 1 hour when the sunlight is weak. This is dependent on the rate of water evapotranspiration.

The degree of solar withering is observed visually. When the luster of second leaf (or first leaf of banjhi leaves) disappears, the leaf will show a wavy appearance, giving a soft hand-feel. The greenish odor disappears; with appearance of pleasant (fragrant) tea aroma. This is considered proper solar withering. The fresh leaf loses 8–12% of its weight.

2.3.2.3 Indoor wilting

The purpose of indoor sitting and shaking is to continue the fermentation action induced by solar withering. The tea leaf continues to ferment and induce the complicated chemical reactions that form the characteristic taste and aroma of New Zealand Oolong tea. Shaking is conducted by lightly turning over the tea leaf with both hands so that the circumferences of the tea leaf can rub against each other and inconsequence the cells around the circumference will be damaged. By doing so, air can enter the cells easily and induce fermentation; at the same time, water can be evaporated evenly due to this turnover. [21]

After solar or hot air withering, tea leaf is moved into the indoor withering room and spread on bamboo basket with a loading density of 0.6-1.0kgm^{-2}. The tea leaf is left sitting for 1-2 hours. Due to loss of moisture; leaf circumference shows a slight wavy shape. The first shaking is then initiated with light motion for about 1-5min. The shaking activity is more vigorous with further shakings and increase in shaking time. Leaf thickness for subsequent spreading is increasing with longer sitting time. In general, tea leaf will be shaken 3-5 times, followed by 90-180 minutes sitting after each shaking. The last shaking usually is around midnight with sudden drop in temperature, thus the spreading should be thicker. During early spring or winter, the temperature is usually lower. Tea leaf, after shaking, is usually piled up to 60 cm or higher in bamboo cages for the sitting period. This procedure will increase the leaf temperature and thus the fermentation reaction with production of characteristic taste and aroma. The sitting after the last shaking is 120-240 minutes with disappearance of the greenish odor and production of pleasant tea aroma. The leaf is then ready for panning.

The first and second shaking should be light, just turning over the tea leaf. If the shaking is too severe at the beginning, the leaf can be easily damaged, causing dull, dark appearance with bitter taste for the brew liquor. If the shaking is

inadequate, the aroma of New Zealand Oolong tea is not strong enough, with off-greenish odor in extreme cases. Therefore, indoor withering time and number of shakings should be appropriately adjusted depending on the tea cultivar, nature of the green tealeaf, season, and weather condition.

2.3.2.4 Fixation

The purpose of fixation (panning) is to inactivate the enzymes by high temperature, inhibiting further fermentation and guaranteeing the characteristic taste and aroma of New Zealand Oolong tea. Tea leaf loses large amount of moisture during panning and thus softens, making the rolling into string shapes and dehydration easier. [20]

Temperature of the rotary pan surface should be 160–180°C, or 380°C on the temperature indicator of the fixation machine. At the beginning of fixation, there is a ‘‘paat-paat’’ sound. The fixation period is 700rpm. At the end of the fixation process, tea leaf has no greenish odor; leaf texture is soft and pliable to hand-holding with a strong pleasant (fragrant) aroma. It is critical not to over fixation New Zealand Oolong tea: this result in prickly leaf circumference or burnt odor. Tea leaf unloaded too early from the pan will be under fixated and have a greenish odor and red central vein. Dried leaves are then left to rest overnight.

2.3.2.5 Rolling and shaping

The purpose of rolling and shaping is to produce its characteristic flavor and appearance through a special mass rolling process. During mass rolling, control of repanning temperature, rerolling pressure, and moisture content of tea leaf has significant effects on the appearance and flavor of this semispherical-type New Zealand Oolong tea. [20, 22]

Next day, the leaves are wrapped inside large cloths to form 9kg balls of tea. Each bag is tightened and then rolled in a special rolling machine to bruise and squeeze

the leaves. The bag is then opened and the compacted leaf is separated and immediately wrapped into a ball again. Trying and rolling the bags is repeated at least 36 times and sometimes up to 60 times until the leaves are tightly rolled up into rough green pellets.

2.3.2.6 Baking and drying

The purpose of drying is to inactivate the residual enzyme activities, terminate further fermentation, and at the same time stabilize tea leaf quality. [20] Drying is conducted by a tea dryer. This procedure will improve the aroma and taste of tea leaf, remove the greenish off-odor, reduce the astringency, and give the brew liquor a clear and beautiful color. The moisture content in tea leaf is reduced to 4%. Both volume and weight are reduced. This is convenient for packaging, storage, and transportation.In this stage the semi-balled tea is finally dried in large ovens operating at approximately 100°C to stop any remaining fermentation active and fix the tea quality. Water content at this stage is less than 4%. (The drying machine determined and display)

2.4 Brewing tea

The first important decision when choosing a tea is whether to buy teabags or loose-leaf. Many people prefer teabags because they are easier to handle, convenient and quick to use. The tea is already measured, the bags are easily removed a form the brew once the correct strength has been achieved, and there is not the problem of how to dispose of wet leaves. Some teabags are extremely disappointing because they contain a small amount and have poor quality of leaf. However, many companies offer very good teabags containing high quality leaf. The latest trend is for bags made of nylon gauze (often called ‘crystal’) or muslin. These bags usually contain larger grades of leaf and allow the tea enough room to swell and release its flavour and colour more successfully into the water [8]

The water used for brewing plays an enormously important role in the final flavour, clarity and colour of liquor. A tea brewed in particular water may taste dull and flat but wonderfully brisk and bright when brewed in water from a different location. All the ingredients in the water play their part in the brewing process-the natural minerals, added chemicals such as chlorine and fluoride, the amount of oxygen, etc. [16, 17]

To remove unwanted soluble compounds, mains water needs to be filtered. Some filtration systems add various salts and minerals to the water, which can create different problems. The most effective filters use ‘reverse osmosis’ where the water is passed through a very fine membrane unwanted chemicals and other deposits are removed, producing water that is approximately 99.4 per cent pure. [16]

Lu Yu (A.D. 780) recommended spring water as the best for tea because of its purity, freshness and high oxygen content. Water that has stood for any time has become lifeless and flat and is not suitable for tea. [22]

Hard water, which has a high calcium or magnesium content, is poor for most types of tea; it deadens the flavour and causes a scum to form on the surface in the cup when calcium carbonate reacts with oxygen to become calcium bicarbonate. Adding acid to the tea helps eliminate the bicarbonate ions, so adding a drop or two of lemon juice prevents scum being formed. Adding sugar to tea also reduces scum development but most teas are better without sugar. [16]

If soft water and permanently hard water (that contains calcium sulphate) are used for tea, the resultant liquor is usually bright and clear and the flavour brisk and lively. A PH of 7 is ideal if bottled water is used. However, many bottled water contain salts and other minerals that can spoil the flavour of tea and must be

selected carefully [8, 16].

2.5 Composition in the tealeaves and manufactured tea

The chemical composition of tea leaves varies greatly depending on origin, age and processing. In fermented teas 38-41%of the dry matter is soluble in hot water. This is significantly more than for roasted coffee. Table 1 provides data on the constituents of fresh and fermented tea leaves. [23]

Table 2. 1: Composition (%, dry weight) of fresh and fermented tea leaves and of tea brews

Constituent	Fresh flush	Black tea	Black tea brew[a]
Phenolic compounds[b]	30	5	4.5
Oxidized phenolic compounds[c]	0	25	15
Protein	15	15	+[d]
Amino acids	4	4	3.5
Caffeine	4	4	3.2
Crude fiber	26	26	0
Other carbohydrates	7	7	4
Lipids	7	7	+
Pigments[e]	2	2	+
Volatile compounds	0.1	0.1	0.1
Minerals	5	5	4.5

[a] Brewing time 3 min. [b] Mostly flavanols. [c] Mostly thearubigins. [d] Traces. [e] Chlorophyll and carotenoids. [23]

2.5.1 Phenolic compounds

Polyphenols make up 25-35% of the dry matter content of young, fresh tea leaves and 80% of the polyphenols are flavanol compounds. The rest are proanthocyanidins, phenolic acids, flavonols and flavones (Table 2). During fermentation, flavanols are oxidized enzymatically to compounds that give the colour and flavour to black tea. The reddish-yellow colour of black tea extract is largely due to theaflavins and thearubigins, while flavour intensity is correlated with the total content of phenolic compounds and polyphenol oxidase activity. [24]

The enzymes are inactivated in green tea, which stops flavanol oxidation. The greenish or yellowish colour of green tea is due to flavonols and flavones. Thus, tea processed into green or black tea is chemically readily distinguishable mainly by the composition of the phenolic compounds. [23, 24]

Table 2. 2: Phenolic compounds in fresh tea leaves (% dry matter)

Compound	Content	Compound	Content
(-)-Epicatechin	1-3	Flavonols and flavonolglycosides	+
(-)-Epicatechingallate	3-6	(quercetin,kaempherol,etc.)	
(-)-Epicatechindigallate	+[a]	Flavones	+
(-)-Epigallocatechin	3-6	(vitexin,etc.)	
(-)-Epigallocatechingallate	9-13	Leucoanthocyanins	2-3
(-)-Epigallocatechindigallate	+	Phenolic acids and esters	˜5
(+)-Catechin	1-2	(gallic acids, chlorogenic acids)	
(+)-Callocatechin	3-4	p-Coumaroylquinicacid, theogallin	
Phenols, grand total	25-35		

[a] Quantitative data are not available. + Inhibited [24]

2.5.2 Catechins in the Oolong tea

Catechins may constitute up to 30% of the dry mass of tea leave. They consist of six major groups that the figure 1 shows; epicatechin (EC), epicatechingallate (ECG), epigallocatechin (EGC), catechin (C), gallocatechingallate (GCG), and epigallocatechingallate (EGCG). These catechins along with caffeine and several amino acids including theanine are important in tea quality and taste. Catechins are known for their pharmacological properties including antioxidant activity, anticancer, anti-hypertension, anti-vascular diseases, and anti-inflammatory action.[25]

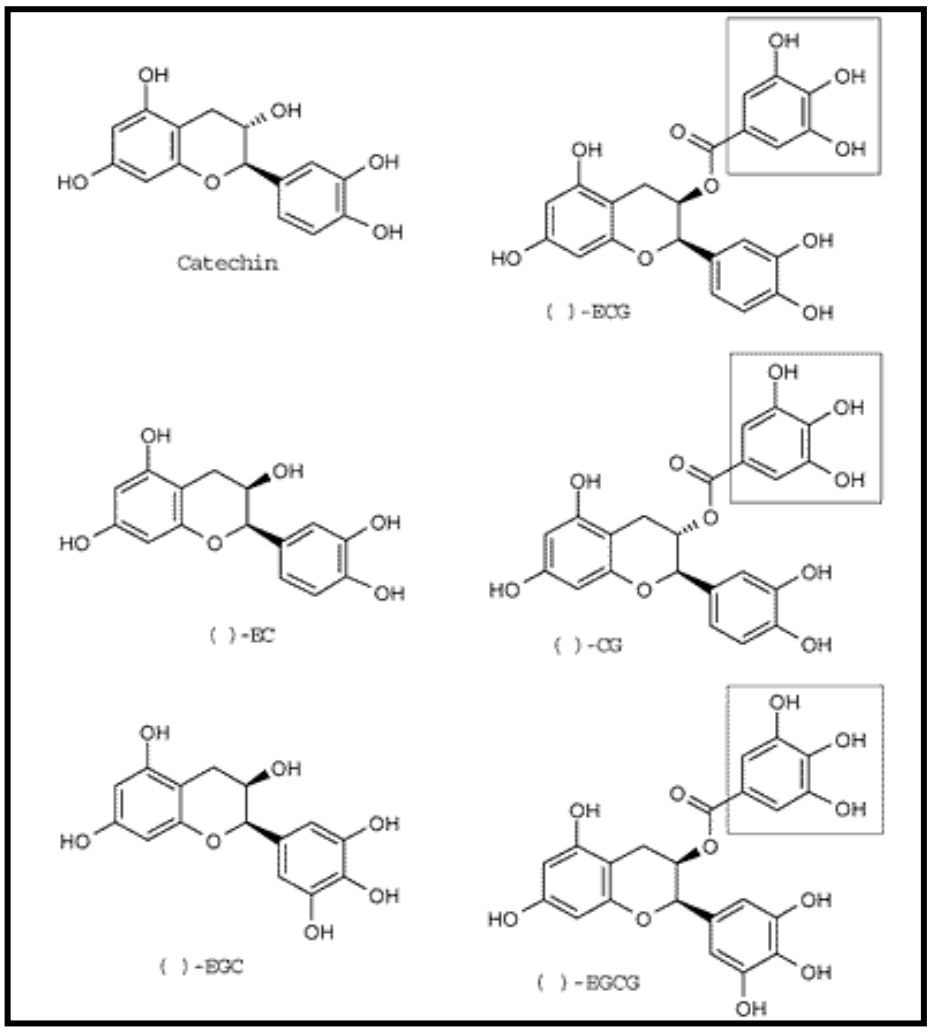

Figure2. 1: Chemical structure of catechins [25]

2.5.3 EGCG in Oolong Tea

Excess body weight is a major health problem in most developed nations and is increasing in both prevalence and severity [26]. It has been known for some time that EGCG helps to control obesity, and this is a common belief in the Chinese society, in which obese people are seldom found in long-term tea-drinking individuals [27]. Moreover, it is generally accepted that drinking tea for a long time will keep one living long to stay in good health generally.

Based on biochemical and pharmacological studies, the mechanisms of action of EGCG in preventing obesity may be through stimulating hepatic lipid metabolism [28] inhibiting gastric and pancreatic lipases [29] stimulating thermogenesis [30] [31] modulatingappetite,10 promoting synergism with caffeine and theanine [32], and finally suppressing fatty acid synthase.

Green tea has been found to possess a high antioxidant activity owing to its high content of EGCG. Processes used in the manufacture of oolong tea are known to decrease the levels of monogenic catechins to a much greater extent of polymerization that leads to the formation of theaflavins and thearubigins [33]. This fermentation process converts catechins to theaflavins, mainly theaflavin (TF-1), theaflavin-3-gallate(TF-2a), theaflavin-3'-gallate (TF-2b), and theaflavin-3,3'-digallate (TF-3), and thearubigins, consequently decreasing the catechin content [33].

It has been demonstrated that the body weight of rats and their plasma triacylglycerol, cholesterol, and LDL cholesterolare significantly reduced by feeding of oolong, black, pu-erh, and green tealeaves to the animals. The results have shown that the suppression of bodyweight of tea leaves–fed groups is in the order of: oolong tea >pu-erh tea > black tea > green tea [34]. Oolong tea could lower the levels of triacylglycerol more significantly than green tea and black tea.

It seemed that the fermented teas, including oolong, black, and pu-erh teas, are more effective than unfermented green tea in suppressing the body weight and lipogenesis in rats. It has been suggested that the inhibition of growth and suppression of lipogenesis may occur through down regulation of fatty acid synthase gene expression in the nucleus and stimulation of cell energy expenditure in the mitochondria. The experimental data indicate that the molecular mechanisms of fatty acid synthase gene suppression by tea polyphenols (EGCG, theaflavins) may be rendered through signal transduction pathways. The possible association of hypolipidemic and antiobesity effects of tea polyphenols with the expression of the uncoupling factor UCP-1, UCP-2, and UCP-3 genes in adipose tissues deserves further studies [35]. Therefore, according to my literature review, that is an incentive to control the level of EGCG in New Zealand Oolong to maxima the flavour and health benefits.Table2.3 show the EGCE content of several different kinds of oolong tea.

Table 2. 3: The EGCG content of several kinds of oolong tea (dry base)

	EGCG content (mg/g)
Pu-erh	1.99
Fujian Oolong	22.2
DaHongPao	5.71

2.6 HPLC Analysis

2.6.1 HPLC Description

High-performance liquid chromatography (sometimes referred to as high-pressure liquid chromatography), HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. [36]

HPLC typically utilizes different types of stationary phases such as a conventional C_{18} or C_8, a pump that moves the mobile phase(s) and analyte through the column, and a detector to provide a characteristic retention time for the analyte. The detector may also provide additional information related to theanalyte, (i.e. UV/Vis spectroscopic data for analyte if so equipped). Analyte retention time varies depending on the strength of its interactions with the stationary phase, the ratio/composition of solvent(s) used, and the flow rate of the mobile phase. It is a form of liquid chromatography that utilizes smaller column size, smaller media inside the column, and higher mobile phase pressures.[36, 37]

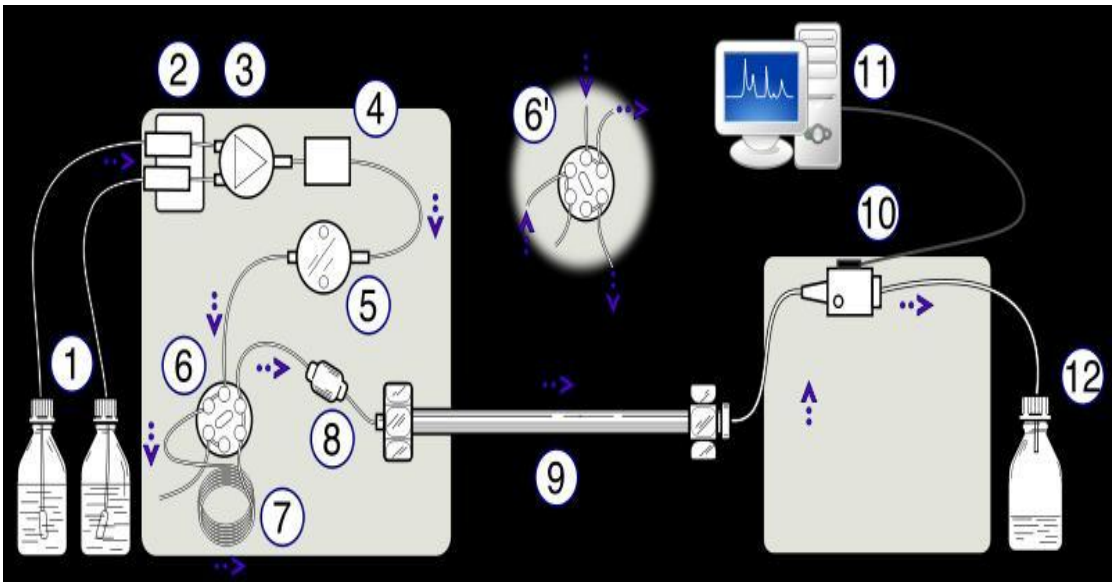

Figure 2. 2: HPLC apparatus (1) Solvent reservoirs, (2) Solvent degasser, (3) Gradient valve, (4) Mixing vessel for delivery of the mobile phase, (5) High-pressure pump, (6) Switching valve in "inject position", (6') Switching valve in "load position", (7) Sample injection loop, (8) Pre-column (guard column), (9) Analytical column, (10) Detector (i.e.IR, UV), (11) Data acquisition, (12) Waste or fraction collector. [37]

With HPLC, a pump (rather than gravity) provides the higher pressure required to move the mobile phase and analyte through the densely packed column. The increased density arises from smaller particle sizes. This allows for a better

separation on columns of shorter length when compared to ordinary column chromatography. [37, 38]

2.6.2 Identification of flavour/aroma compounds using HPLC

The flavour of New Zealand Oolong Tea comes from the phenolic compounds that are the resultant which the EGCG of New Zealand Oolong Tea have been fomented. The concentration of EGCG of the fresh tea leaves and finished tea can be identified by reversed phase HPLC (RP-HPLC or RPC) that has a non-polar stationary phase and an aqueous, moderately polar mobile phase. One common stationary phase is silica which has been treated with RMe_2SiCl, where R is a straight chain alkyl group $C_{18}H_{37}$ or C_8H_{17}. With these stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily. [38, 39]

RPC operates on the principle of hydrophobic forces, which originate from the high symmetry in the dipolar water structure and play the most important role in all processes. RPC allows the measurement of these interactive forces. The binding of the analyte to the stationary phase is proportional to the contact surface area around the non-polar segment of the analyte molecule upon association with the ligand in the aqueous eluent. [38] This solvophobic effect is dominated by the force of water for "cavity-reduction" around the analyte and the C_{18}-chain versus the complex of both. The energy released in this process is proportional to the surface tension of the eluent and to the hydrophobic surface of the analyte and the ligand respectively. The retention can be decreased by adding a less polar solvent (methanol) into the mobile phase to reduce the surface tension of water. Gradient elution uses this effect by automatically reducing the polarity and the surface tension of the aqueous mobile phase during the course of the analysis. Therefore, the UV lamp can give the good peak separation that I can get the concentration of EGCG. [39]

2.6.3 Previous studies using HPLC to measure EGCG

The HPLC technique was developed to analyse black and green tea constituents in the 1970s and showed good separations of phenolic compounds in tea. [40]

However, the quantitative reproducibility of HPLC analysis was still very poor until the late 1980s before photodiode array(PDA) detection was incorporated into the HPLC system for the study of thearubigins in a model in vitro fermentation system [41-44].The latter improved HPLC analysis has since contributed effective separation and identification of the black tea pigments from the chemical oxidation of green tea polyphenols, epigallocatechin gallate (EGCG) and epicatechingallate (ECG) [45-49]. Recent studies showed that a similar system of HPLC analysis can be used to determine (simultaneously), catechins (including their gallates), caffeine and gallic acids in green, oolong, black and pu-erh teas. [49-51]

2.7 Summary

The literature review has indentified that:

- Oolong tea grow in NZ was unique characteristics.
- The EGCG found in tea has desirable sensory and health benefits.
- HPLC technology can be used to measure the amount of catechins in tea leaves.
- Desirable to control EGCG content.

Therefore the aim of this work is to

- Use HPLC to determine the content of EGCG in fresh tea leaves of New Zealand Oolong tea.
- The rate of oxidation of EGCG as a result of the tea processing stages.
- Develop guidelines to optimise early processing stages of New Zealand Oolong tea to maximize EGCG content.

Chapter 3: Materials and Methods

3.1 Plant materials

The tea samples (shoots), consisting of one apical bud and two adjoining leaves, were hand-plucked from the fields of Zealong tea farm at Hamilton, New Zealand, during harvest seasons from October 2010 to March 2001 (tea is harvested half-yearly in New Zealand).

Figure 3. 1: The fresh tea leaves that is a bud and three leaves

3.2 Equipment

3.2.1 HPLC System

A ÄKTA™ Purifier HPLC system was used which consists of a computer-controlled system with upgraded UNICORN™ software and an SCL-10A VP System controller. Other accessories were a an m-925 Mixer, two P-903 Liquid Chromatography Pumps, an PU908 Auto Injector, a CTO-10A Column Oven, and an UV-900 Detector. Supelco Analytical C_{18} reversed-phase packing column (4.5 mm×25 cm, 5 µm) was used for separation throughout this study. Globe science.26mm Minisart Syringe Filters (0.2um Pore Size with Luer Lock Outlet) were used to filter the tea leaves extract.

Figure 3. 2: The HPLC system

3.2.2. Degas System

The degas system was made up with a sonic bath and a vacuum machine as shown in Figure 3.3.

Figure 3. 3: The degas system

3.3 Chemicals

EGCG reference samples were purchased from Sigma Chemical Co. New Zealand (Figure 3.4).The solvents used for the mobile phase were methanol (MeOH), ethanol (EHOH) and ortho-phosphoric acid, and were of HPLC grade reagent from Pharmco. Products INC. All solution preparations were made using distilled and then deionized water from Millipore Milli-Q purification system. All the other chemicals were of analytical reagent grade and were used without further purification.

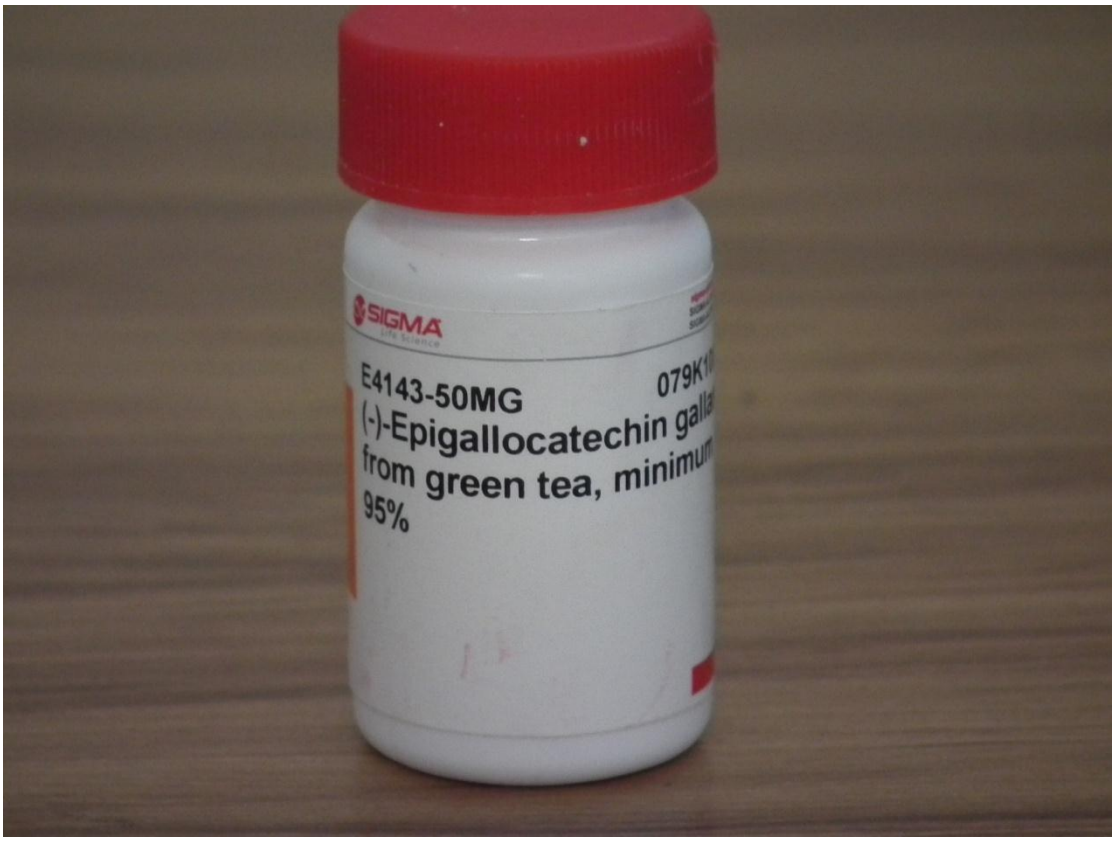

Figure 3. 4: The HPLC system

3.4 Determination of Rate of Evaporation at each processing

The trail was based on the tea manufacture processing which included the flowing six processing: harvesting, solar wilting, indoor wilting, fixation, rolling, and drying. Every trail had different starting time, temperature and moisture content, but they are general consistent. In the last two processing the all trail's teas were mixed to manufacture.

3.4.1 First processing-Harvesting of Raw Materials

The raw materials were selected by hand before two o'clock, as show in Figure 3.5. At that time the environment temperature was 15-27 °C that is suitable quality control of intake fresh leaf. This avoided reddening of fresh leaf due to high temperature and muggy environment, resulting in ‘‘dead’’ leaf.

Figure 3. 5: The raw materials were selected by hand

3.4.2 Second processing-Solar Withering

The loading density was 0.8kgm^{-2}. Temperature on the tea leaf was maintained at 30–40°C. During the withering process, the leaf had been turned over two times to provide even withering. Withering time was one hour. The luster of second leaf shown in Figure3.1 had disappeared, the leaf showed a wavy appearance, giving a soft hand feeling.

Figure 3. 6: The solar withering in factory

3.4.3 Third processing-Indoor wilting

The loading density was 0.8kgm^{-2} (Figure 3.7). The tea leaf was left sitting for 1 hour. The first shaking was then initiated with light motion for about 5 minutes. The Subsequent shakings were more vigorous and shaking times were increased. Tea leaf had been shaken 4 times, followed by one and 2 hours sitting after each shaking. It was to ensure that wilting was as uniform over the leaf surface as possible.

The final shaking was around midnight with sudden drop in temperature, thus the spreading was thicker. Tea leaf, after shaking, was piled up to 60 cm or higher in bamboo cages for the sitting period. This procedure increased the leaf temperature and thus the fermentation reaction with production of characteristic taste and aroma. The sitting after the last shaking was 5 hours with disappearance of the greenish odor and production of pleasant tea aroma.

Figure 3. 7: In door wilting on bamboo basket

3.4.4 Fourth processing-Fixation.

Fixation was conducted using fixation machine (Figure 3.8). Temperature of the rotary pan surface was 160–180°C. The fixation machine revolving speed was 700rpm.

Figure 3. 8: The fixation machine

3.4.5 Fifth processing-Rolling.

After fixation the tea leaves were rolled into balls in the rolling machine (Figure 3.9). After being unloaded from the rolling machine, the tea leaf was turned over by hand 3 times to release the hot vapor in tea mass, followed by loading into the drum of the rolling machine (roller) to proceed with the rolling process. Tying and rolling the bags was repeated 49 times until the leaves are tightly rolled up into rough green pellets. The tying and rolling times were so much, because the pressure was not too heavy to damage the leaves or make them twine.

Figure 3. 9: Rolling machine

3.4.6 Sixth processing-Drying

Drying was conducted by a chain-belt–type tea dryer. Incoming hot air at 100°C was introduced to dry the tea leaf for 30 minutes. In this stage the semi-balled tea was finally dried for 1 hour in large ovens operating at 100°C (Figure 3.10).

Figure 3. 10: Drying machine

3.5 The tea samples mass determination and stored

In every processing of the trail, five samples of which included twenty leaves were selected. The samples masses and average masses were measured at each trail, in order that the rates of evaporation (*R*) might be determined for each processing of the trail.

The rate of evaporation was expressed as:

$$R_{i,j} = \frac{(M_{i,j} - M_{i,j-1})}{M_{i,1}} \qquad (3.1)$$

Where: *M* was the average mass of the sample, *i* was the trail index (i.e. A. B. C. or D) and *j* was the six processing index (i.e. 1, 2, 3, 4, 5, or 6)

Finally the samples were wrapped in washed calico and packed in dry ice in polystyrene foam boxes. All samples were then delivered with dry ice to the laboratory at University of Waikato. After arrival, all the samples were stored in a freezer at -23°C prior to HPLC analysis. This protocol was developed in an endeavour to prevent oxidation of tea polyphenols by the enzymes present in the fresh shoots.

3.6 Sensory evaluation by trained tea tasters

Three grams of tea samples that include New Zealand Oolong Tea (dry matter, DM) were infused with 150 ml freshly boiled water for 5 minutes. The sensory quality of the tea was estimated and scored by professional tea tasters from the South China Botanical Garden of the Chinese Academy of Sciences. This is a ‘blind’ taste test that the samples were all presented in a random order in un-marked cup. The grading system was based on a quality score (QS) of 100, of

which 10% was awarded for the tea appearance score, 40% for the tea aroma score, 40% for the tea taste score (TS) and 10% for the infused leaf score (Table 4.24). This grading system is commonly used to evaluate the Oolong tea quality in China. [15]

3.7 Solvent extraction

The dried tea leaves that pass the third stage (1g) were mixed with milli-Q water (100ml,100°C) and was conducted by shaking for 10min in 100 °C water bath. The extract was filtered (pore diameter: 11_m, Whatman, UK, 26mm Minisart Syringe Filters) and diluted with Milli-Q water to 100mL. Approximately 1ml sample solution was made up to a final volume of 10 ml with Milli-Q water.

For fresh tea shoots that didn't pass the third stage, blending for 5 min with methanol at a ratio of 1:18 (w/v) maximised the extraction of EGCG. [31] Approximately 5ml solution was filtered and 100µl sample was made up to a final volume of 10ml with Milli-Q water. Immediate analysis by HPLC of the methanol extract showed higher separation efficiency. This method showed a good repeatability and recovery rate.

3.8 Standard solution

Stock solution, approximately 2 mg of EGCG reference standard were accurately weighed into a 25-ml volumetric flask, dissolved in water by sonication for 1 min, and made to volume with Milli-Q water. Working standard solutions were prepared as 1-64 fold dilution of the stock solution with water prior to HPLC analysis. All buffer solutions were passed through 0.2µm filter.

3.9 HPLC Analysis

3.9.1 The mobile phase

Mobile phases consisted of 0.1% ortho-phosphoric acid in water (v/v) (eluent A) and 0.1% ortho-phosphoric acid in methanol (v/v) (eluent B). The gradient was as follows: 0–5 min, 20% B; 5–7 min, linear gradient from 20 to 24% B; 7–10 min, 24% B; 10–20 min, linear gradient from 24 to 40% B. Post-run time was 5 min. Elution was performed at a solvent flow rate of 0.5 ml/min. Detection was accomplished with a diode array detector and chromatograms were recorded at 210 nm. The column was maintained at 30°C. The sample injection volume was 20µl. Peaks were identified by comparing their retention times and UV spectra in the 200–400 nm range with authentic standards and by checking the purity of the peaks. And the flowing peaks and areas were performed by the software.

3.9.2 Standard solution analysis

The concentrations of the standard EGCG solution were 0.00125(mg/l), 0.0025(mg/l), 0.04(mg/l), and 0.08(mg/l). The standard solutions were passed through the HPLC system and the peaks were obtained.

3.9.3 Tea samples analysis

The tea samples solutions were prepared as described in section 3.4. Then the samples solutions were passed through the HPLC system and the peaks were obtained.

Chapter 4: Results and Analysis

4.1 Analysis of HPLC trials

The samples of fresh tea leaves were extracted by methanol. [43] The solution was then diluted to 25 ml with methanol. But in initial trials, the peak separation was not satisfactory. Therefore, 100µl samples that had been filtered and were made up to a final volume of 10ml with Milli-Q water were used. Immediate analysis by HPLC of the methanol extraction showed higher separation efficiency.

Two mobile phases, one containing acetonitrile /0.1% ortho-phosphoric acid (w/v) in water and the other containing methanol /0.1% ortho-phosphoric acid (w/v) in water were tested. These mobile phases were used for separating catechins from the tea mixtures. The latter phase gave a complete separation of EGCG. Similar separation profile was obtained by employing methanol/0.1% ortho-phosphoric acid in water as mobile phase for separating tea catechins but as they employed isocratic separation, the run time was 2 hours. With this gradient program, complete separation of tea catechins was achieved in 90 minutes. Since acetonitrile is highly toxic, the mobile phase that was used in subsequent runs was methanol /0.1% ortho-phosphoric acid (w/v) in water and the gradient program was used.

Initially helium was used to degas the mobile phase; however, the efficiency was not sufficient. There were lots of bubbles in the mobile phase, the base line was not good and had some noise. Subsequently a vacuum machine was used to degas the mobile phase; however, results were still not satisfactory. The third time a sonic bath was used to degas the mobile phase, until the bubbles disappeared. But the result was not good either. Finally, both sonic bath and the vacuum machine were used to degas the mobile phase, which produced satisfactory results.

In this study, the tea leaves samples were stored in freezer at -23°C. The samples that went through the fixation stage were convenient to analyse. Some of other fresh tea leaves that had not been through the fixation stage showed evidence of significant oxidation, and were not satisfactory. Therefore, it is recommended that the tea leaves samples that didn't go through the fixation stage must be stored in freezer at a lower temperature..

If the HPLC machine can stand the more pressure the flow rate should be 1ml/s. Therefore, the trails time should be shortened and the separation efficiency should be better.

4.2 The sensory quality score of different oolong tea

The sensory quality score of the tea samples are summarised in Table 4.1. The New Zealand Oolong Tea had highest quality score in four different oolong teas. This confirmed that the special processing was suitable for the New Zealand Oolong tea manufacture. This was consistent with the workers experience and the objective of the manufacturing process.

Table 4. 1: Sensory quality score of the tea samples assessed by the tea tasters (n-3, mean±S.D.[2])

Samples	Appearance score	Aroma score	Taste score	Infused leaf score	QS
Fujian	8.2±0.2	31.8±0.8	31.2±0.3	8.3±0.1	79.5±0.2
Jinjunmei	7.1±0.1	27.0±0.5	27.7±0.8	7.1±0.1	68.8±0.2
Zealong tea	7.9±0.1	32.5±0.5	32.1±0.7	8.2±0.1	80.7±0.2
Dahongpao	7.3±0.2	31.3±0.6	29.4±1.9	7.5±0.1	75.5±0.2

4.3 The mass of samples in every process and stage

4.3.1 Sample masses at harvest (First process)

The results from the first process which started at the data and time are presented in Table 4.2.

Table 4. 2: The samples mass (g) of every stage in the harvesting process

						Average ($M_{i.1}$)
Trail A (Time: 10:00AM, Temperature: 30°C, Humidity: 56%)	19.9	20.54	19.32	17.45	16.96	18.834
Trail B (Time: 11:15AM, Temperature: 25°C, Humidity: 44%)	16.96	19.12	17.83	18.68	16.34	17.786
Trail C (Time: 12:45PM, Temperature: 36°C, Humidity: 34%)	17.02	15.74	16.64	16.7	17.66	16.752
Trail D (Time: 1:30PM, Temperature: 31°C, Humidity: 33%)	16.68	16.48	16.24	18.34	23.22	18.192

4.3.2 Sample after solar wilting (Second process)

The results from the No.2 process which started at the data and time are presented in Table 4.3.

Table 4. 3: The samples mass (g) of every stage in solar wilting process

						Average ($M_{i.2}$)
Trail A (Time: 11:00AM, Temperature: 28°C, Humidity: 43%)	15.6	12.66	13.66	15.36	14.28	14.312
Trail B (Time: 12:15AM, Temperature: 27°C, Humidity: 33%)	15.91	15.26	17.97	18.11	17.91	17.032
Trail C (Time: 1:45PM, Temperature: 31°C, Humidity: 26%)	14.84	16.3	14.56	13.83	13.61	14.628
Trail D (Time: 2:30PM, Temperature: 20°C, Humidity: 24%)	16.65	16.29	17	15.35	18.23	16.704

4.3.3 Sample masses after indoor withering (Third process)

The results from the No.3 process which started at the data and time are presented in Table 4.4.

Table 4. 4 :The samples mass (g) of every stage in indoor withering.

						Average ($M_{i.3}$)
Trail A (Time: 1:00AM, Temperature: 5°C, Humidity: 40%)	13.55	12.6	11.5	13.93	11.92	12.7
Trail B (Time: 2:15AM, Temperature: 5°C, Humidity: 40%)	15.1	14.05	12.2	14.08	14.07	13.9
Trail C (Time: 3:45AM, Temperature: 10°C, Humidity: 40%)	14.29	13.59	14.24	14.62	14.25	14.198
Trail D (Time: 4:30AM, Temperature: 9°C, Humidity: 40%)	11.49	14.19	15.08	15.1	13.98	13.968

4.3.4 Sample masses after fixation (Fourth process)

The results from the No.4 process which started at the data and time are presented in Table 4.5.

Table 4. 5: The samples mass (g) of every stage in the fixation process

						Average ($M_{i.4}$)
Trail A (Time: 1:20AM, Temperature: 5°C, Humidity: 40%)	8.05	7.93	9.53	7.92	5.75	7.836
Trail B (Time: 2:35AM, Temperature: 5°C, Humidity: 40%)	7.89	7.06	6.03	8.09	7.32	7.278
Trail C (Time: 4:05AM, Temperature: 10°C, Humidity: 40%)	9.87	11.97	8.25	7.94	8.68	9.342
Trail D (Time: 4:50AM,Temperature: 9°C, Humidity: 40%)	9.05	8.85	9.12	9.05	9.33	9.08

4.3.5 The mass of samples after rolling (Fifth process)

The results from the fifth stage are presented in Table 4.6.

Table 4. 6: The samples mass (g) of every stage in the rolling process

						Average ($M_{i.5}$)
All trail mixture (Time: 7:40AM, Temperature: 15°C, Humidity: 48%)	3.75	3.47	3.68	3.76	3.47	3.63

4.3.6 The mass of samples after drying (Sixth process)

The results from the sixth stage are presented in Table 4.7

Table 4. 7: The samples mass (g) of every stage in the drying process

						Average ($M_{i.6}$)
All trail mixture (Time: 6:40PM, Temperature: 23°C, Humidity: 34%)	2.4	2.35	2.65	2.98	2.44	2.56

4.4 The rate of water evaporation at each processing of trail

Water evaporation occurred at each stage of the New Zealand Oolong tea manufacture processing. The rate of water evaporation was calculated from the changes of samples mass in every process and stage that from the data that shown in Table 4.2 to 4.7. Using Equation 3.1, the rates of water evaporation of every stage are summarized in table 4.8.

Table 4. 8: The rate of water evaporation of every stage in each processing (%)

	A stage	B stage	C stage	D stage	Average
Second process	24	2.4	13	8.2	12
Third process	8.6	8.1	2.6	13	8.1
Fourth process	26	37	29	27	30
Fifth process	22	21	34	30	27
Sixth process	5.7	6	6.4	5.9	6

The water evaporation is a very important change. It was closely connection with the EGCG fermentation. Shown in Table 4.8, the fixation process had the highest rate of water evaporation that was 30%. It is probable that the fixation deactivated most of the enzymes and controlled the activity of the remainder, and also reduced the oxidation rates due to the high temperature and the high rate of water evaporation. After this stage the content of EGCG maintain 112mg/g(Section 4.6).In the first stage the rate of water evaporation is 11.9%, and the content of EGCG hadn't significant change, because the membrane of cell is not been destroyed.

The rate of water evaporation in indoor wilting stage was lowest. This ensured that the fermentation process was well going. The content of EGCG was reduced from 124mg/g to 112mg/g (Section 4.6). Therefore, in this stage the moisture and temperature control was very important. The rate of water evaporation of the whole processing was about 83%. The common level of water evaporation is between 80%-86%.

4.5 The HPLC results of EGCG standard solution (calibration)

The HPLC graphs came from the software of the HPLC system. The peaks' areas and heights were related to the concentration of the detection solution. 'mAU' was the Absorbance unit.

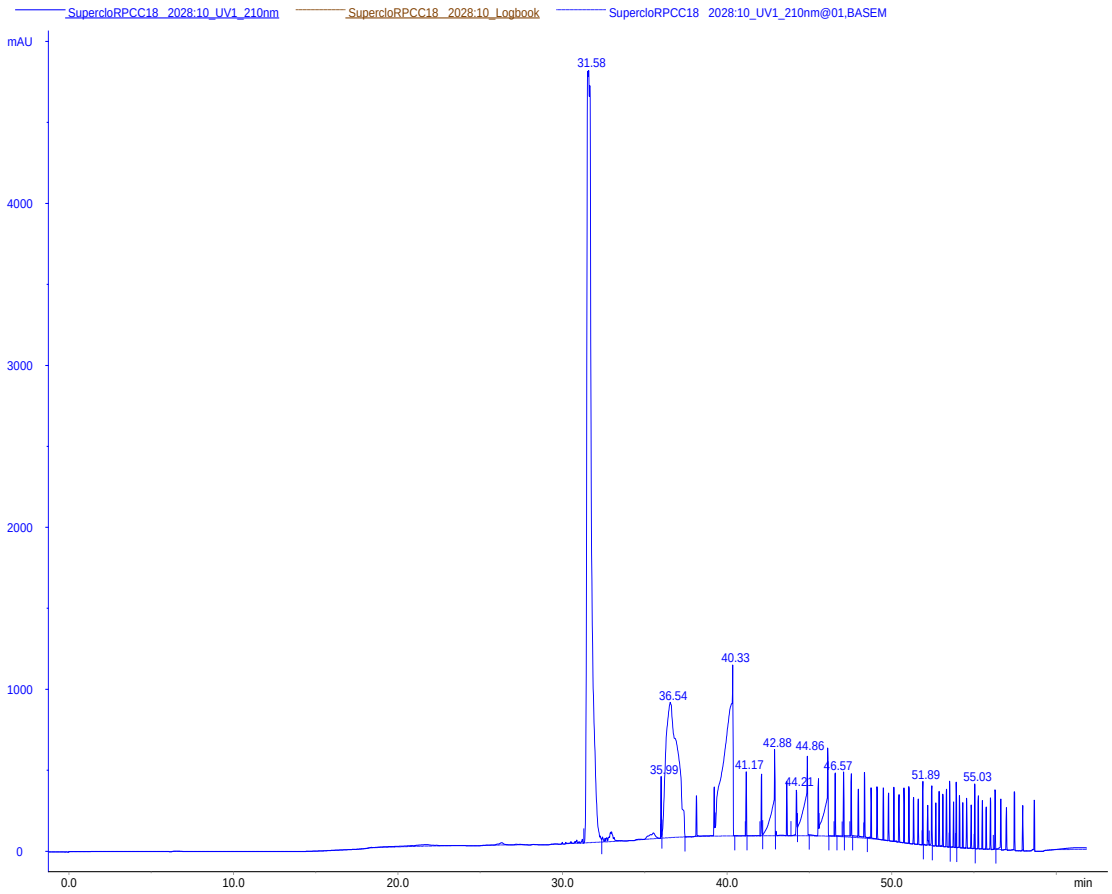

Figure 4. 1: The peak of EGCG standard solution (0.08mg/l)

Table 4. 9: The Retention, area and height of EGCG standard solution peak (0.08mg/l)

Retention (min)	Area (mAU*min)	Height (mAU)
31.58	1311.9625	4634.952

As shown in Table 4.9, the peak was appearing at 31.58 min for the 0.08 mg/l solution. The peak area is 1311.9625mAU*min and the height was 4634.952mAU.

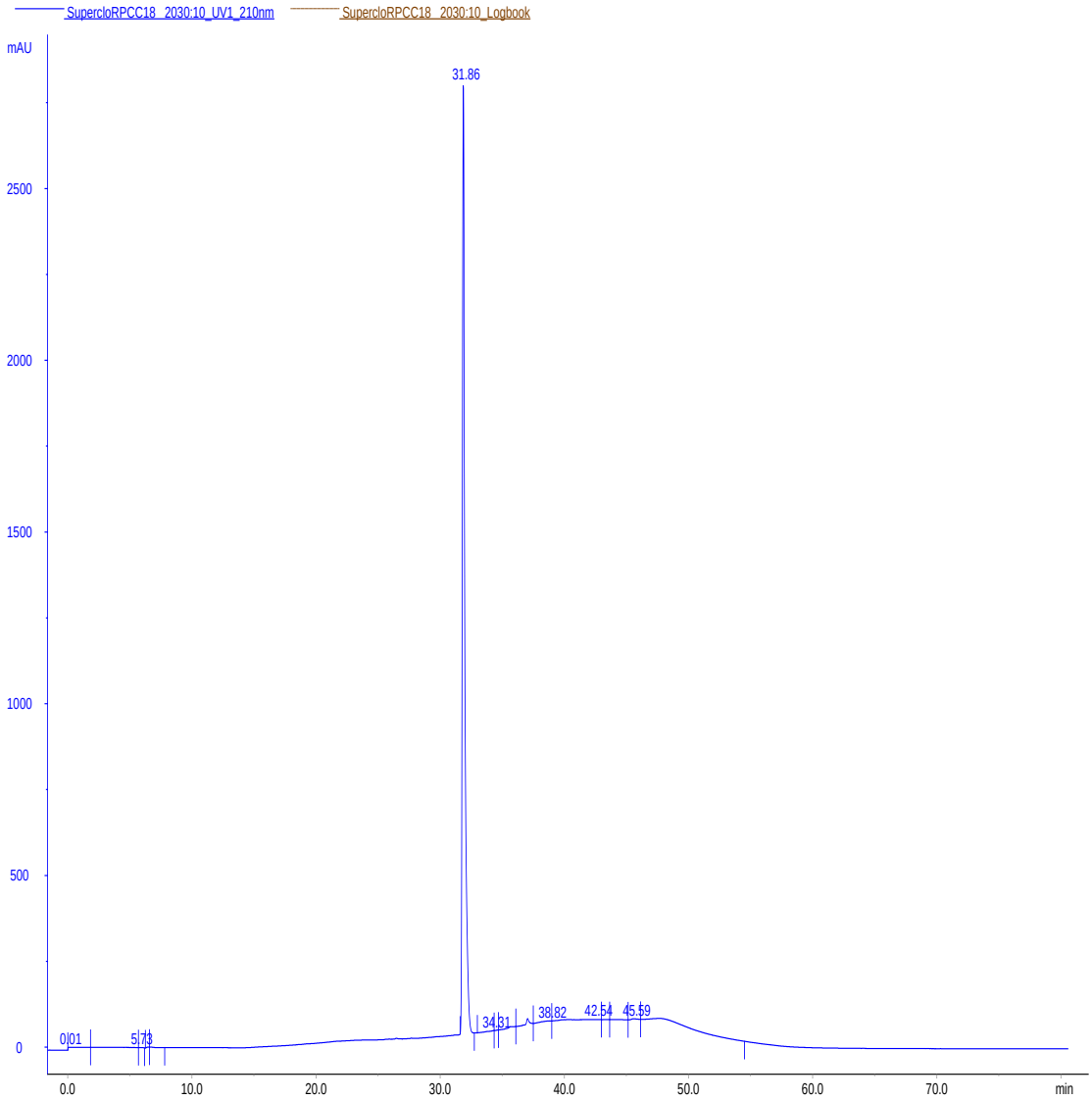

Figure 4. 2: The peak of EGCG standard solution (0.04mg/l)

Table 4. 10: The Retention, area and height of EGCG standard solution peak (0.04mg/l)

Retention (min)	Area (mAU*min)	Height (mAU)
31.86	656.9776	2767.417

As shown in Table 4.10, the peak was appearing at 31.86 min for the 0.04 mg/l solution. The peak area was 656.9776mAU*min and the height was 2767.417mAU.

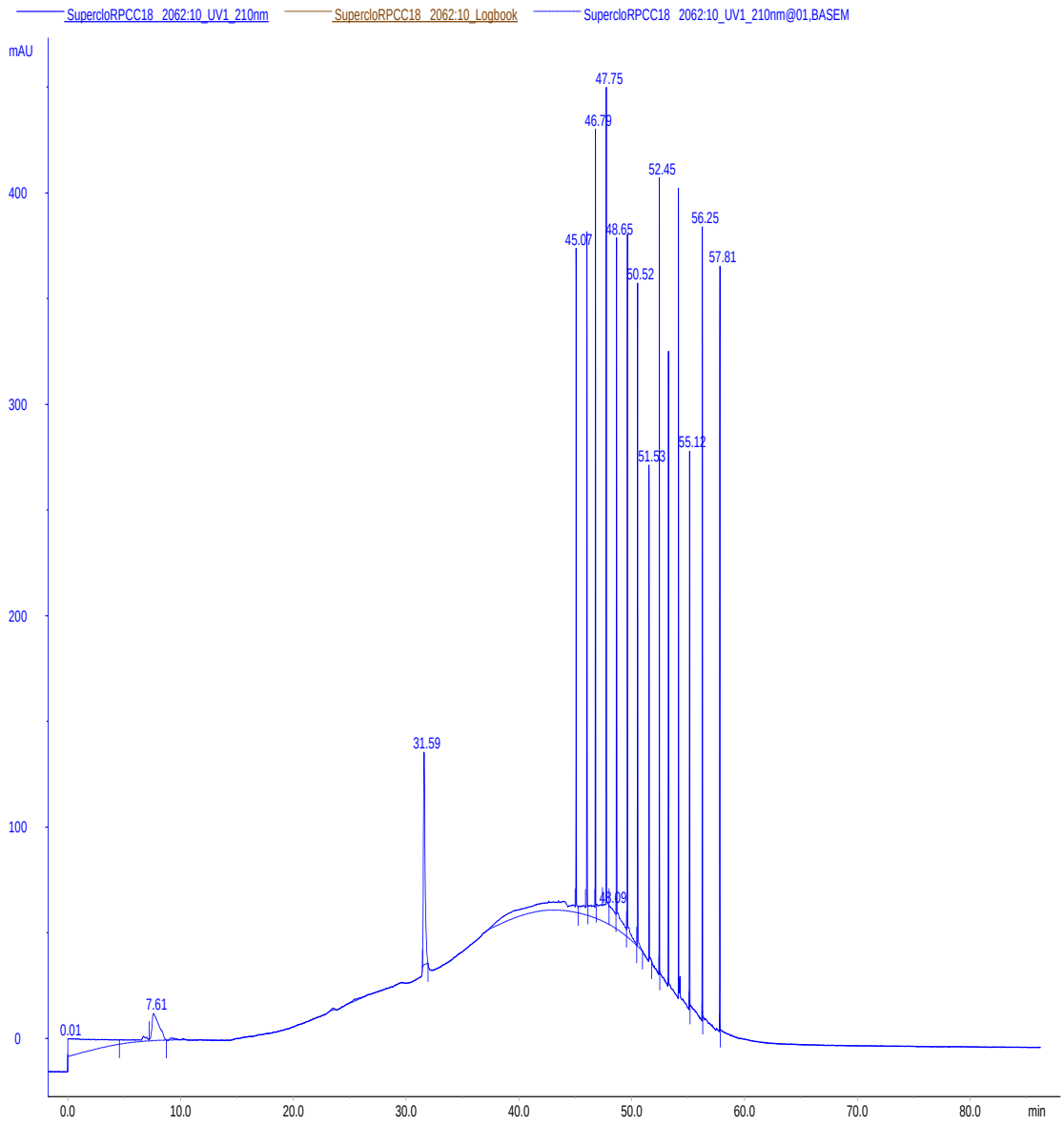

Figure 4. 3: The peak of EGCG standard solution (0.0025mg/l)

Table 4. 11: The Retention, area and height of EGCG standard solution peak (0.0025mg/l)

Retention (min)	Area (mAU*min)	Height (mAU)
31.79	20.5298	100.755

As shown in Table 4.11, the peak was appearing at 31.79 min for the 0.0025 mg/l solution. The peak area was 20.5298mAU*min and the height was 100.755mAU.

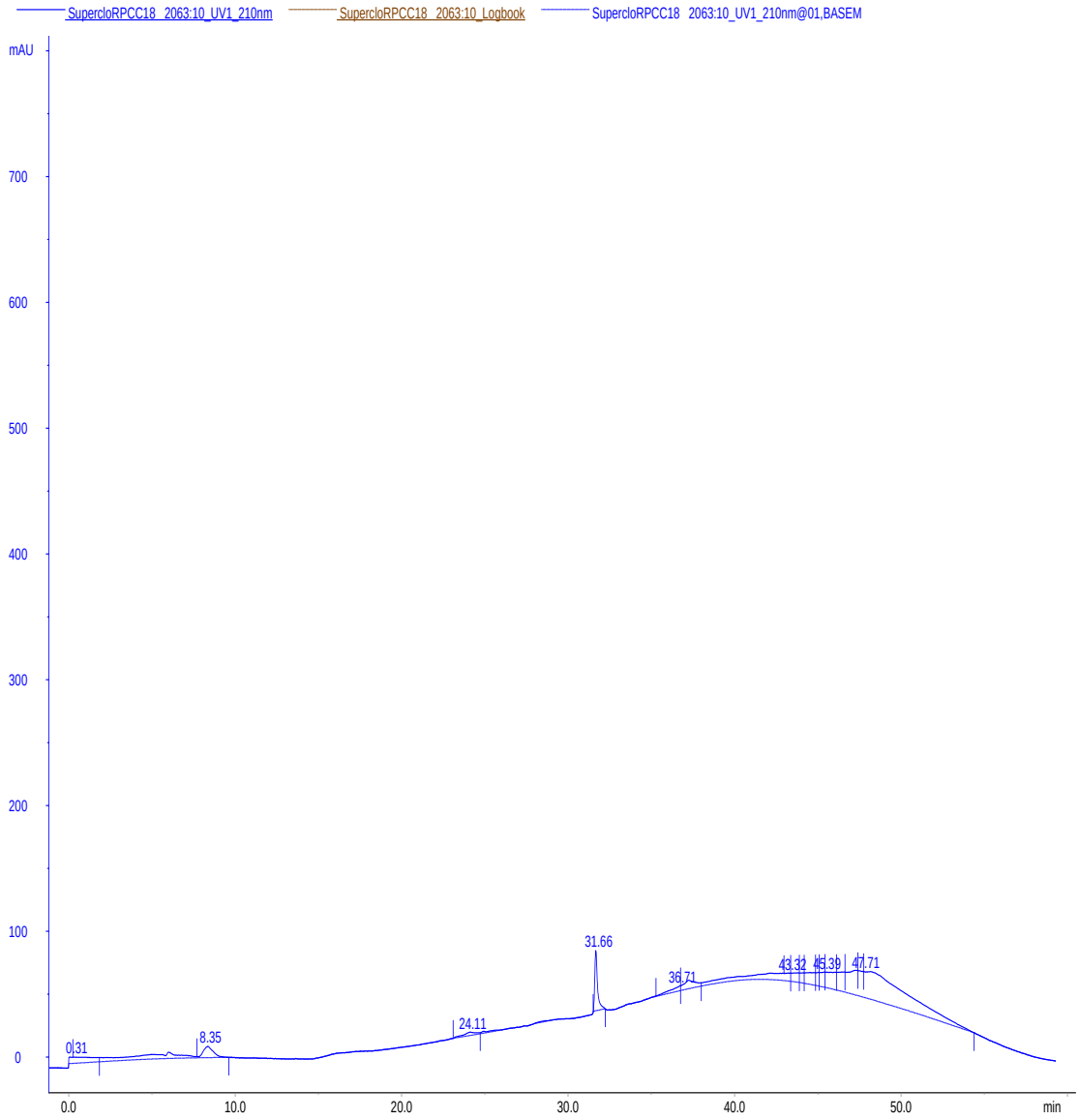

Figure 4. 4: The peak of EGCG standard solution (0.00125mg/l)

Table 4. 12: The Retention, area and height of EGCG standard solution peak (0.00125mg/l)

Retention (min)	Area (mAU*min)	Height (mAU)
31.58	10.1256	48.045

As shown in Table 4.12, the peak was appearing at 31.58 min for the 0.00125 mg/l solution. The peak area was 10.1256mAU*min and the height was 48.045mAU.

From the data in Tables4.9 to 4.12, the relationship of concentration of standard solution and the peak area can be constructed that shown in Table 4.13.

Table 4. 13: The relationship of concentration of standard solution and the peak area

Concentration (mg/l)	0.00125	0.0025	0.04	0.08
Peak area (mAU*min)	10.1256	20.5298	656.9776	1312.962

From Table 4.13, a standard solution curve can be constructed that shown as Figure 4.5. This standard curve can help me to get the concentration of any sample solution.

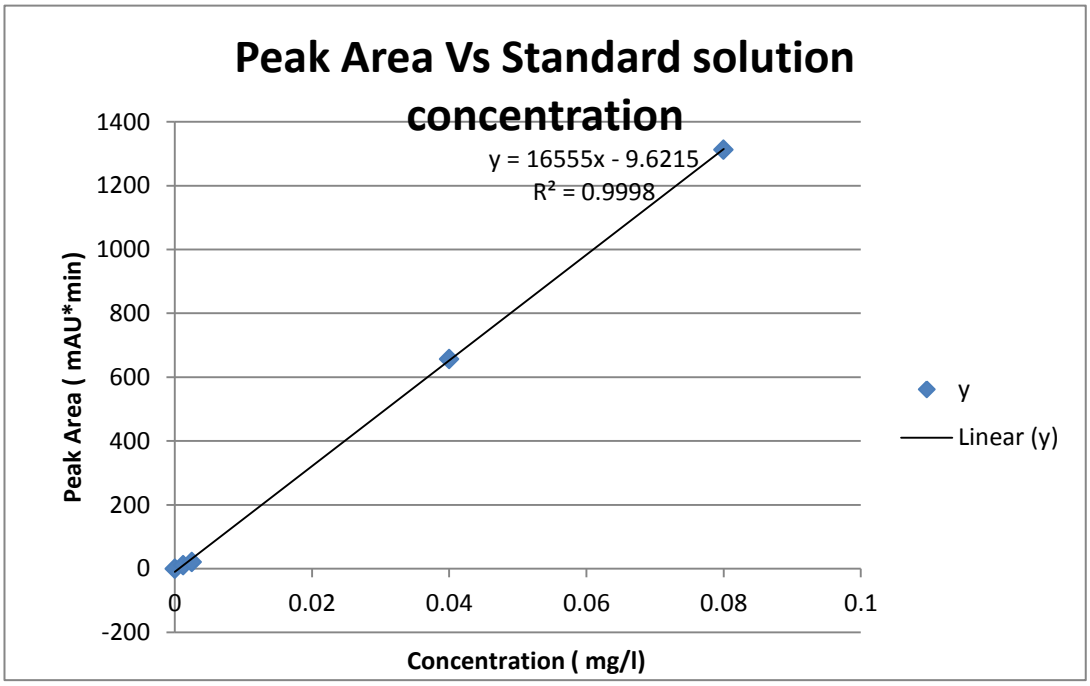

Figure 4. 5: Peak Area Vs Standard solution concentration

Using linear regression a calibration curve between the peak area from the HPLC chromatograph and EGCG concentration was derived (Equation 4.1):

$$y = 16555x - 9.6215 \qquad (4.1)$$

Where: y is the peak area (mAU*min) and x is the EGCG concentration (mg/l).

4.6 The HPLC results of dried tea samples extraction solution

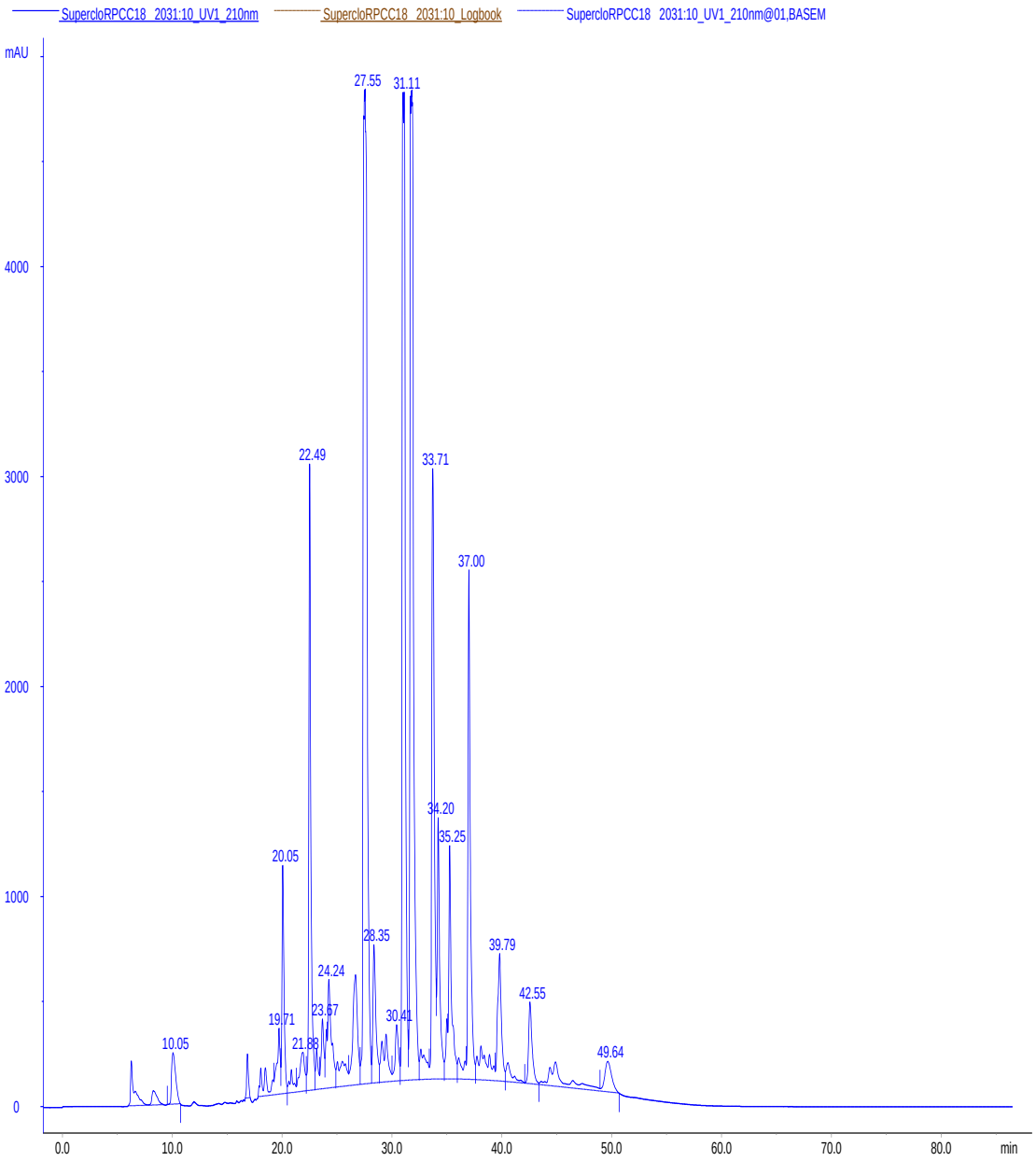

Figure 4. 6: The peak of dried tea sample solvent extraction

Table 4. 14: The Retention, area and height of tea sample solvent extraction peak

Retention (min)	Area (mAU*min)	Height (mAU)
31.81	1845.1369	4714.132

As shown in Table 4.14, the peak was appearing at 31.81 min. The peak area is 1845.1369mAU*min and the height is 4714.132mAU.

Using the calibration curve (Equation 3.1) when the peak area is1845mUA*min, the concentration (C_s) of EGCG in the dried tea is 0.112g/l. The dried tea samples solution (V_s) was 10ml.The mess of EGCG in original dried tea samples solution (M_s) could be determined from:

$$M_s = C_s V_s = 0.112 \frac{g}{l} \times 0.01l = 1.12 \times 10^{-3} g \qquad (4.2)$$

The dried tea sample's mass (M_0) was 1g; hence the dry basis content of EGCG in the dried tea is 1.12×10^{-1}g/1g.=112mg/g.

4.7 The HPLC results of fresh tea samples extraction solution

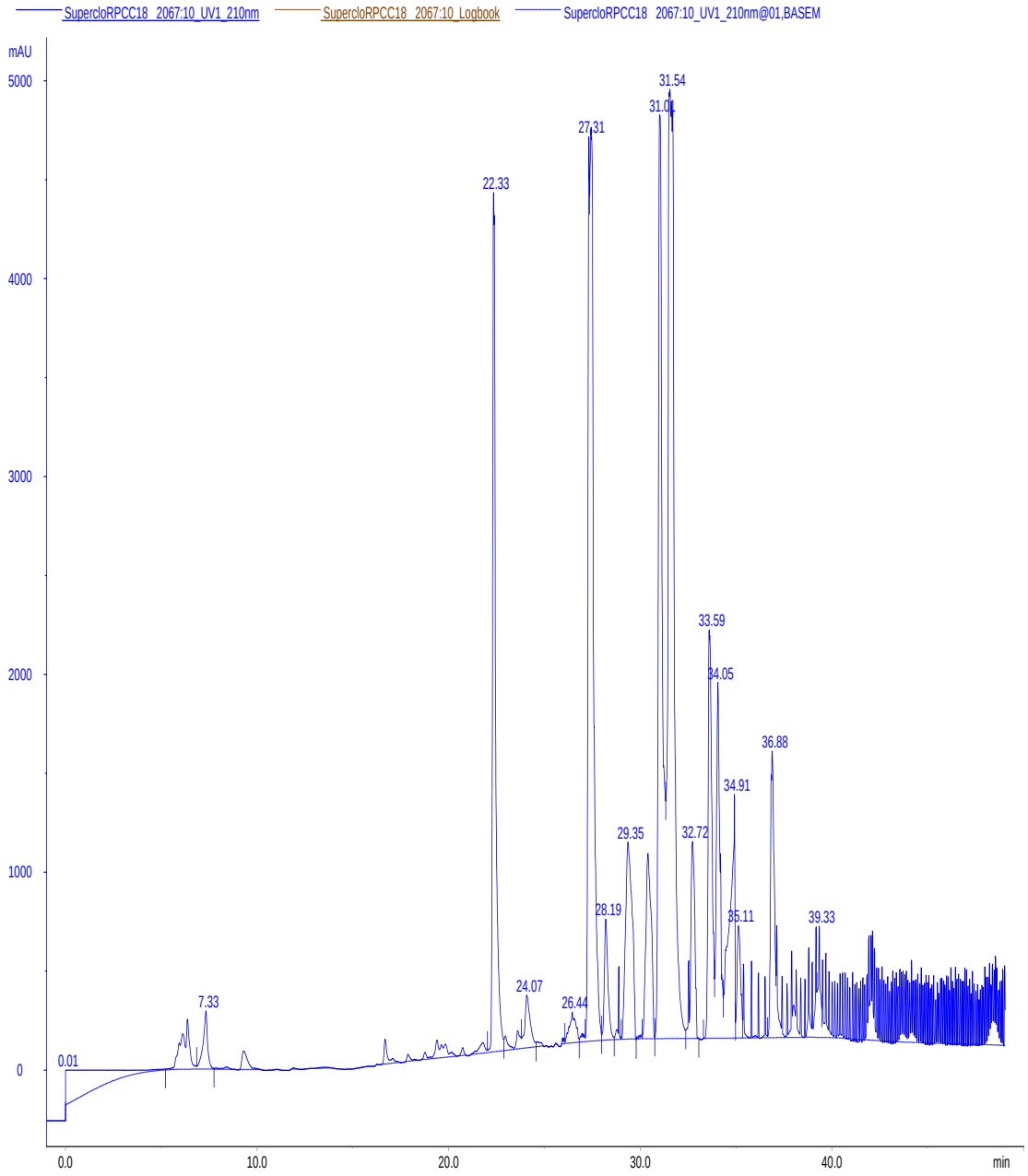

Figure 4. 7: The peak of fresh tea (just before indoor withering) sample solvent extraction solution

Table 4. 15: The Retention, area and height of undried tea sample solvent extraction solution peak

Retention (min)	Area (mAU*min)	Height (mAU)
31.80	1149.4041	4670.396

Shown in Table 4.15, the peak was appearing at 31.80 min. The peak area is 1149.4041mAU*min and the height is 4670.396mAU.

Using the calibration curve (Equation 3.1) when the peak area is 1149 mUA*min, the concentration (C_s) of EGCG in the fresh tea is 6.93×10^{-2}g/l. The fresh tea samples solution (V_f) was 10ml.The mess of EGCG in 100ml original fresh tea samples solution (M_f) could be determined from:

$$M_f = C_s V_f = 0.0693\frac{g}{l}\times 0.01l \times \frac{5ml}{0.1ml} = 3.47\times10^{-2}\,g \qquad (4.2)$$

The fresh tea sample's dry mass (M_{f0}) was 0.28g; hence the dry basis content of EGCG in the dried tea is 3.47×10^{-2}g/0.28g.≈124mg/g.

4.8 Implications for New Zealand Oolong tea processors

According the HPLC analysis the concentration of EGCG in fresh tea leaves is about 124mg/g ($p<0.05$), and the concentration of EGCG in finished tea and processing tea is about 112mg/g ($p<0.05$).

Thus, the rate of EGCG oxidisation was

$$R = \frac{124-112}{124}\times 100\% = 9.7\% \qquad (4.4)$$

The product of EGCG oxidation is theaflavin, and it has a direct effect on the color and taste of tea. [15] As show in Table 4.1, of New Zealand Oolong tea thad the high taste score (TS) and quality score (QS), even though only 9.7% EGCG had been oxidized. It is possible therefore, that the level of fermentation of New Zealand Oolong tea can be increased to produce more product more flavonoids

that make the tea's colour and flavor might be further improved. Even without further oxidation, the content of EGCG of New Zealand Oolong tea (112mg/g in finished tea) is high enough for the health benefit that had described Section 2.5.3 to be gained.

Chapter 5: Conclusions and Recommendations

5.1 Conclusions

This study used HPLC to determine EGCG the content of leaves used to make New Zealand Oolong tea. In the fresh tea leaves that have not been through the indoor wilting stage, the content of EGCG is 124mg/g. In finished tea the content of EGCG is 112mg/g. As shown in Table2.3, it is significantly higher than other teas such as Pu-erh, Fujian Oolong tea, DaHongPao Black tea.

The results indicated that the early stages of the current tea manufacturing process are critical for controlling the EGCG content and extend of EGCG oxidation in the finished product.

5.2 Recommendations for future research

New Zealand has the advantage of being in the southern hemisphere and could access the off-season market in the northern hemisphere. This study showed that high content of EGCG in the fresh tea leaves was not significantly affected by storage at -23℃. It would be valuable to investigate whether the tea could be stored at an even higher temperature, which would reduce costs.

Further studies could focus on improving the level of the fermentation in New Zealand Oolong tea to control the content of the EGCG, make the teas at a good balance. We can improve the situation of the trials to quickly determinate the content of EGCG and control the tea quality.

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