# **Optimising the Quality of NSW Central Coast Green Tea**

By

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A thesis submitted for the degree of Doctor of Philosophy, Food Science

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### **Statement of Originality**

This thesis contains no material previously accepted for the award of any other degree or diploma in any university or tertiary institution. Furthermore, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference has been made in the text.

However, I acknowledge that the work embodied in this thesis has been done in collaboration with other researchers and has been carried out in part at other institutions. Where necessary, I have indicated within the thesis the extent and type of collaboration, and acknowledged the contributing parties.

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James C Krahe

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# List of Abbreviations

| ~                 | approximately  |
|-------------------|--|
| %                 | per cent   |
| μg                | microgram(s)   |
| AF                | auto focus   |
| C.                | Camellia   |
| DI                | deionised  |
| EC                | Epicatechin  |
| ECG               | Epicatechin Gallate                                    |
| EGC               | Epigallocatechin                                       |
| EGCG              | Epigallocatechin Gallate                               |
| GCG               | Gallocatechin Gallate                                  |
| HPLC              | High-Performance Liquid Chromatography                 |
| IS                | internal standard                                      |
| MJ/m <sup>2</sup> | mega joules of energy per square metre of surface area |
| mL                | millilitre   |
| mM                | millimole per litre                                    |
| NIR               | Near Infrared  |
| NSW               | New South Wales  |
| nm                | nanometre(s)   |
| PPO               | polyphenol oxidase                                     |
| QA                | quality assurance                                      |
| RIRDC             | Rural Industries Research and Development Corporation  |
| S                 | second(s)  |
| UV                | ultraviolet  |
| Vis               | visible  |

### **Synopsis**

The green tea industry on the Central Coast of New South Wales (NSW) is an agricultural endeavour in the early stages of its establishment. It is a venture that, encouraged through public and private sector funding, has begun the propagation and production of Japanese styled green tea products for eventual export into the premium markets of Japan.

The working hypothesis for this project is that through a greater understanding of green tea production in the Australian conditions and environment and a deeper understanding of the relationship between green tea quality and its bioactive constituents, methods to improve Australian green tea could be developed. The resulting optimised products would be better suited to the heavy competition in the overseas markets of Asia, especially in Japan.

The main aim of the study was to develop a quality-assessment technique, the Quality Score, to highlight areas of strength and weakness within NSW Central Coast green tea products as they are currently produced, so that future developments can be planned for product optimisation. The concentrations of bioactive constituents in the products - that is, theanine, caffeine and the catechins: Epicatechin (EC), Epigallocatechin (EGC), Epigallocatechin gallate (EGCG), Gallocatechin gallate (GCG) and Epicatechin gallate (ECG) - were measured by High-Performance Liquid Chromatography (HPLC) and these values were related to the tea's corresponding retail-market quality categorisation.

The Quality Score testing procedure involved the development of five Quality Indices and their relationship to the levels of low, medium and high retail market quality. These Quality Indices were: the total catechin concentration, which showed the concentration was inversely related to retail quality; the theanine: total catechin ratio, which showed a positive correlation with increased retail quality; the theanine: caffeine ratio, which also showed a positive correlation with increased retail quality; the EGCG: EGC ratio, which again showed a positive correlation with increased retail market quality; and, finally, the EGCG: GCG ratio, which showed a negative correlation with increased market quality. These five Quality Indices were then combined to produce a Quality Score, which was used to categorise the green tea products in the study, according to similar products available in the Australian boutique green tea retail market.

All field studies were conducted at one of the two Gosford Primary Industries Institute tea research sites, at Somersby and Narara, NSW. The studies in the following chapters used, whenever appropriate, a treatment versus control study design. However, in the case of studies on environment factors, a time-based repeated measures study design was utilised. All collected samples of green tea were heat inactivated and dried immediately after harvesting or frozen immediately and heat inactivated and dried upon thawing to minimise oxidation of target bioactive constituents. All samples were brewed into green tea infusions and analysed for their content of theanine, caffeine and the catechins (EC, EGC, EGCG, GCG and ECG) using HPLC. Based on these quantitative analysis outcomes, the samples were subsequently scored for quality according to retail quality levels and categorised.

The Quality Score and Quality Indices were used to analyse historically produced green teas from the Central Coast region in an attempt to determine areas of product strength and weakness. This is reported in Chapter 6. The findings confirmed the importance traditionally placed upon green tea products; with the greatest market value assigned to 'First Harvest' and shaded or 'Gyokuro' green tea products. It was also concluded that a greater understanding of the environment and seasonal variations within the Central Coast region would contribute to product optimisation. Furthermore, it is argued that, as they also offer potential benefit, best practice agricultural techniques in regards to shading should be the focus of in-depth studies. Research into post-harvest processing also has potential for optimising NSW Central Coast green tea products.

Based on the findings of Chapter 6, green tea products were monitored across a complete harvesting season from September to May. This is reported in Chapter 7. Peak product quality was found to occur at 'First Harvest' and showed a slow natural decline throughout the remaining harvesting season. This decline was observed in both the leaves and stems, but the data showed that this decline was delayed slightly in the stems. This indicates that the inclusion of stems in teas from later harvests, particularly from the 'Second Harvest', could improve the quality of the resulting product. It was

found that the most abundant catechin in green tea infusions in the early part of the harvesting season was EGCG, but that towards the end of the growing season, after the seasonal peak in temperature, the most abundant catechin was EGC. Furthermore, it was revealed that the leaf concentration of EGCG is affected by solar intensity via a relationship with chlorophyll content and that this may contribute to a seasonal change in catechin concentration.

An investigation of the use of shading in the production of green tea showed that the Quality Score tool was able to distinguish between variations arising from the use of different shading techniques. The greatest improvement in Quality Score observed for short-term shading, up to 14 days, was when the available ambient light was reduced to one per cent. However, the most significant finding was the effect of long-term shading (up to 11 weeks) on the concentrations of theanine and caffeine within the stems of green tea plants that were shaded to 10 per cent ambient light levels for periods of seven to 11 weeks. These results indicate that utilisation of the stems is particularly important in the production of shaded green tea products after the 'First Harvest'.

Another important finding of this study was that the Quality Score was unable to measure the quality decline brought on by delays to the post-harvest polyphenol oxidase (PPO) enzymes inactivation. This was due to the teas developing characteristics of semi-fermented teas. Since the Quality Score was not calibrated to analyse semi-fermented teas, the tool was unable to provide an accurate measure of product quality. The use of raw concentration measures for the important target constituents including theanine, caffeine, EGCG, EGC and total catechins might prove more useful for future studies because they provided a better indication of fermentation.

The major conclusion from this study is that it is possible to use variations in the concentrations of target green tea bioactive constituents to quantify the green tea market quality objectively. It was also found that understanding the effect of growing and environmental conditions on the green tea crops, and tailoring farm practices to suit the production of particularly high quality and high value target green tea products, could improve the quality of the green tea being produced in the NSW Central Coast region and thereby improve its economic future.