Extraction and Preparation of Bioactive Components from Green Teas

Quan Van Vuong
Msc. in Food Technology

Thesis submitted for the degree of
DOCTOR OF PHILOSOPHY
STATEMENT OF ORIGINALITY

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

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DECLARATION OF AUTHORSHIP

I hereby certify that this thesis is submitted in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a signed statement from each co-author; and endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

..................................

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LIST OF PUBLICATIONS INCLUDED AS PART OF THE THESIS


**Paper V:** Quan V. Vuong and Paul D. Roach. Caffeine in green tea: its removal and isolation. Submitted to *Food Research International* on 30 June 2012.


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LIST OF ADDITIONAL PUBLICATIONS, ACHIEVEMENTS AND AWARDS

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BOOK CHAPTER


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4. Quan V Vuong, Minh H Nguyen, Costas E Stathopoulous, John B Golding, Paul D Roach. Optimising conditions for extracting catechins from green tea using hot water. *Proceedings of The Australian Institute of Food Science Technology(AIFST), Sydney, NSW, Australia (2011).*

5. Quan V Vuong, John B Golding, Minh H Nguyen, Paul D Roach. Production of partially decaffeinated instant tea from Australian grown green tea. *Proceedings of The Australian Institute of Food Science Technology(AIFST), Sydney, NSW, Australia (2011).*


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6. Travel grant given by the Australian Nutrition Society for attending the annual conference in Queenstown, New Zealand, November 2011.

7. Best poster presentation award granted by the Australian Institute of Food Science and Technology at the 45th Annual AIFST Convention in Adelaide, July 2012.
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>°C</td>
<td>Degree Celsius</td>
</tr>
<tr>
<td>µL</td>
<td>Microliter(s)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>C</td>
<td>Catechin</td>
</tr>
<tr>
<td>CG</td>
<td>Catechin Gallate</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
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<tr>
<td>EC</td>
<td>Epicatechin</td>
</tr>
<tr>
<td>ECG</td>
<td>Epicatechin Gallate</td>
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<tr>
<td>EGC</td>
<td>Epigallocatechin</td>
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<tr>
<td>EGCG</td>
<td>Epigallocatechin Gallate</td>
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<tr>
<td>et al.</td>
<td>and others</td>
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<tr>
<td>g</td>
<td>gram (s)</td>
</tr>
<tr>
<td>GC</td>
<td>Gallocatechin</td>
</tr>
<tr>
<td>GCG</td>
<td>Gallocatechin Gallate</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
</tr>
<tr>
<td>IS</td>
<td>Internal Standard</td>
</tr>
<tr>
<td>MAE</td>
<td>Microwave Assisted Extraction</td>
</tr>
<tr>
<td>MAP</td>
<td>Modified Atmosphere Packaging</td>
</tr>
<tr>
<td>min</td>
<td>Minute (s)</td>
</tr>
<tr>
<td>MIPs</td>
<td>Molecularly Imprinted Polymers</td>
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<tr>
<td>mL</td>
<td>Millilitre (s)</td>
</tr>
<tr>
<td>mM</td>
<td>millimolar</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>PPO</td>
<td>Polyphenol oxidase</td>
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<tr>
<td>RP-HPLC</td>
<td>Revered-phase HPLC</td>
</tr>
<tr>
<td>RSM</td>
<td>Response Surface Methodology</td>
</tr>
<tr>
<td>sec</td>
<td>Second (s)</td>
</tr>
<tr>
<td>SFE-CO2</td>
<td>Supercritical Fluid Extraction with Carbon dioxide</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid Phase Extraction</td>
</tr>
<tr>
<td>SWE</td>
<td>Subcritical Water Extraction</td>
</tr>
<tr>
<td>UAE</td>
<td>Ultrasound Assisted Extraction</td>
</tr>
<tr>
<td>UHPE</td>
<td>Ultrahigh Pressure Extraction</td>
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<tr>
<td>UV-Vis</td>
<td>Ultraviolet – Visible</td>
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<tr>
<td>v/v</td>
<td>Volume by Volume</td>
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ABSTRACT

Background

Green tea is a rich source of the strong antioxidant compounds, the catechins, and the unique amino acid, theanine, which have been linked with health benefits such as prevention of certain types of cancers and cardiovascular diseases, decrease in obesity and improvement of the immune system. However, epidemiological studies suggest that the volume of green tea required to obtain health benefits is rather large, ranging from 5-10 cups a day. Therefore, it is questionable whether individuals, especially in western countries where they are not used to drinking green tea, can consume a large enough quantity of green tea to obtain the levels of the green tea bioactive compounds needed for health benefits.

Therefore, extraction of the catechins and theanine from green tea to provide concentrated preparations for use as food supplements or as additives for functional foods has been considered as a way to increase the consumption of these green tea bioactive compounds. In addition, green tea extracts and powders can be utilised in various foods to prolong their shelf-life. Green tea also contains a high level of caffeine, which can work as a mild central nervous stimulant. However, caffeine can cause some negative effects in some people and therefore, its removal from green tea products needs to be addressed. Furthermore, as a consequence of the concerns relative to the use of organic solvents, which are usually used for extractions of plant materials in the food industry, water is the only solvent which should be used.

Another way of increasing the intake of the beneficial green tea components is to ensure that they are well extracted when people prepare their green tea themselves. A low extraction of the compounds could be one of the reasons why large amounts of the beverage appear to be needed to obtain the health benefits.

Hypothesis and Aims

The current study hypothesised that the aqueous extraction of the three bioactive components, the catechins, theanine and caffeine, from loose leaf green tea or green tea in tea bags, could be improved and that aqueous extractions could be used to prepare decaffeinated, normal caffeine and caffeine-enriched green tea catechin powders.

The overall aims were to 1) improve the aqueous extraction of the three main bioactive components, catechins, theanine and caffeine from loose leaf green tea, 2) to prepare decaffeinated,
normal caffeine and caffeine-enriched green tea catechin powders from freshly harvested young and old green tea leaves using water as the only solvent for the extractions and freeze drying and spray drying to dry the aqueous extracts, and 3) to improve the extraction of the three green tea bioactive components form green tea in teabags using water and the microwave oven.

**Results**

The results showed that the extraction of the catechins from loose leaf green tea could be improved by brewing ground green tea ($\leq 1$ mm) twice: once at $80^\circ$C for 30min with a water-to-tea ratio of 12:1 mL/g and once at $80^\circ$C for 30min with a water-to-tea ratio of 8:1 mL/g. The extraction of the theanine from loose leaf green tea could be also improved by brewing ground green tea (0.5-1 mm) at $80^\circ$C for 30min with a water-to-tea ratio of 20:1 mL/g. Water was also found to be effective for decaffeinating freshly harvested young (apical bud to fourth leaf on the growing shoot) and old tea leaves (the fifth to tenth leaves down the stem). Blanching the young tea leaves at 100°C for 4 min at a water-to-tea ratio of 20:1 mL/g removed 83% of the caffeine while retaining 94% of the catechins whereas blanching the old tea leaves at 100°C for 10 min at a water-to-tea ratio of 20:1 mL/g removed 80% of the caffeine while retaining 83% of the catechins.

Three types of green tea powders: decaffeinated, normal caffeine and caffeine-enriched green tea powders were also prepared by brewing, filtering, concentrating extracts and then either freeze drying or spray drying them into powders. Both freeze drying and spray drying were found to be suitable for drying the green tea aqueous extracts. However, in terms of cost-effectiveness, spray drying was considered as a method of choice and its optimal conditions were found to be $180^\circ$C for the inlet temperature and $115^\circ$C for the outlet temperature. These green tea powders had catechin levels of 174-197 mg/g and theanine levels of 7-22 mg/g. The caffeine levels were 6.1-7.3 mg/g for decaffeinated powder, 21.3-21.8 mg/g for normal caffeine powder and 94.8 mg/g for caffeine-enriched powder. In addition, these green tea powders had excellent physical properties such as high water solubility ($\geq 96\%$) and low moisture content ($<2.5\%$).

Finally, the results indicated that brewing teabags for 3 min at room temperature in 200 mL of boiled water, as suggested by the manufacturers, was not efficient as only 62% of the catechins, 76% of the caffeine and 80% of the theanine were extracted from the teabags. However, the extraction of these three bioactive components could be improved by first brewing the teabags in freshly boiled water for 0.5 min at room temperature followed by irradiation for 1 min in a microwave oven. This method improved the extraction of the catechins, caffeine and theanine by 34%, 29% and 14%, respectively, in comparison with the common brewing method of 3 min in 200 mL of boiled water.
Conclusions

In conclusion, the hypothesis was supported and the aims were achieved. The aqueous extraction of the three main bioactive components, the catechins, theanine and caffeine from green tea was optimised and improved. In addition, using water as the only solvent, this study developed methods to prepare decaffeinated dried green tea and decaffeinated, normal caffeine and caffeine-enriched green tea catechin powders from freshly harvested young and old green tea leaves. Finally, this study developed a method using the microwave oven to improve the extraction of the three green tea bioactive components from green tea in teabags.