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A METABONOMICS STUDY OF *CAMELLIA SINENSIS* (TEA)

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INTRODUCTION

Objectives of this Study:

- **Collect accurate mass LC/MS data for infusions of differing varieties of *Camellia sinensis* (tea).**
- ***Process the LC/MS data sets and then analyze using multivariate analysis techniques, i.e., Principle Component Analysis (PCA) and Orthogonal Partial Least Squares (OPLS).***
- ***Determine the statistically most significant analytes between tea groups and identify them.***

Tea History Highlights:

- **Origin of *Camellia sinensis* is unknown, but likely evolved from the jungles of the eastern Himalayas.[1]**
- **Likely that tea was "chewed" by early Humans around 60-100 thousand years ago.[1]**
- **Legend suggests that tea was first consumed as a beverage in China with the discovery that a tea leaf dropped into boiling water made a pleasant drink nearly 5000 years ago.[1,2,3]**
- ***Camellia sinensis* plant was domesticated in China.[1]**
- **Tea arrived in Europe first in Amsterdam (1610), then France in 1630's, and later to England in 1650's.[1,2]**
- **Tea bag serendipitously invented in 1908 by Thomas Sullivan, a New York importer.[3]**

Tea Varieties Studied:

(1) Japanese Green Tea, (2) Lotus Green Tea, (3) Traditional English Tea, (4) Earl Grey Tea, and (5) Chai Tea

METHODS

Instruments and Software Utilized:

Hardware	Software
Waters® LCT Premier™ (ESI+/ESI-)	Waters® MassLynx™ 4.1
Waters® ACQUITY UPLC™	MarkerLynx™
ACQUITY UPLC™ BEH™ C18 1.7µm 2.1 x 100 mm	Waters eLab Notebook™ 3.0
	Umetrics SIMCA-P 11

Sample Preparation

The teas were brewed using hot water in a typical cup volume (240 mL) as per package directions. Prior to analysis by LC/MS, the samples were diluted 1:4 with MilliQ water.

Waters® ACQUITY UPLC™ Conditions

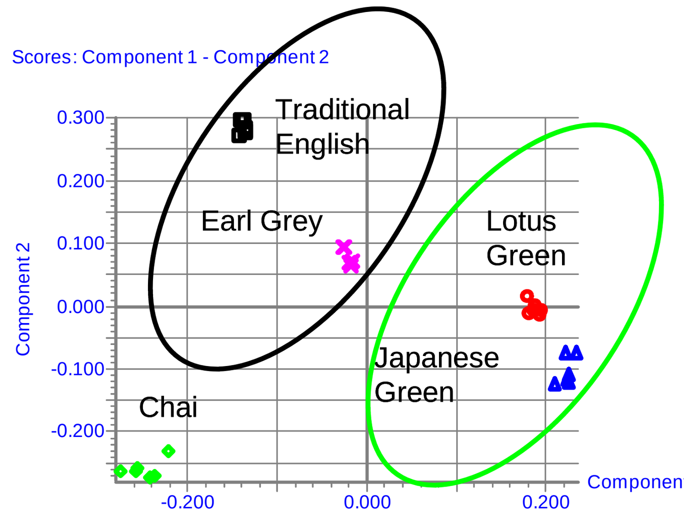
Flow Rate:	770 µL/min
Mobile Phase Solvent A:	0.1 % Formic Acid in Water
Mobile Phase Solvent B:	0.1% Formic Acid in Acetonitrile
Injection Volume:	5 µL
Sample Temperature:	10 °C
Column Temperature:	40 °C
Gradient:	0% B to 0.36 min; 12% B to 0.60 min; 21% B to 2.10 min; 25% B to 2.40 min; 95% B to 3 min.

RESULTS AND DISCUSSION

A Global View of the Tea LC/MS Data

MarkerLynx was used to perform component detection, exact mass / retention time pair alignment, and PCA of the LC/MS data. The five tea varieties studied are easily distinguished in the PCA scores plot (Figure 1). The black and green teas formed two distinctive groups. Chai tea is typically a black tea that is blended with herbs such as coriander, cumin seeds, curry leaves, *etc.*, and as a result did not fit into either the green or black tea groups.

Figure 1. PCA scores plot of 5 freshly brewed tea infusions from the 3 minute LC/MS dataset. Black teas form a separate group from the green teas.

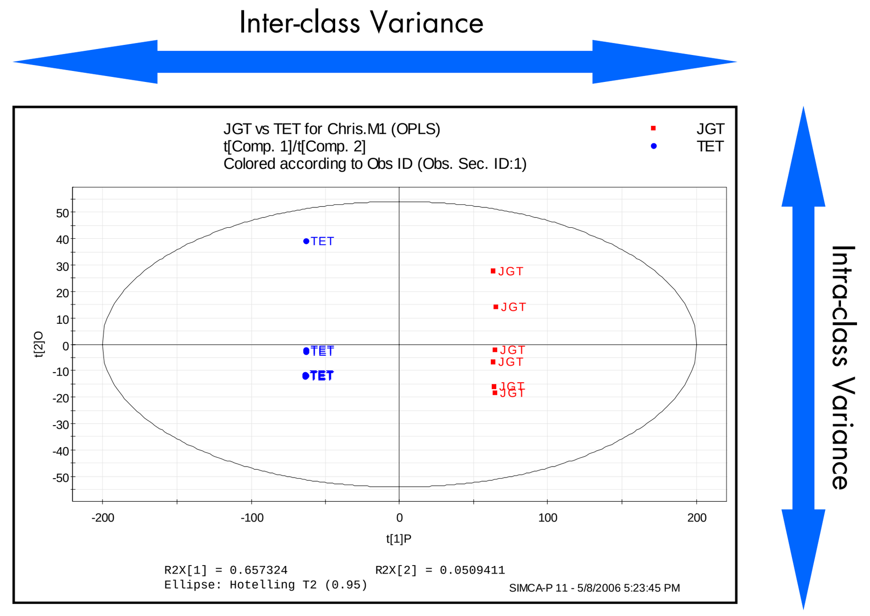


Targeted Comparison of Tea Samples

Green and black teas both originate from the *Camellia sinensis* plant. The difference between the tea types arises during processing. All teas go through a 4 step process: (1) Withering, (2) Rolling, (3) Oxidation, and (4) Drying.[2] The oxidation time is minimized for green teas.[2] In contrast, oxidation time is optimized in black teas to allow the enzymes present in the leaves to turn the leaves dark.[2]

The difference in "oxidation time" between a green and black tea was investigated by OPLS. Japanese Green Tea and Traditional English tea were studied. The aligned data matrix was exported directly from MarkerLynx into SIMCA-P using the integrated SIMCA-P interface button. The OPLS Plot (Figure 2) highlights the inter-class variance between the Traditional English and Japanese teas. The intra-class variance is also shown in the OPLS plot (Figure 2) and probably results from both analytical and sample variation.

Figure 2. OPLS plot comparing Traditional English Tea (TET) and Japanese Green Tea (JGT) from SIMCA-P.



The S-plot (Figure 3) shows the ions responsible for the variance shown in the OPLS plot (Figure 2). The ions highlighted in red (Figure 3) are at a higher abundance in the Japanese Green Tea over Traditional English tea. Conversely, the ions highlighted in blue (Figure 3) are at a lower abundance in Japanese Green tea versus Traditional English tea. Although these ions (Table 1) are present in both tea samples, the "oxidation time" has apparently affected the relative concentrations between the Japanese Green tea and the Traditional English tea.

Figure 3. S-Plot indicating the ions with high (•) and low (•) abundance in Japanese Green tea with respect to Traditional English tea from SIMCA-P.

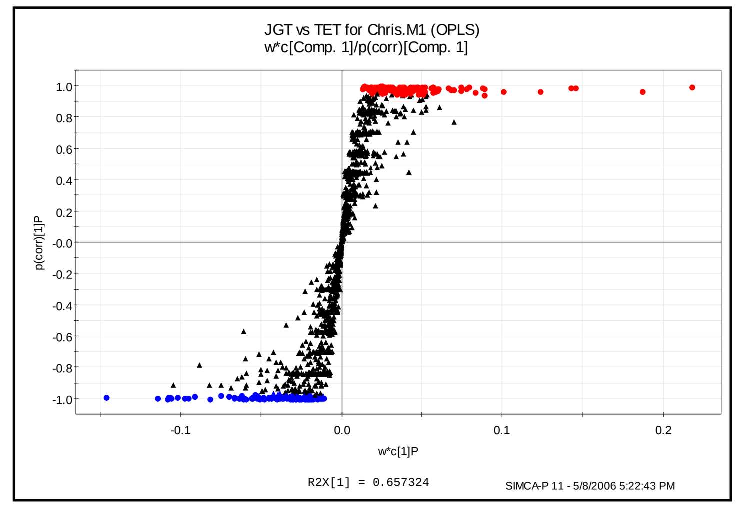


Table 1. Statistically significant ions with a greater abundance in Japanese Green Tea over Traditional English Tea.

Var ID (Primary)	M1.w*c[1]P	M1.p(corr)[1]P
1.20_307.0826	0.217835	0.994708
1.50_459.0961	0.186689	0.968157
1.98_443.0996	0.145054	0.989954
1.50_291.0898	0.142461	0.989955
1.76_773.2222	0.123329	0.967247
0.70_337.1618	0.100362	0.96748
1.50_460.0989	0.0887719	0.942665
1.20_308.0863	0.0877556	0.9906
2.02_757.2227	0.0831028	0.96128
1.36_579.1522	0.0794745	0.99542

Identifying Statistically Important Ions with eLN

Table 1 lists the statistically significant ions that are found at a relatively greater abundance in Japanese Green tea over Traditional English tea. A database search from within an electronic laboratory notebook (eLN) provided a list of potential identities for the statistically significant ions (Figure 4). A search for nominal masses below 307 (Figure 4) illustrates that many of the polyphenols present in tea are isomers. A few of the ions were positively identified by using authentic standards (Figure 5) and listed in Table 2.

Figure 4. Performing a mass search using the Chemistry Browser in eLab Notebook.

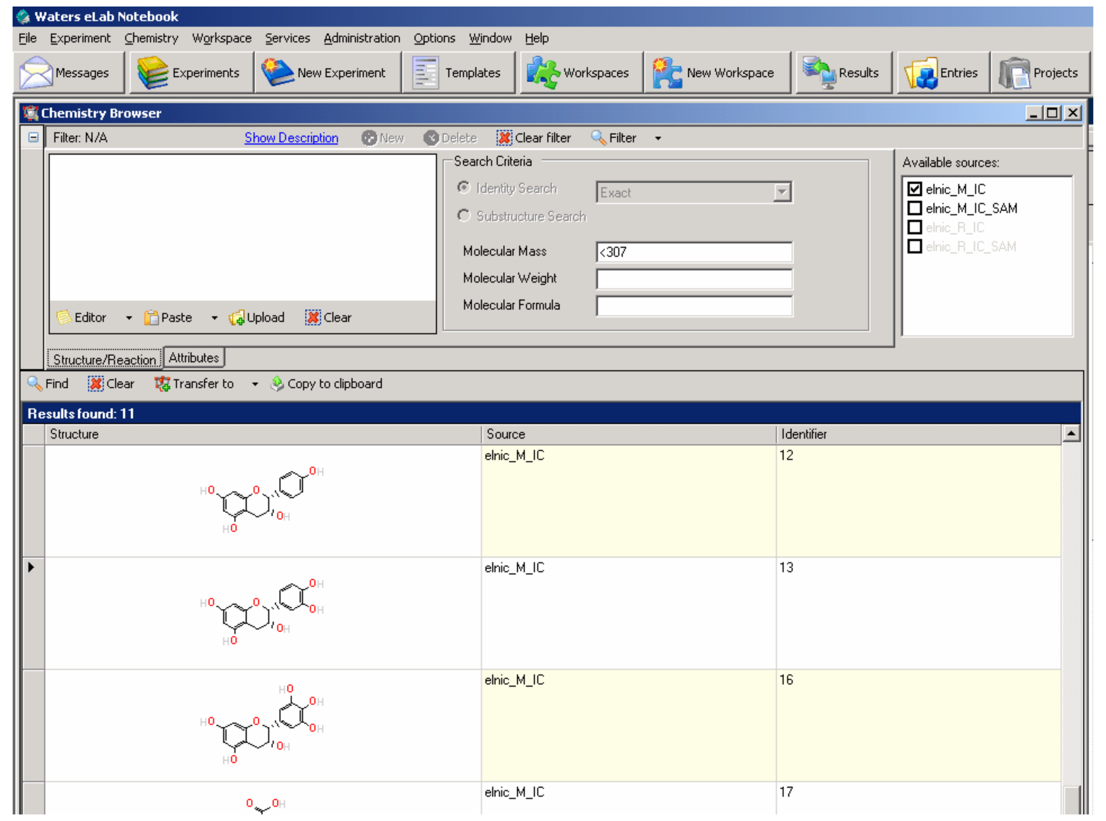


Figure 5. Transferring database hit results into eLab Notebook experiment.

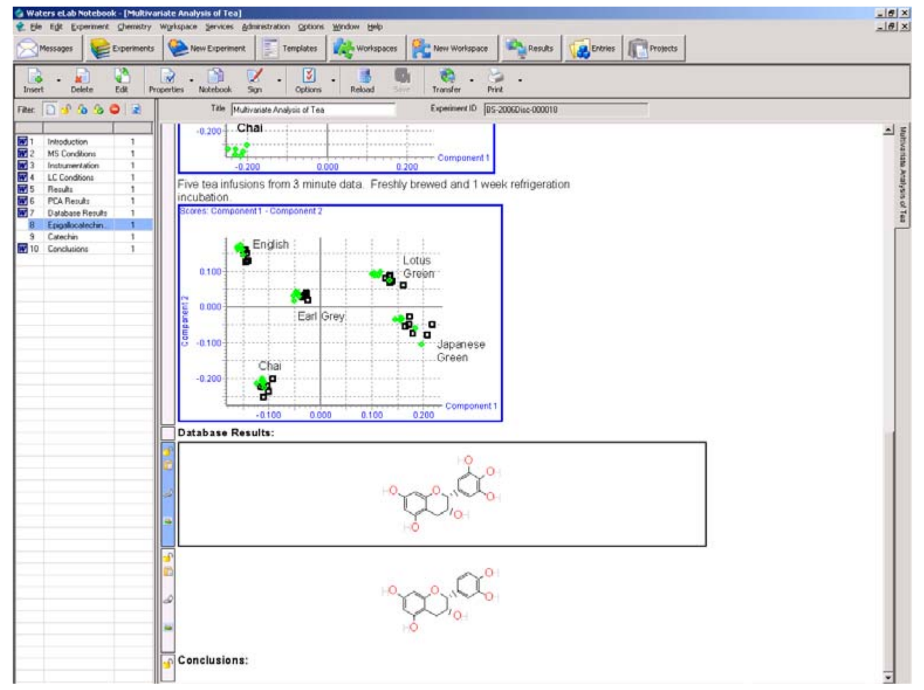


Table 2. Identities for top 4 ions with a greater abundance in Japanese Green Tea over Traditional English Tea.

Var ID (Primary)	M1.w*c[1]P	M1.p(corr)[1]P	Analytes
1.20_307.0826	0.217835	0.994708	Epigallocatechin
1.50_459.0961	0.186689	0.968157	Epigallocatechin gallate
1.98_443.0996	0.145054	0.989954	Catechin gallate
1.50_291.0898	0.142461	0.989955	Catechin

CONCLUSION

- **Multivariate analysis of UPLC / MS data easily distinguishes among the 5 tea varieties.**
- **Statistically significant ions predominantly found in Japanese green tea over Traditional English tea were identified.**
- **This approach may be applied to other areas of food science and natural products.**

References

1. Macfarlane, Alan; and Macfarlane, Iris "The Empire of Tea: The Remarkable History of the Plant that Took over the World", The Overlook Press: NY, 1st Edition, 2004.
2. History of Tea found on http://www.twinings.com.
3. From Wikipedia, the free encyclopedia on http://en.wikipedia.org/wiki/Iced_tea

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