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A Multi-Omics Approach to Finding Biomarkers in Philippine Civet Coffee

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Abstract: Civet coffee, obtained from the feces of civet cats feeding on coffee cherries, is an exotic, high-priced beverage. But due to issues regarding counterfeiting, animal abuse, and disease spread, physico-chemical characterization and authentication studies have become necessary. In this paper, separation with mass spectrometric methods for the analyses of metabolites, endogenous oligosaccharides, proteins, and endogenous peptides were developed and carried out. Results showed that two metabolites (2-ketoadipic acid and pentitol) and three peptides associated with a pathogenic-response protein (GVKSVEILEGDGGVGTIKLT, GVKSVEILEGDGGVGTIK, and GVKSVEILEGDGGVGT) may serve as potential bioamarkers. A glycomics approach methods was also developed.

Key Words: omics, biomarkers, mass spectrometry, civet coffee

1.INTRODUCTION

Arguably the most expensive coffee in the world, civet coffee is said to have a more flavorful and richer aroma compared to regular roasted coffee beans. It has been surmised that the enzymes inside the gastrointestinal tract of the civet cat ferment the raw beans thereby giving it an exotic and exquisite aroma upon roasting.

The steep price of civet coffee, which goes from $20/kg from the source to as high as $700/kg when it reaches the end consumer, is associated with claims that it is richer in aroma. The dilemma, from the point of view of science, lies on the lack of sufficient chemical and physical information on this type of coffee that may distinguish it from other types. Currently, there are less than a handful published scientific articles on this. So far the most extensive investigations include scanning

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electron microscopy of the coffee beans, SDS-PAGE on green (raw unroasted) coffee beans, and electronic nose experiments on volatile compounds from the brew of Indonesian and Ethiopian civet coffee (Marcone, 2004). Another report applied a metabolomics approach and identified malic acid, citric acid and the inositol/pyroglutamic acid ratio as discriminant markers for the authentication of Indonesian civet coffee (Jumhawan et al, 2013). The study aims to characterize the physical and chemical properties of Philippine civet coffee. Specifically, the study aimed to (1) examine surface morphology of and determine possible microbial presence/contamination in civet coffee beans; (2) recognize distinguishing characteristics by analyzing volatile and non-volatile metabolites, including aroma compounds and several important soluble and non-soluble constituents; and (3) develop methods for and identify possible biomarkers from the protein, endogenous oligosaccharide, and endogenous peptide profile, that may be critical in establishing the distinction of civet coffee from other types. The physicochemical characterization of civet coffee may not only provide key information for coffee aficionados and professionals/expert, but also extend to aid in addressing issues concerning its trade and sale, particularly since it involves an endangered animal.

2.METHODOLOGY

2.1. Sampling

Civet and non-civet Robusta (C. canephora) and Excelsa (C. liberica var. dewevrei) coffee samples were obtained first-hand from coffee plantations in Alfonso, Cavite (civet droppings obtained from wild civet cats that are not held in captivity), during the harvest periods of January-February 2015 (for metabolomics, proteomics, and peptidomics). For the glycomics experiment, Philippine C. canephora harvested on January-February 2015, and Brazil C. arabica and Rwanda C. arabica, harvested within the respective countries' harvest seasons of 2014, were used.

Upon harvesting, only wet fermentation was applied on the coffee cherries.

2.2. Roasting and Brewing/Extraction

Green beans were roasted using a Nesco™ Professional Coffee Roaster at temperatures and roasting times based on Specialty Coffee Association of America (SCAA) protocol, to produce light, medium, and dark roasts. The beans were ground using a KruppsTM 203-42 Fast Touch Coffee Grinder for 20 seconds each. Brewing/extraction, likewise were carried out using SCAA specifications, with drip filter method employed.

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Aliquots of the brews were stored in cryovials at 4°C until further use.

2.3 General Sample Preparation

Polyvinylpolypyrrolidone (PVPP) were added to the brew aliquots to reduce the amount of polyphenols that would interfere with the analyses. Repeated votexing and centrifugation were done to produce visibly clear extracts. The supernatants were then subjected to the appropriate sample preparations for each omic method.

2.4. Metabolomics

Coffee grinds were prepare based on West Coast Metabolomics Center (University of Californian-Davis) protocol (O. Fiehn et al, 2008). Briefly, the dried grounds were mixed with fatty acid methyl esters of C8-C30 as internal retention index markers in chloroform and subsequently trimethylsilane(TMS)-derivatized for GC-MS analyses.

2.5. Glycomics.

Aliquots of coffee brews were cleaned with C8 SPE prior to reduction with $NaBH_4$, followed by PGC SPE prior to LC-MS/MS analysis.

2.6. Proteomics/Peptidomics

Endogenous peptides were extracted from dried coffee grounds using ascorbic acid solution with PVPP. The supernatant was repeatedly vortexed and centirfugated at -20°C and then cleaned with C18 SPE prior to LC-MS/MS analysis.

3.RESULTS and DISCUSSION

3.1. Metabolomics

Factorial analysis at One-Factor Level, revealed several compounds that may serve as potential discriminant markers for coffee in terms of (1) species; (2) treatment; (3) category (Tables 1-3).

Compound	Species	
	Liberica	Robusta
Tyrosine	Low	High
Trisaccharide	High	Low
Chlorogenic Acid	High	Low
Caprylic Acid	High	Low
Beta-sitosterol	High	Low
Benzoic Acid	Low	High
Aconitic Acid	High	Low
3,4-dihydroxyhydrocinnamic acid	Low	High
3,4-dihydroxycinnamic acid	High	Low
2-deoxyerythritol	High	Low
Citric Acid	High	Low
Palmitic Acid	High	Low
Oleic Acid	High	Low
Maleic Acid	High	Low
Linoleic Acid	High	Low

Table 1. Possible discriminant markers based on different coffee species.

Compound	Category	
	Civet	Non-Civet
2-ketoadipic acid	High	Low
Pentitol	Low	High

Table 2. Possible discriminant markers based on effect of civet digestion (category).

Compound	Treatment	
	Green	Roasted
Arabinose	Low	High
Allantoic Acid	High	Low
Glucose Acid Lactone	High	Low
Galactonic Acid	High	Low
Mannose	High	Low
Tyrosine	High	Low
Fructose	High	Low
Ferulic Acid	High	Low
Trisaccharide	High	Low
Cis-gondoic Acid	Low	High
Chlorogenic Acid	High	Low
Caprylic Acid	High	Low
Benzoic Acid	Low	High
Alanine	High	Low
Aconitic Acid	High	Low
3,4-dihydroxyhydrocinnamic acid	Low	High
3,4-dihydroxycinnamic acid	High	Low
2-ketoadipic acid	Low	High
2-hydroxyhexanoic acid	Low	High
Thymine	Low	High
2-hydroxybutanoic acid	Low	High
2-deoxytetronic acid	Low	High
2-deoxyerythritol	High	Low
Citric Acid	High	Low
1-Monoolein	Low	High
Threonic Acid	High	Low
Threitol	High	Low
Succinate Semialdehyde	Low	High
Pentitol	High	Low
3,4-dihydroxybenzoic acid	Low	High
Oleic Acid	Low	High
N-acetyl-D-galactosamine	High	Low
Mucic Acid	High	Low
Methylmalonic Acid	High	Low
Maleimide	Low	High
Maleic Acid	High	Low
Lysine	High	Low
Linoleic Acid	Low	High
Leucine	High	Low
Hydroxylamine	High	Low

Table 3. Possible discriminant markers based on roasting levels (treatment).

It can be inferred that treatment, or level of roasting, is the most critical factor in determining coffee cup quality, followed by species, and much less in terms of animal digestion.

3.2. Glycomics

A method for the analysis of endogenous oligosaccharides using hot water extraction was carried out. Chromatographic and tandem MS data (Figure 1), and after subsequent filtering for non-glycans and duplicates, revealed around 163

glycans (Figure 2). Further characterization of each glycan is on-going.

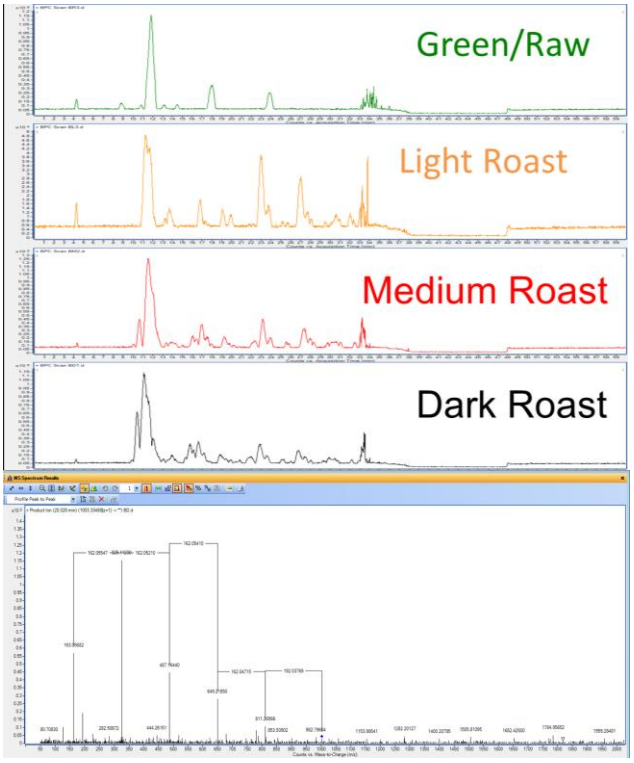

Figure 1. Representative chromatrograms and TandemMS data of Brazil *arabica* coffee.

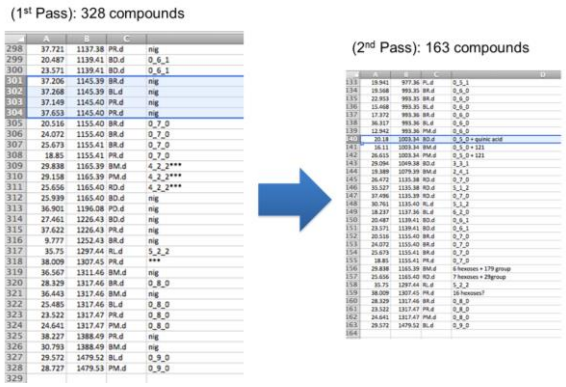

Figure 2. Partial list of characterized glycans after data processing.

3.3. Proteomics/Peptidomics

A simple extraction method for endogenous peptides in coffee was developed, involving ascorbic acid solution and PVPP. Results compared well with several methods based on literature. The procedure provided a simple yet effective way for LC-MS/MS determination of such peptides. Results showed three peptides associated with a pathogenic-response protein (GVKSVEILEGDGGVGTIKLT, GVKSVEILEGDGGVGTIK, and GVKSVEILEGDGGVGT) (Figure 3). Figure 4 further demonstrated that peptides are almost non-existent in roasted coffee, making these (ROASTED coffee) virtually irrelevant for biomarker discovery.

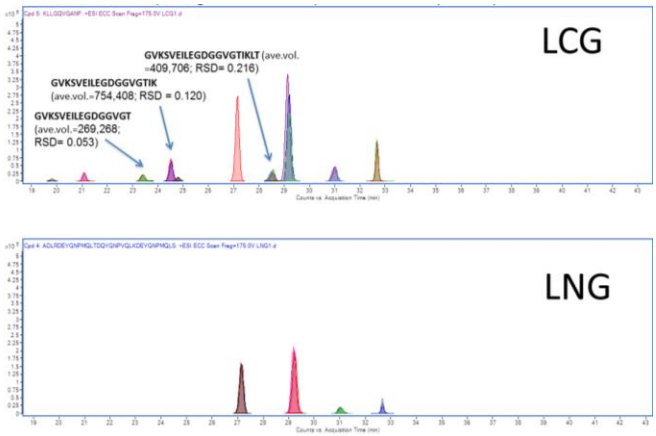

Figure 3. Extracted compound chromatograms (ECC) of endogenous peptides in GREEN *C. liberica* coffee:

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(LCG-Liberica Civet Green; LNG-Liberica Non-civet Green).

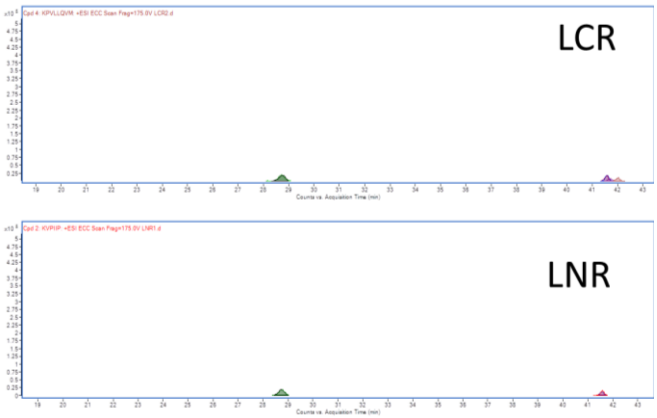

Figure 4. Extracted compound chromatograms (ECC) of endogenous peptides in ROASTED C. liberica coffee: (LCG-Liberica Civet Roasted; LNG-Liberica Non-civet Roasted).

4. CONCLUSIONS

Authentication biomarkers from green beans may be more reliable than from roasted beans. Peptidomics may be more advantageous in establishing authentication biomarker/s for civet coffee

5. ACKNOWLEDGMENTS

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