The Spectro-Electro Array: A Novel Platform for the Measurement of Secondary Metabolites in Botanicals, Supplements, Foods and Beverages - Part 3: Metabolomics

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Overview

Purpose: Investigate the use of a Spectro-Electro Array platform to generate metabolic patterns that could be interrogated using chemometric modeling software. This metabolomic approach was then used to differentiate wines and teas, and to study adulteration and the effects of geography and varietals using fruit juice as the example.

Methods: Gradient HPLC with diode array detection (DAD) and electrochemical (EC) array detection with chemometric modeling.

Results: Principal component analysis (PCA) easily differentiated a variety of wines and teas. Fruit juice adulteration using dilution with another juice, or the inclusion of orange peel or pulp-wash was readily detected. Orange juice samples can be classified by varietal and geographical region.

Introduction

Plants contain an extraordinary diverse range of secondary metabolites including polyphenols, alkaloids, and terpenoids. Although these compounds are not involved in normal growth, development, and reproduction, they still play a crucial role in the organism. For example, polyphenols act as pigments and can protect against disease. Secondary metabolites are also thought to be responsible for the purported health benefits associated with the consumption of botanicals, supplements, some foods, and beverages. Interestingly, polyphenols are associated with the quality and sensory characteristics of tea, wine, and beer.

Gradient HPLC with Spectro-Electro Array detection is both selective and sensitive and can be used to simultaneously measure hundreds of known and unknown secondary metabolites in a sample. Such metabolite profiles contain a wealth of useful information. Changes in the pattern of metabolites, when evaluated using chemometric modeling software, can be used to study: product adulteration, contamination, composition and stability, and in the case of wine and juice, the effects of growing region and differences between varietals used in their production. The application of this metabolomic approach to the study of wines, teas, and fruit juice adulteration will be discussed in greater detail.

Methods

Liquid Chromatography

Pump:	Thermo Scientific Dionex LPG-3400 BM with SR-3000 Solvent Rack
Autosampler:	Thermo Scientific Dionex WPS-3000TBSL
UV Detector:	Thermo Scientific Dionex DAD-3000RS Diode-Array Detector Channel 1: 218 nm Channel 2: 240 nm Channel 3: 254 nm Channel 3: 275 nm
EC Detector:	Thermo Scientific Dionex CoulArray Detector with Thermal Organizer
EC Parameters:	16 channel array from 0 to +900 mV in +60 mV increments
Mobile Phase A:	20 mM monobasic sodium phosphate, 3% acetonitrile, 0.2% tetrahydrofuran, pH 3.35
Mobile Phase B:	20 mM monobasic sodium phosphate, 50% acetonitrile, 10% tetrahydrofuran, pH 3.45
Mobile Phase C:	90% methanol
Gradient:	0-2 min: 2%B/3%C. 30 min: 97%B/3%C, 45 min 97% B/3%C. Curve 7 (concave).
Analytical Column:	Thermo Scientific Acclaim 120, C18, 3x150 mm, 3 µm
Flow Rate:	0.65 mL/min
Injection:	10 or 20 µL

Data Analysis and Processing

Data were analyzed using Thermo Fisher Dionex Chromeleon Chromatography Data System 6.8 and CoulArray software 3.1. EC-array data were transferred to Pirouette® software for chemometric analysis using a CoulArray™ version 2.0 Software Utility (Pattern Recognition Setup Wizard). UV data were tabularized prior to transfer to Pirouette.

Standard Preparation

Stock standards, depending on solubility, were prepared in ethanol, methanol or methanol/water solutions at 1000 µg/mL or 100 µg/mL. Working standards were prepared at 0.2, 0.5 and 1.0 µg/mL in 5% methanol containing 0.02% ascorbic acid.

Sample Preparation

Supplements, culinary herbs, and spices were prepared for analysis by extracting 100 mg of the material with 20 mL methanol. The samples were placed in an ultrasonic bath for 30 minutes and subsequently centrifuged to obtain a clear solution. The solution was further diluted 5x with a preservative solution (10% methanol containing 0.2% ascorbic acid with 0.02% EDTA) prior to analysis by the HPLC System. Wine samples were diluted 1:50 v/v and beer samples 1:1 v/v with the preservative solution. Tea was prepared by steeping 0.5 g of tea with 75 mL of boiling water for 15 min. This solution was further diluted 10x with the preservative solution. Orange juice samples were centrifuged and then filtered through a 0.2 µm filter at 4 °C prior to analysis.

Results and Discussion

The Spectro-Electro Array makes use of both spectrophotometric and electrochemical data. While UV data provides identification and quantitation of the major components in a sample, EC array detection provides additional information. First, the EC array is incredibly sensitive, with low pg LOD. Second, it voltammatrically resolves compounds that co-elute chromatographically. Third, the EC array is fully gradient compatible thereby extending the number of analytes that can be measured in a sample. Fourth, the redox behavior of a compound reacting across the array provides qualitative information and can be used for analyte identification/authentication.

Wine Analysis

A simple experiment examining the metabolite profiles of a selection of red wines was used to evaluate the application of the Spectro-Electro Array platform to metabolomic studies. The general polyphenol method was used to analyze five red wines (four Cabernet Sauvignon samples and one Burgundy sample). Several hundred analytes, including both known (e.g., see Table 1) and unknown compounds, were measured in each sample (Figures 1). Principle component analysis (PCA) was then used to differentiate samples (Figure 2). Although this study is preliminary it does show the capability of the system to differentiate samples by grape varietal/blend and by growing region. This approach is important when trying to authenticate a sample or for identifying product adulteration.

FIGURE 1. A) Wine Sample 1 (Cabernet Sauvignon, Argentina) analyzed by UV at 254 nm. B) Same sample analyzed using EC array detection (presented at low sensitivity). Note that compounds that co-elute by UV detection are fully resolved using EC array detection (e.g., quercetin/cis-resveratrol; kaempferol/isorhamnetin).

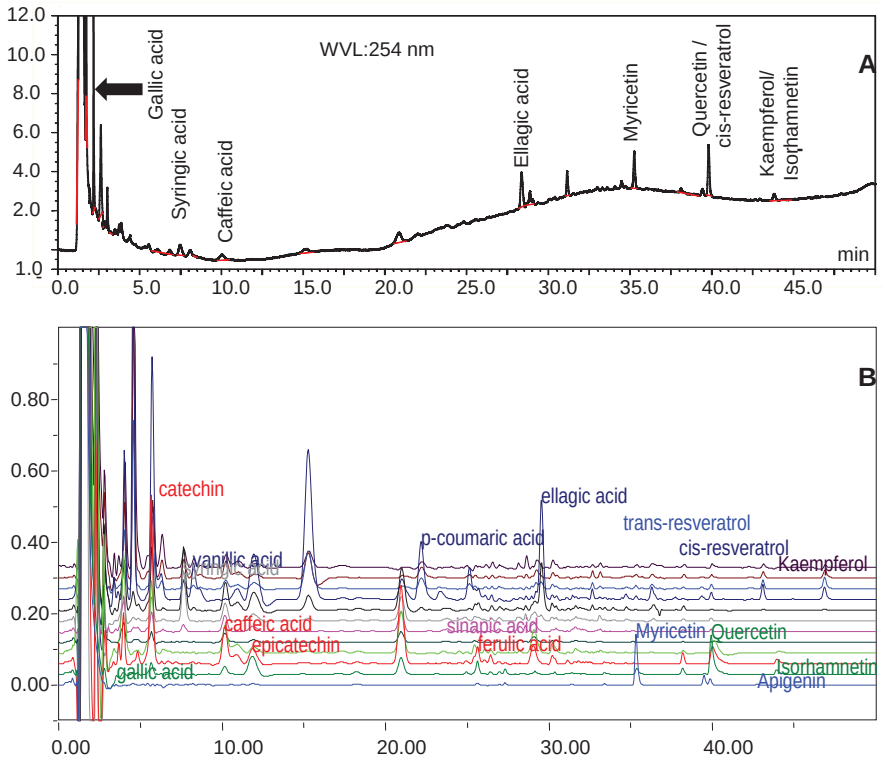
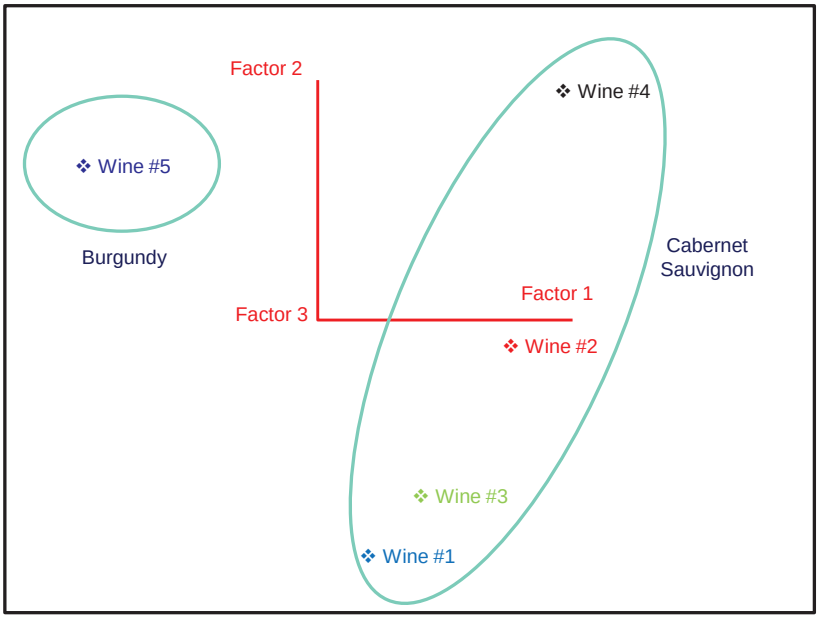


Table 1. Some of the more abundance analytes measured in different wine samples and in good agreement with literature. Wine 1: Cabernet Sauvignon, Argentina; Wine 2: Cabernet Sauvignon, South Africa; Wine 3: Cabernet Sauvignon, US; Wine 4: Cabernet Sauvignon, Chile; Wine 5: Hearty Burgundy, US.

Compound	Wine #1 mg/L	Wine #2 mg/L	Wine #3 mg/L	Wine #4 mg/L	Wine #5 mg/L
Apigenin	16	17.5	9.5	13	41
Caffeic acid	8	13	5	17	3
Catechin	37	26	26.5	24	22
Ellagic acid	52	133	84	94	100
Epicatechin	19	15	16.5	11	4
Ferulic acid	1	1	2	3	2
Gallic acid	57	33.5	37	35	29.5
Isorhamnetin	6	5.5	2.5	6.5	2
Kaempferol	0.5	0.5	0.5	1	1
Myricetin	11	11	5	8	1.5
p-coumaric acid	8.5	16	2.5	14.5	3.5
Quercetin	13.5	15.5	3	14	4
Resveratrol – cis	1	1.5	0.5	2	0.5
Resveratrol – trans	2.5	2	1	2.5	1.5
Sinapic acid	2	2	2	2	2
Syringic acid	19	9.5	9	12	7
Vanillic acid	6.5	4.5	2.5	8	4

FIGURE 2. Initial study showing principal component analysis of wines.



Tea Analysis

A similar approach was also applied to tea analysis to see whether the Spectro-Electro Array platform could differentiate between samples of green, white, and black tea and the bergamot-flavored black tea, Earl Grey. Several hundred analytes were simultaneously measured in each sample, including both known (Table 2) and unknown analytes. A typical EC array chromatogram is presented in Figure 3. To test the reproducibility of the extraction method and the chromatographic method, tea samples were extracted and analyzed several days apart. PCA was then used to differentiate samples (Figure 4). As can be seen, the approach clearly distinguishes between the metabolite profiles of green, white, and black teas. Although black tea and flavored black tea showed some similarity, they did show distinct clustering of samples by PCA. It is unclear whether such difference in their metabolite patterns is a reflection of the addition of flavoring (bergamot orange extract) or differences between the flavored and unflavored black tea base. Regardless, subtle changes in metabolite profiles are easily identified using this approach.

FIGURE 3. Green tea EC array chromatogram presented at low sensitivity showing the highly abundant catechins.

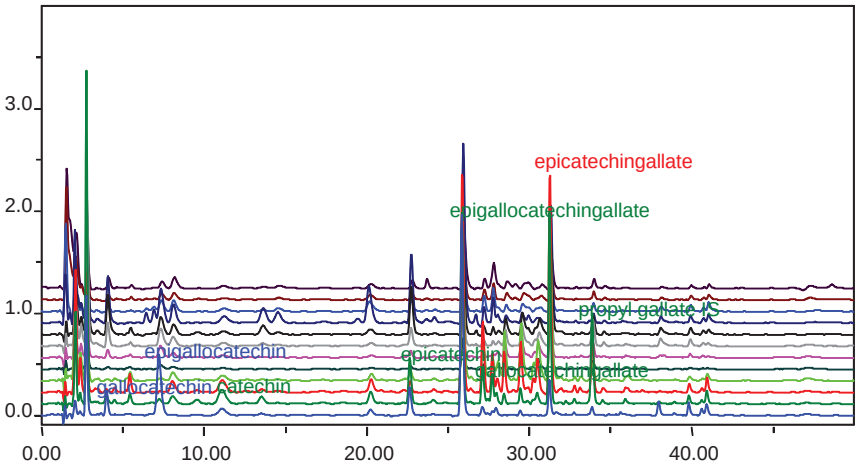
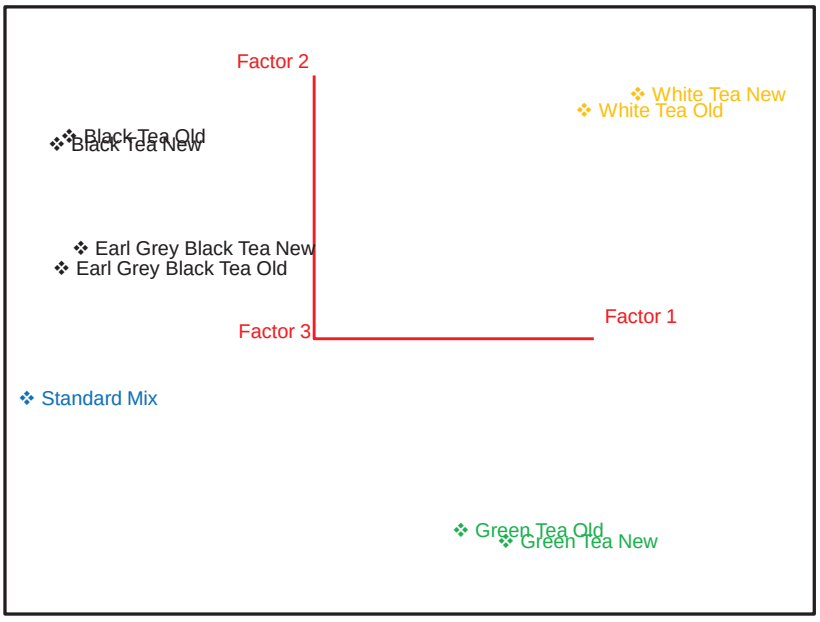


Table 2. Abundance of catechins in different teas. Data are in good agreement with the literature.

Compound	Green Tea mg/g	Black Tea mg/g	White Tea mg/g
Catechin	3.7	3.0	8.1
Epicatechin	50.8	9.3	39.8
Epicatechingallate	65.3	40.6	95.9
Epigallocatechin	49.2	2.5	32.3
Epigallocatechingallate	180	31.3	211
Gallocatechin	18.8	3.2	22.0
Gallocatechingallate	5.9	7.0	3.0

FIGURE 4. Initial study showing PCA of teas.



Juice Analysis

A similar gradient HPLC Spectro-Electro Array analytical method to the polyphenol method described above was used to study fruit orange juice adulteration.[1] The intention of the study was to use this approach along with PCA to try to see the lowest level of adulteration (by blending with other juices, or through the addition of orange peel or pulp-wash) that could be detected in orange juice samples. Figure 5A shows distinct clustering of apple, grapefruit, and orange samples. Blending of as little as 10% grapefruit juice into orange juice could easily be measured. Similarly, blending as little as 10% orange peel or 10% pulp-wash into orange juice could be detected (Figure 5B).

Finally, data from the Spectro-Electro Array platform can be analyzed and displayed as a nearest neighbor dendrogram showing the relationship between orange varietals and geographic location of where the oranges were grown (Figure 5C).

FIGURE 5. Measurement of orange juice adulteration by blending with other juices (A) or by addition of orange peel or pulp wash (B) using the Spectro-Electro Array and PCA. Nearest neighbor dendrogram classifying orange juice samples by varietal and geography (C). Key – Figures 5A and B: GF – grapefruit; OJ – orange juice; OJ10%GF – orange juice blended with 10% grapefruit juice. POOL – equal blend of several orange juice samples.

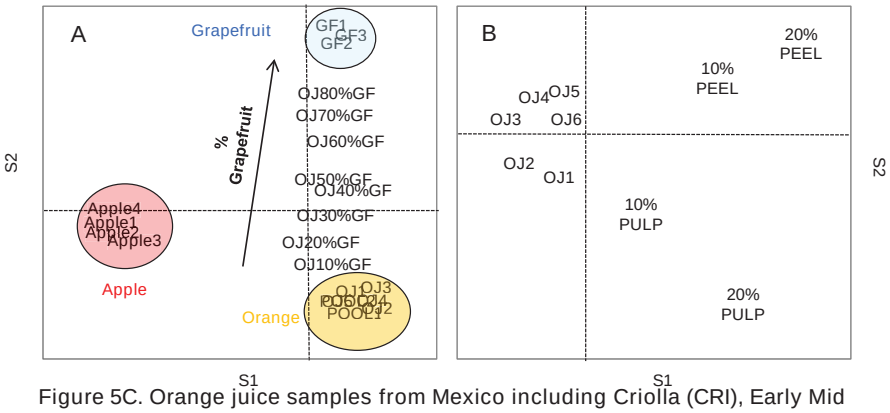
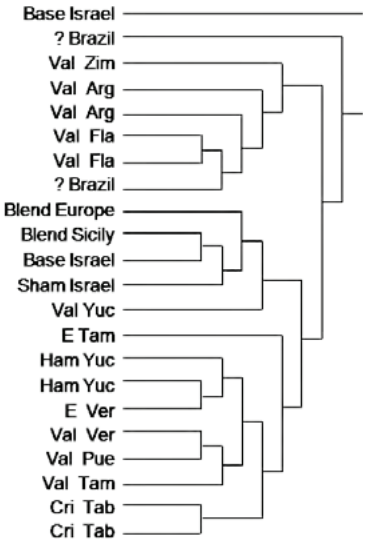


Figure 5C. Orange juice samples from Mexico including Criolla (CRI), Early Mid (E), Hamlin (HAM), and Valencia (VAL) varieties from Puebla (PUE), Tabasco (TAB), Tamaulipas (TAM), Veracruz (VER) and Yucatan (Yuc) regions. SHAM – Shamouti variety; Zim – Zimbawe; ARG – Argentina. Bold – adulteration detected.



Conclusion

- Gradient HPLC with Spectro-Electro Array detection is a simple approach that can be used to generate both targeted and information-rich metabolomic data. Metabolite profiles are generated with sensitive three-dimensional electrochemical array data.
- Metabolomic data can be imported into pattern recognition software and with principle component analysis used to readily identify product adulteration and authenticity.
- Although its application to beverages was highlighted in this poster, this method has major application to other fields including botanical/supplement testing, fuel/oil testing, drug testing, and product counterfeit identification.

Reference

1. Gamache, P.; Acworth, I.; Lynch, M.; and Matson, W. Coulometric Array Detection for HPLC In the analysis of Juice Products in Methods to Detect Adulteration of Fruit Juice Beverages; Nagy, S., Wade, R., Eds.; AGSCIENCE USA, Inc.,.1995; Vol. 1, pp 120-144..

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