Evaluation of Herb and Fruit Juice Adulteration and Authenticity by Coulometric Array Detection and Pattern Recognition Analysis

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Overview

Purpose: Coulometric array detection was used to generate untargeted metabolomic fingerprints of either herbs or fruit juice that could then be interrogated using pattern recognition analysis. The use of this approach to identify potential adulteration was evaluated using oregano herb and orange juice samples.

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

Results: This approach could readily detect as little as 5% adulteration of oregano (intentionally blended with typical adulterants including thyme and marjoram), and of orange juice (either blended with another juice, or by the addition of orange peel or pulp-wash). The technique can also be used to classify the oranges used in juice samples by varietal and geographic growing region.

Introduction

Although the adulteration of herb and fruit juice is a frequent phenomenon, there are few simple methods available for the screening of large numbers of commercial batches of product. The challenge arises from the complexity and variability of genuine material combined with unrelenting conduct of adulteration. Herb and fruit variety, growing region, season, ripeness, and processing methods all contribute to the variability of the authentic product, making unambiguous characterization difficult. Currently, one of the most reliable and applicable authentication methods is based on analytical chemical fingerprinting (untargeted metabolomic) techniques. Gradient HPLC with coulometric electrochemical array detection is particularly suitable for generating information rich metabolite fingerprints of enedogenous electroactive metabolites termed the "redoxome". The analytes that form the redoxome reflect an organism's health or disease state, and in addition for herbs and juices, is comprised of compounds that influence color, flavor, nutritional value, stability and aroma. Such fingerprints can be interrogated using pattern recognition and unsupervised statistical programs such as principal-component analysis (PCA) to evaluate the authenticity or geographic origin of a given sample by comparing its chromatogram with a compiled population of authenticated reference samples in the database. In order to test the applicability of our technique, a generic gradient HPLC method with coulometric electrochemical array detection was developed that was capable of simultaneously measuring several hundred analytes in a single sample. Data were then interrogated using PCA to determine the minimal level of adulteration that could be detected. Three sample sets were chosen to test our approach. First, intentional blending of authentic oregano herb with typical adulterants; second, blending of pure orange juice with other fruit juices; and third, adulteration of orange juice with either peel or pulp wash.

Methods

Liquid Chromatography

Pump:	Thermo Scientific™ Dionex™ UltiMate™ 3000 LPG-3400BM with Solvent Rack SR-3000
Autosampler:	Thermo Scientific™ Dionex™ UltiMate™ 3000 WPS-3000TBSL
EC Detector:	Thermo Scientific™ Dionex™ CoulArray™ Detector with Thermal Organizer Module
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
Analytical Column:	Thermo Scientific™ Acclaim™ 120,C18, 3 × 150 mm, 3 μm
Flow Rate:	0.65 mL/min
Injection:	10 or 20 μL
Data Station:	Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System Software 6.8 SR9 and Coularray™ software 3.1 Pirouette® software V4.5

Sample Preparation

Herbs 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 by a factor 5 with a preservative solution (10% methanol containing 0.2% ascorbic acid with

0.02% EDTA) prior to analysis by the HPLC System. Orange juice samples were thawed and diluted with deionized (DI) water to a Brix value of ~11.8. Samples were centrifuged (10,000 g, 4 °C, 5 min.) through a 0.22 µm filter prior to analysis.

Results and Discussion

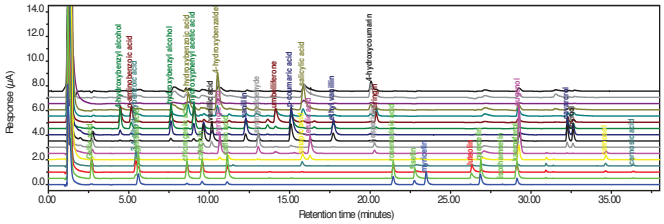
Botanicals, such as herbs and fruit juices, contain an amazingly diverse range of secondary metabolites, many of which are electroactive. Thus coulometric electrochemical array detection is particular useful for generating data rich metabolomic fingerprints. Electrochemical detection is renowned for being both highly sensitive and selective. The coulometric electrode array (CoulArray) detector also offers unique resolution that comes from the high sensitivity of the flow-through porous graphite working electrodes. When these electrodes are used in series in an array, compounds are resolved based on their voltammetric behavior. Compounds detected by the CoulArray detector are resolved based both on their chromatographic and voltammetric (redox) behavior. Furthermore, these parameters can be used to identify and authenticate a compound. The CoulArray detector is fully gradient compatible, thereby extending the number of compounds that can be measured in a sample.

As part of method development, we targeted 37 compounds that can occur endogenously in the samples under evaluation. These compounds showed a wide variety of chemical structures. Their name, retention times, and dominant electrochemical channel are listed in Table 1. Figure 1 shows a typical chromatogram of these standards and illustrates analyte separation and voltammetric resolution by a 16-channel CoulArray detector. The measurement of these compounds was used to check the performance of the analytical system.

TABLE 1: Standard mixture - identification, retention time, and array channel.

Peak No.	Compound	Retention Time minutes	Dominant EC Channel
1	Gallic acid	2.68	2
2	4-Hydroxybenzyl alcohol	4.44	10
3	p-Aminobenzoic acid	5.08	11
4	3,4-Dihydroxybenzoic acid	5.44	4
5	Gentisic acid	5.57	2
6	2-Hydroxybenzyl alcohol	7.57	10
7	Chlorogenic acid	8.64	2
8	4-Hydroxybenzoic acid	8.71	13
9	p-Hydroxyphenyl acetic acid	9.05	10
10	Catechin	9.57	2
11	Vanillic acid	10.16	8
12	4-Hydroxybenzaldehyde	10.52	13
13	Syringic acid	10.66	6
14	Caffeic acid	11.13	2
15	Vanillin	12.27	9
16	Syringealdehyde	12.95	7
17	Umbelliferone	14.1	11
18	p-Coumaric acid	15	9
19	Sinapic acid	15.78	5
20	Salicylic acid	15.85	13
21	Ferulic acid	16.28	6
22	Ethyl vanillin	17.6	9
23	4-Hydroxycoumarin	20.1	16
24	Hesperidin	20.28	7
25	Naringin	20.4	11
26	Rosemarinic acid	21.34	2
27	Fisetin	22.77	2
28	Myricetin	23.5	1
29	Luteolin	26.3	3
30	Quercetin	26.7	2
31	Kaempferol	29	2
32	Isorhamnetin	29.15	2
33	Eugenol	29.2	6
34	Cavarcrol	32.3	9
35	Thymol	32.6	8
36	Carnosol	34.5	5
37	Carnosic acid	36.37	4

FIGURE 1: Standard mixture with 16-channel coulometric electrode array detection.



After some preliminary data processing (peak alignment and data concatenation) using the pattern recognition wizard in CoulArray software, the 16-channel EC array data were then imported into Pirouette software for PCA and cluster analysis. PCA is a mathematical procedure that uses an orthogonal transformation to convert a set of observations of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. It is mostly used as a tool in exploratory data analysis and for making predictive models. The greatest variance by any projection of the data is found in the first coordinate (Factor 1), the second greatest variance is found on the second coordinate (Factor 2), and so on. Thus samples with different patterns in their electrochemical profile are distinguished from each other based on their relative position in three dimensional space. The entire electrochemical fingerprint of all samples was used to investigate authenticity and geographical growing region.

Oregano Herb Analysis

The method described above was then used to study oregano herb adulteration. Either marjoram or thyme were used as the adulterants and were blended into oregano at various percentages (5, 10, 20 30, 40, 50, 60, 70, 80 and 90%). This approach was used to identify the lowest possible level of adulteration that could be uncovered using PCA. Figure 2 presents typical CoulArray chromatograms of a) oregano, b) marjoram and c) thyme. As shown in Figure 3, PCA analysis easily classified majoram-blended oregano sample and thyme blended ones into two distinguishable groups. As little as 5% blending of marjoram and thyme was easily distinguished from 100% oregano.

FIGURE 2. EC array chromatogram of oregano herb (low sensitivity presented for clarity)

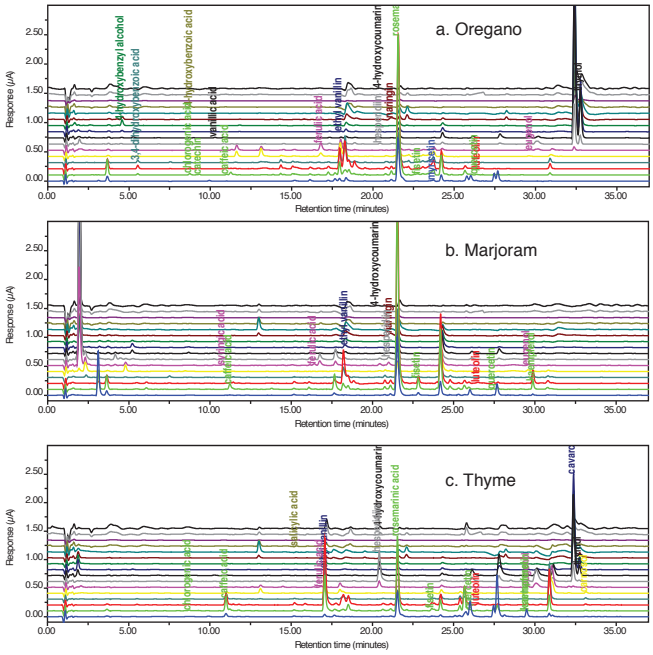
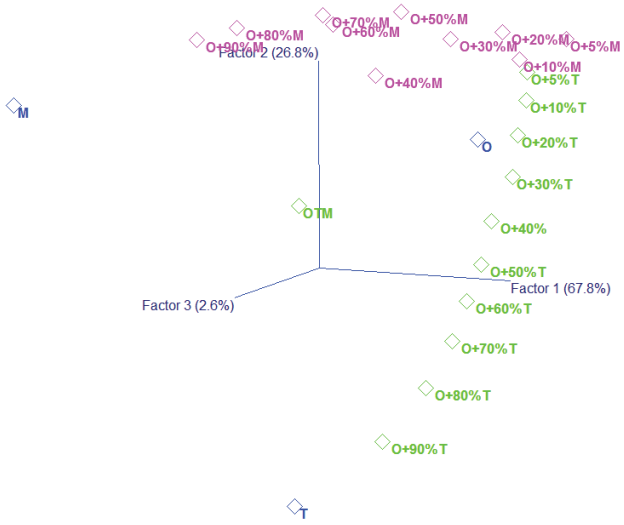


FIGURE 3. PCA plot showing measurement of oregano adulteration by blending with thyme or marjoram (OTM = equal blend of oregano, marjoram and thyme)

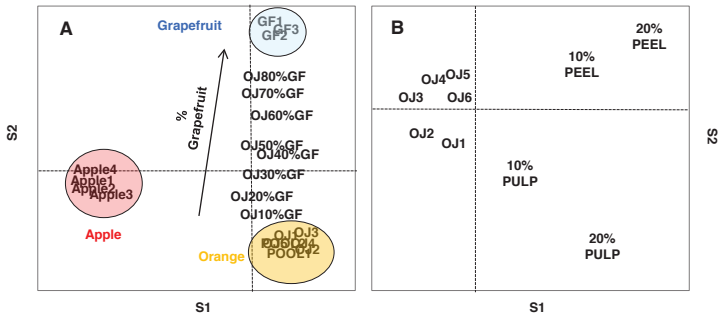


Juice Analysis

A similar gradient HPLC-EC Array analytical method to the polyphenol method described above was used to study orange juice adulteration (by blending with other juices, or through the addition of orange peel of pulp-wash). Figure 4A 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 4B).

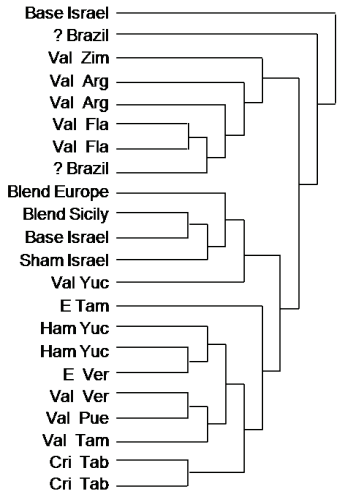
Data from the EC Array platform can also be analyzed and displayed as a nearest neighbor dendrogram and can be used to show the relationship between orange varietals and the geographic location where the oranges were grown (Figure 4C).

FIGURE 4A and B. PCA analysis from measurement of orange juice adulteration by blending with other juices (A) or by addition of orange peel or pulp wash (B) using the CoulArray data with Pirouette software. Nearest neighbor dendrogram classifying orange juice samples by varietal and geography (C).



Key: GF – grapefruit; OJ – orange juice; OJ10%GF – orange juice blended with 10% grapefruit juice. POOL – equal blend of several orange juice samples

FIGURE 4C. 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



Conclusions

- Gradient HPLC with electrochemical array detection is a simple approach that can be used to generate information-rich metabolite fingerprint data. Metabolite profiles are generated with the sensitive three-dimensional electrochemical array.
- Metabolomic data can be imported into pattern recognition software and with PCA analysis used to readily identify product adulteration and authenticity.
- Using this approach, blending as little as 5% marjoram or thyme into oregano herb could easily be identified. Adulteration of orange juice with grape fruit juice, orange peel or pulp-wash could also be detected at as low as 10% level.
- This technique can also be used to classify the oranges used in juice samples by varietal and geographic growing region.

References

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, 120-144.

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