Analysis of Organic Marker Compounds and Hazardous Organic Compounds by GC/MS to Identify Contamination, Counterfeiting and Adulteration of Spices

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ABSTRACT

Food adulteration and counterfeiting continues to grow as a worldwide issue of food safety and economic concern. Spices are one of most commonly adulterated and counterfeited agricultural products in the US. Our previous study determined there were extensive elemental and heavy metals contamination and adulteration in spices. Many of our spice products were identified possibly being highly adulterated or contaminated by metals. In our follow-up Organic study, we focused on the organic markers and toxic organic compounds in our common spices and botanicals (Black Pepper and Cinnamon) in various forms (i.e. spices, teas, condiments and supplements) to determine if these products appeared to be adulterated from an organic compound standpoint as well as an elemental standpoint.

Cryogenic grinding and microwave extraction were employed in sample processing. Samples were extracted for the primary and secondary marker compounds native to each spice group and for any potentially toxic organic compounds (dyes, preservatives, pesticides & industrial residual chemicals). The concentration and identity of compounds were compared across the groupings and to cited concentration references for each marker or compound. Low concentrations of critical markers were found in low cost spice and botanical samples indicating potential adulteration. Samples that were previously suspect by ICP/MS examination were confirmed to be adulterated or economically compromised by reduced or absent concentration of these critical primary and secondary marker compounds. High levels of potentially toxic chemicals were also found in some of the previously suspect spice and spice product samples.

METHODS & MATERIALS

Samples

Samples were purchased from several types of locations including online, health food stores, grocery stores, retain chain stores and discount or dollar stores. The samples ranged in price from a dollar per bottle to more than $20 per ounce. Some products were designated as 'Organic". The products represented seven different spice groups and a multitude of different products including supplements, teas, sauces, mixes, condiments, ground and whole spices. The sample breakdown was as follows:

- Black Pepper (piper nigrum): Whole & Ground Spices
- Cinnamon (Cinnamomum sp.): Whole & Ground Spices, Supplement, Tea

Cinnamon species often used in the cinnamon spices include four different species from different geological locations in the world. The different species are considered to be of varied qualities with the most expensive cinnamon species being C. Verum or 'true cinnamon'. The least expensive cinnamon species is C. Cassia or 'Chinese Cassia cinnamon'. More than 70% of the cinnamon sold in the United States is the cheaper Chinese cinnamon.

Table 1. Species of Plants designated as Cinnamon

Species	Type of Cinnamon
C. cassia	Chinese Cassia Cinnamon
C. burmannii	Indonesian Cassia Cinnamon
C. loureiroi	Vietnamese Cassia Cinnamon
C. verum	True Cinnamon

Table 2. Breakdown of Species of Cinnamon represented in the test Samples

Sample	Type of Cinnamon
Dollar E Ground	Cinnamon Unknown Type
Dollar 5 Ground	Cinnamon Unknown Type
Farmers Whole	Cinnamon Unknown Type
Chain GV Ground	Cinnamon Unknown Type
Retail Ground	Cinnamon Unknown Type
Organic Ground	Cinnamon (*Cinnamomum loureirii*)
Retail Whole	Cinnamon Unknown Type
Supplement	Cinnamon Cassia
Tea	Cinnamon (Vietnam 60%, Indonesia 16%, Indian 10%)

Sample Preparation

Initial Sample Preparation:

- Whole spices were ground using SPEX SamplePrep Freezer Mill.
  - Grinding Conditions:
    - 2 g of Spice
    - Program:
      - Precool = 20 minutes
      - Grind for 5 cycles (2 min/cycle)
      - Each cycle = 2 min. cooling
      - Impact rate= 16 impacts/second
- Powdered or ground spices were tested as purchased.
- Supplement capsules were opened and weighed out.
- Teas, sauces and condiments were tested as purchased.

Sample Digestion:

- Samples were extracted using a CEM Mars 5 microwave.
  - Microwave conditions
    - MarsXpress Vessels
    - 1-1.5 g sample
    - 10 mL EtOH
      - 15 minute ramp to 130 C
      - 30 min Hold
      - Stirring used

Materials:

- SPEX CertiPrep Standards:
  - SPEX CLPS-I90
  - Marker Standards
  - *Can-Terp-1 & 2
  - *Marker compounds
    - Primary Marker Compounds
      - Piperine (Black Pepper)
      - Capsaicin (Red Peppers)
      - Cinnaldehyde (Cinnamon)
    - Secondary Marker Compounds
      - a & b-Pinene
      - beta-Carophyllene
      - D-3-Carene
      - D-Limonene
      - Linalool
      - Eugenol
      - Coumarin

Instrumentation:

- Agilent 5890 GC with 5973
  - GC-MS in scan mode with EIC (35-450 m/z)
  - CV-5 capillary column (30 m x 0.25 mm x 0.25 µm)

Method Design:

Our previous study of spices was designed to evaluate the metal content in the spices for evidence of contamination by heavy metals or for adulteration and counterfeiting by notable concentrations of wear or additive metals. This study targeted the same spice samples to quantify the characteristic primary and secondary market compounds for each spice group.

RESULTS

Black Pepper

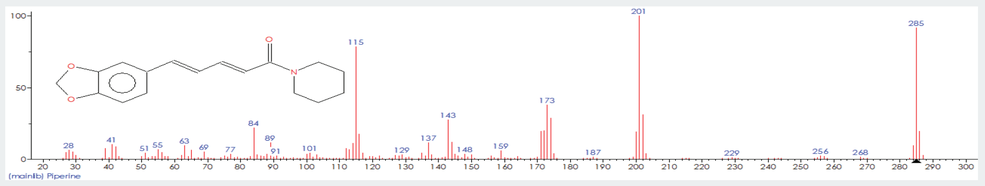


Figure 1. GC/MS Spectrum for Piperine (NIST Database)

The primary marker compound for black pepper is piperine. Piperine and it's isomer, chavacine are alkaloid compounds responsible for the strong acrid pepper odor and flavor. Piperine compounds are found naturally in black pepper in concentrations ranging from 3-10% by mass. The samples of black pepper analyzed contained between 1-10 % piperine. The lower cost ground peppers purchased at the dollar stores and farmer's markets contained the least amount of the primary marker compound (1-4%). The more expensive whole, retail and organic black pepper samples contained between 6-10 % of piperine.

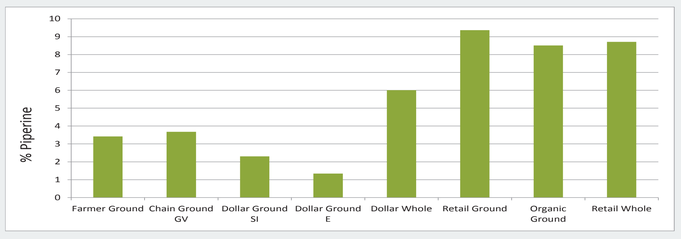


Figure 2. Piperine Content in Black Pepper Samples (mass %)

The secondary marker compounds for black pepper are a mix of common terpenes including: a & b-pinene, b-carophyllene, D-3-carene, and D-limonene. The largest concentration of the secondary marker compounds are found in the retail and organic samples. The less expensive dollar store and farmer's brands have smaller amounts of these secondary compounds including a&b-pinene, 3-Carene, and D-limonene.

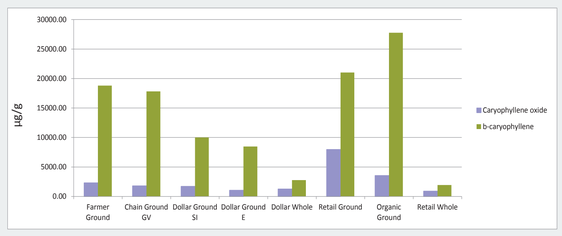


Figure 3. Black Pepper Markers

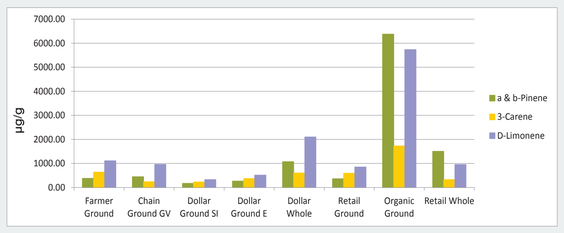


Figure 4. Black Pepper Markers

Cinnamon

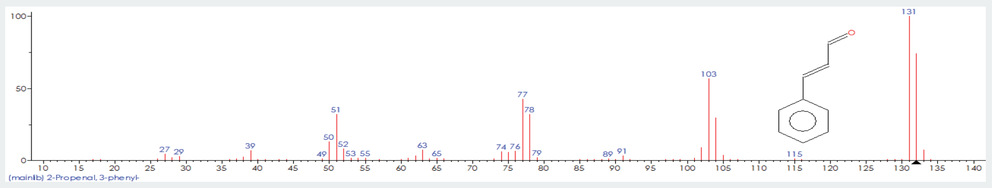


Figure 5. GC/MS Spectrum for Cinnamaldehyde (NIST Database)

The primary marker compound for cinnamon is cinnamaldehyde. Cinnamaldehyde is an unsaturated aldehyde responsible for the characteristic flavor and fragrance of cinnamon. Cinnamaldehyde is cited as being between 1-3% of the bark by mass and can be up to 90% of cinnamon essential oils. The samples of cinnamon contained between 0.5% and 2.1% Cinnamaldehyde. The lowest concentrations were found in the low cost dollar store and farmer's brands. The highest concentrations were found in the organic ground cinnamon and the cinnamon tea. The cinnamon supplement contained just over 1% cinnamaldehyde.

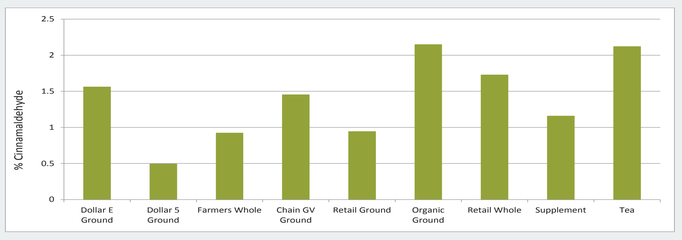
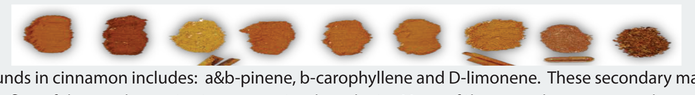


Figure 6. Cinnamaldehyde Content in Black Pepper Samples (mass %)



The secondary marker compounds in cinnamon includes: a&b-pinene, b-carophyllene and D-limonene. These secondary marker compounds are terpenes which often provide fragrant or flavorful secondary notes to many natural products. Many of the secondary compounds were not detected in the samples. Only the whole stick samples retained the majority of the secondary marker compounds. Alpha & beta-pinene were not detected at all in the ground samples except for the cinnamon supplement. The highest levels of b-caryophyllene were found in the ground samples. The cinnamon tea did not contain any of the secondary marker compounds.

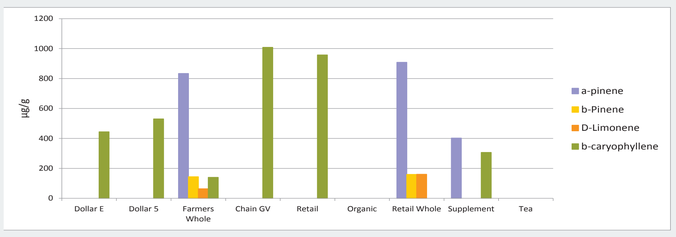


Figure 7. Cinnamon Markers

There are two marker compounds, eugenol and coumarin, present in certain species of cinnamon that could be used to identify the different species of cinnamon. While eugenol is a compound which can give products added flavor and fragrance, coumarin is potentially toxic.

Table 3. Average coumarin content

Species	Type of Cinnamon	Avg. cited Coumarin Content
C. cassia	Chinese Cassia Cinnamon	0.31 g/kg
C. burmannii	Indonesian Cassia Cinnamon	2.15 g/kg
C. loureiroi	Vietnamese Cassia Cinnamon	6.97 g/kg
C. verum	True Cinnamon	0.017 g/kg

The samples tested all contained measurable amounts of coumarin. The samples which contained the highest coumarin levels were the organic ground cinnamon, the retail whole cinnamon and the cinnamon tea. The cinnamon tea was reported to contain C. loureiroi, C. burmannii and 'Indian cinnamon'. These varieties of cinnamon contain the highest reported amounts of coumarin of all the species of cinnamon. The organic brand of cinnamon was reported to be C. loureiroi which has the highest cited amounts of coumarin of the cinnamon species. The retail whole spice did not report a species of cinnamon.