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App Note

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

Abstract

Food adulteration and counterfeiting continues to grow as a worldwide issue of food safety and economic concern. Spices are one of the most commonly adulterated and counterfeited agricultural products in the US. Our previous study determined extensive elemental and heavy metals contamination and adulteration in spices. Many of our spice products were identified as 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, and industrial residual chemicals). The concentration and identity of compounds were compared across the groupings 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, retail 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 various geological locations around the world. The 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 Cassia cinnamon.

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

Species	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 for 20 minutes
    - Grind for 5 cycles (2 minutes per cycle)
    - Each cycle = 2 minute 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 samples
    - 10 mL EtOH
        - 15 minutes ramp to 130 °C
        - 30 minutes hold
        - Stirring used

Materials:

- Spex CertiPrep Standards
  - Spex CLPS-I90
  - Marker Standards
  - Can-Terp-Mix 1 & Mix 2
- Marker Compounds
  - Primary Marker Compounds
    - Piperine (Black Pepper)
    - Cinnaldehyde (Cinnamon)
- Secondary Marker Compounds
  - a & b-Pinene
  - b-Carophyllene
  - d-3-Carene
  - d-Limonene
  - Linalool
  - Eugenol
  - Coumarin

Instrumentation

- Agilent 5890 GC and 5973 MS
  - 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.

Black Pepper

The primary marker compound for black pepper is piperine. Piperine and it 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 farmers 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.

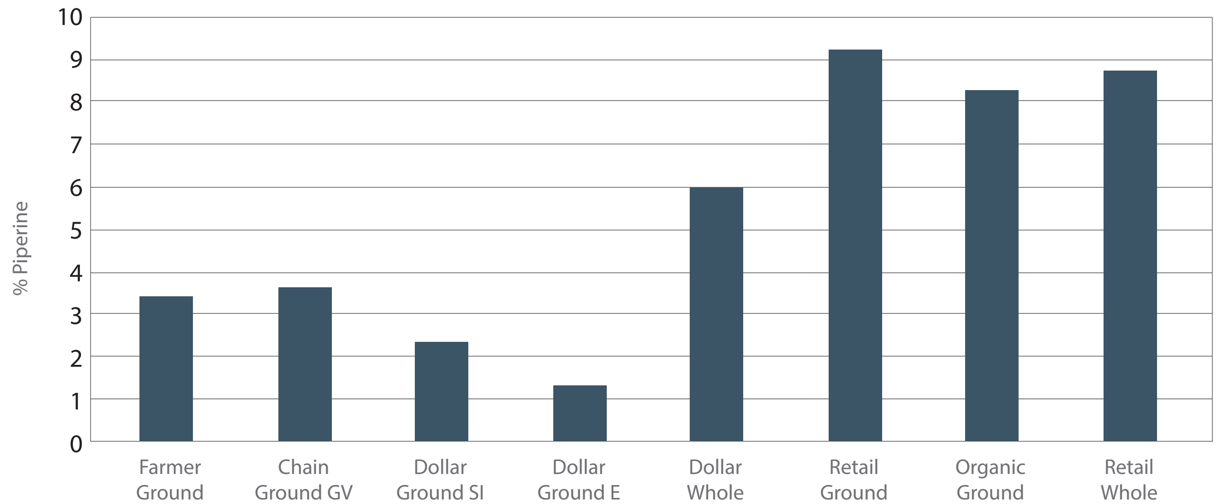

Figure 1. Piperine content in black pepper samples (mass %)

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 farmers brands have smaller amounts of these secondary compounds including a & b-pinene, d-3-carene and d-limonene.

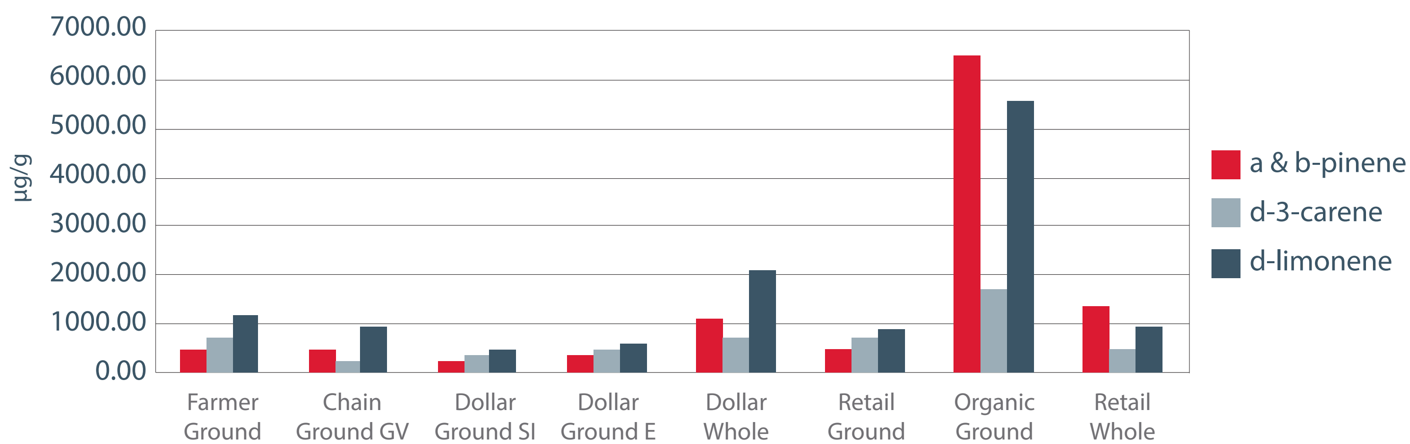

Figure 2. Major Black Pepper Terpene Markers.

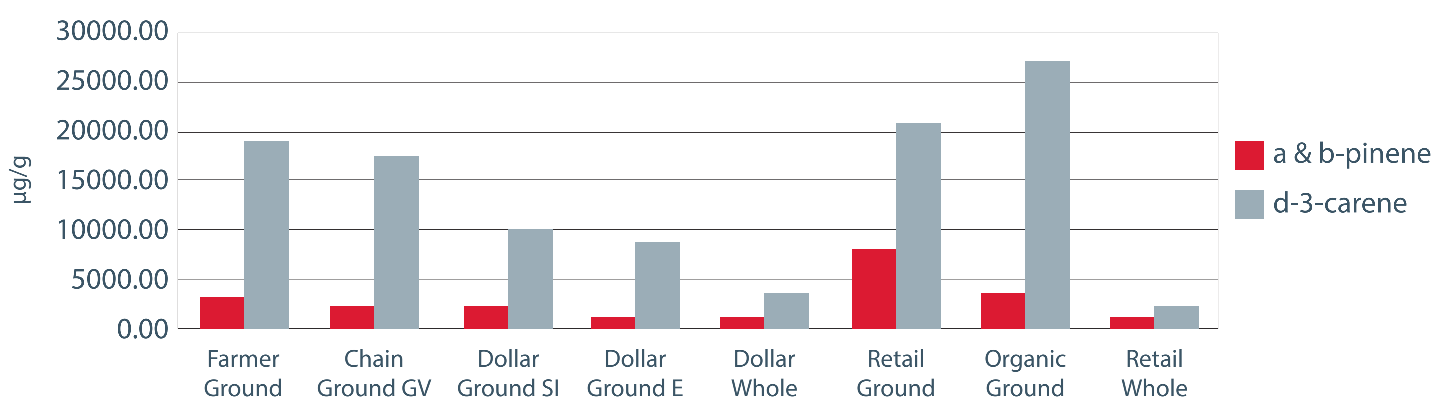

Figure 3. Minor Black Pepper Terpene Markers.

Cinnamon

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 farmers brands. The highest concentrations were found in the organic ground cinnamon and the cinnamon tea. The cinnamon supplement contained just over 1% cinnamaldehyde.

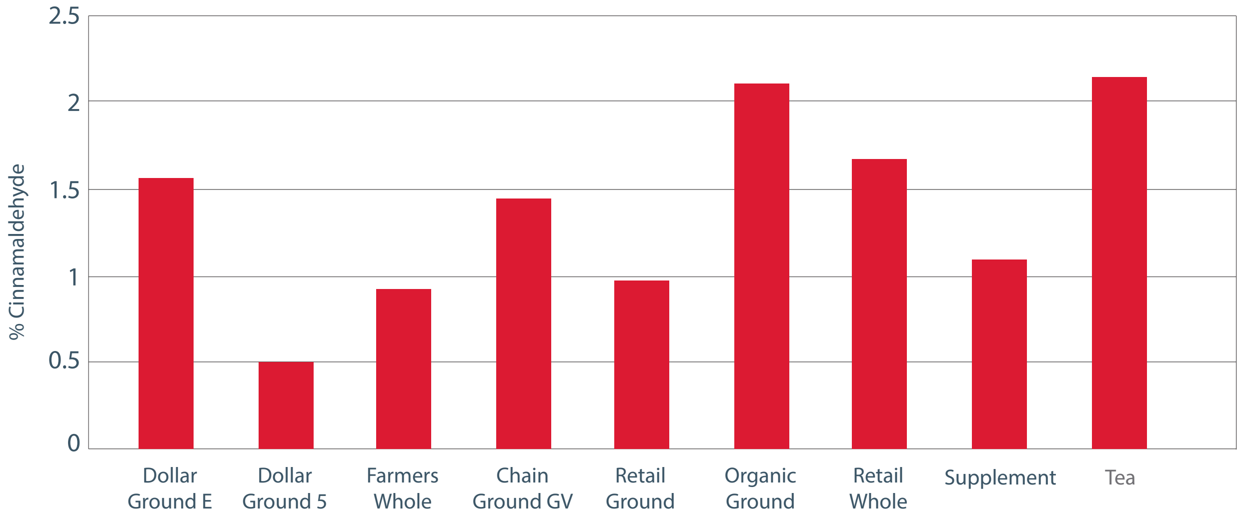

Figure 4. Cinnamaldehyde Content in Black Pepper Samples (Mass %)

The secondary marker compounds in cinnamon includes alpha and beta-pinene, b-carophyllene and d-limonene. These secondary marker compounds are terpenes which often provide fragrance 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 and beta-pinene were not detected at all in the ground samples except for the cinnamon supplement. The highest levels of b-carophyllene were found in the ground samples. The cinnamon tea did not contain any of the secondary marker compounds.

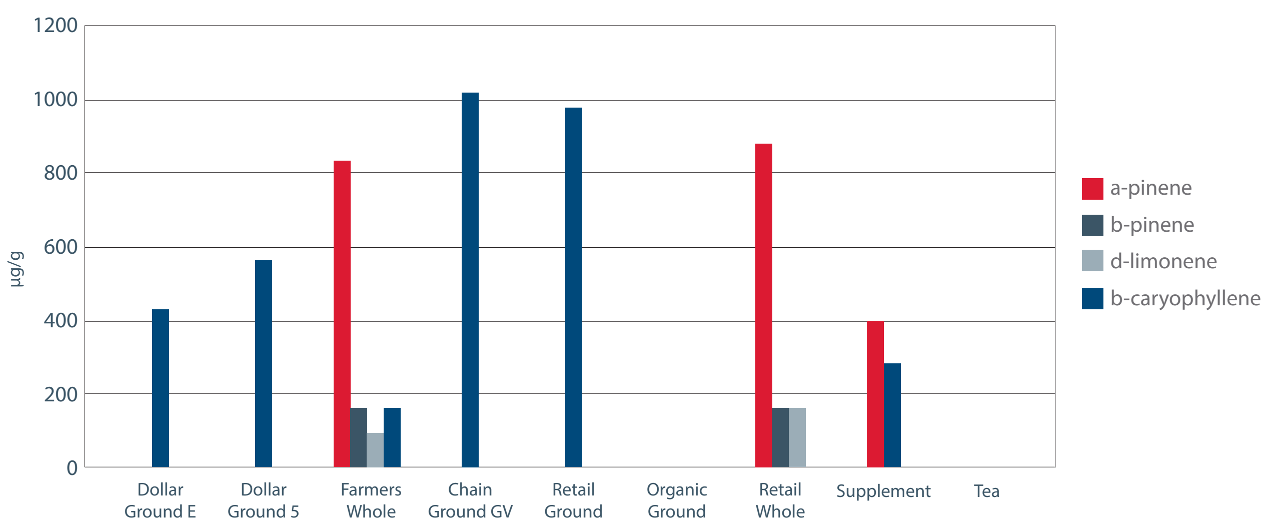

Figure 5. Cinnamon Terpene Markers.

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	Average 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.

Conclusion

The primary marker compound for black pepper, piperine, was found in all samples of black pepper showing some component of the spice was actually the cited product. The lower cost ground spice, however, had a significantly lower level of the piperine compound than the more expensive whole and ground pepper spices. In addition to the lower levels of piperine in the less expensive spices, there were less of the secondary flavor marker compounds - b-caryophyllene, caryophyllene oxide, alpha and beta-pinene, d-3-carene, and d-limonene. This reduction of marker compounds shows a lack of the fine flavor compounds which could be the result of loss through age and grinding or prove adulteration and counterfeiting of black pepper spices.

Cinnamon samples showed a similar pattern when it came to the concentration of the primary marker compound, cinnamaldehyde. The highest amounts of cinnamaldehyde were found in the more expensive spices and spice products. There was a general lack of the secondary marker compounds in all of the ground cinnamon spices and products. The whole cinnamon spices had the greatest variety of secondary marker compounds suggesting that the grinding of the spices was possibly responsible for the loss of the secondary flavor and fragrance notes.

The results suggest that the cheaper spice samples obtained from the dollar store and farmers markets contained the least amount of all the marker compounds. This reduction of marker compounds suggest some of these products could possibly be adulterated in some way which reduced the marker compounds. It is also possible that the age and grinding of the samples caused a reduction of the marker compounds.