NON-TARGETED ANALYSIS OF MUSHROOM-CONTAINING COFFEE PRODUCTS USING ION MOBILITY-HIGH RESOLUTION MASS SPECTROMETRY

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INTRODUCTION

- In recent years, the health benefits of mushrooms—including anti-inflammatory effects[1] and improved cognitive function[2]—have led to huge growth in the number of food and supplement products containing so-called "functional" mushrooms.
- There is a wide variety of coffee products currently on the market that are blended with extracts from an assortment of different mushroom species.
- In this study, we characterized mushroom coffee products containing up to three mushroom species: chaga, cordyceps, and lion's mane. The chemical fingerprints were compared with that of traditional coffee.

METHODS: SAMPLE PREPARATION

- Samples were purchased from retail outlets, including: four mushroom coffee products (each containing two or three of the target mushrooms; Table 1), two traditional instant coffee products (T-A & T-B), and capsules of individual mushroom extracts (chaga, cordyceps, lion's mane).
- Each product was dissolved in hot water following the package directions; powdered supplements were similarly dissolved in water to mimic coffee preparation.
- Prepared samples were centrifuged and diluted 10-fold with water prior to analysis.
- All traditional and mushroom coffee samples were mixed to create a pooled sample. Samples were analyzed in triplicate in random order.

Mushroom Coffee Product Designation	Mushroom Contents	Additional Ingredients
M-A	Lion's Mane	Mint, rosehips, rhodiola extracts
M-B	Chaga & Cordyceps	Eleuthero extract
M-C	Lion's Mane, Chaga & Cordyceps	
M-D	Lion's Mane & Chaga	

Table 1. Mushroom coffee ingredients (according the package labels)

METHODS: LC ANALYSIS

LC: ACQUITY™ UPLC I-Class PLUS
Column: Atlantis™ Premier BEH™ C18AX
(1.7 µm, 2.1 × 150 mm)
MPA: Water + 0.1% formic acid
MPB: Acetonitrile + 0.1% formic acid
Column Temp: 25°C
Flow Rate: 0.3 mL/min

	%A	%B
Initial	100	0
3.0	100	0
6.0	75	25
11.0	2	98
13.0	2	98
13.2	100	0
17.0	100	0

Table 2. LC Gradient

METHODS: MS ANALYSIS

MS: SYNAPT™ XS
Acquisition Mode: HDMS^E
Polarity: Negative
Capillary Voltage: -1 kV
Mass Range: 50 – 1200
Scan Time: 0.15 s
Transfer Cell Collision Energy: 20 – 60 V
IMS Wave Velocity: 1000 – 550 m/s

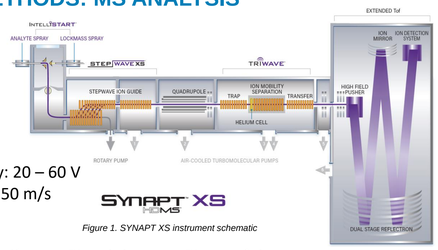

Figure 1. SYNAPT XS instrument schematic

Data were processed using Driftscope™ and the UNIFI™ app of the waters_connect™ software platform. Multivariate analysis was performed using EZinfo.

RESULTS & DISCUSSION

Coffee is a complex matrix with highly polar constituents that are challenging to separate chromatographically (Fig. 2). Orthogonal ion mobility separation was essential for adequately resolving the polar constituents (Fig. 3). IMS also proved useful for identifying chemical signatures, in particular oligosaccharides that were enriched in cordyceps capsules and mushroom coffees (Fig 3).

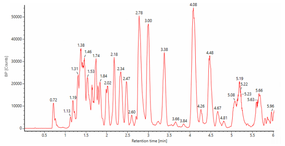

Figure 2. Example base-peak chromatogram for sample M-D showing the chromatographic separation only (RT 1-6 min).

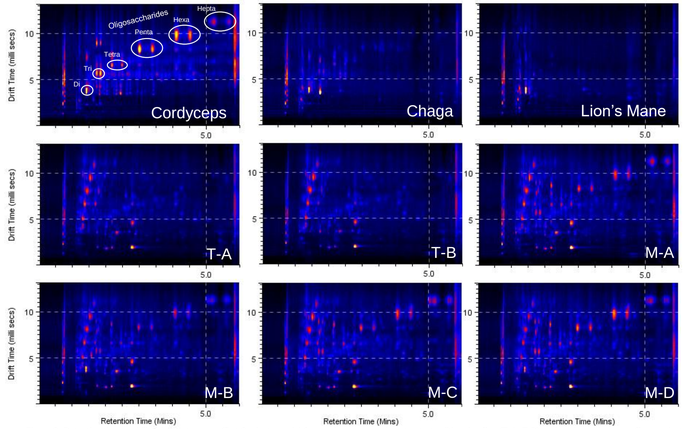

Figure 3. Retention time-mobility chromatograms (first 6 minutes only) for mushroom capsule extracts, traditional coffee (T-A, T-B) and each mushroom coffee product (M-A to M-D); oligosaccharide markers are indicated on the cordyceps sample according to oligomer size; similar signatures were observed for each mushroom coffee.

RESULTS & DISCUSSION

Principal component analysis was performed on all samples (Fig. 4). Replicate injections were closely clustered, indicating reproducibility of the method. Mushroom coffees were clearly distinguished from traditional coffee, as well as the individual mushroom capsules. Whereas the chemical fingerprints of the traditional coffees were essentially identical, some variation among the mushroom coffees was observed.

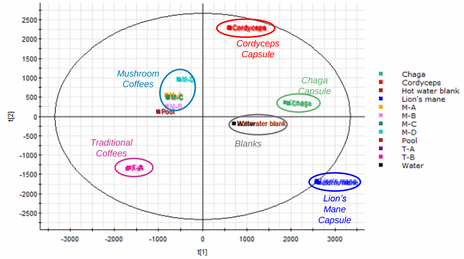

Figure 4. Principal component analysis of tested coffee products, mushroom capsules and blanks (based on 884 markers detected between RT = 1-6 min only).

Comparison of Traditional Coffee vs. Mushroom Coffee

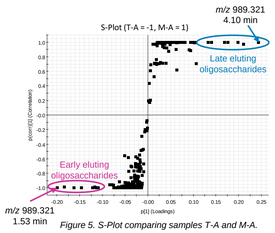

Figure 5. S-Plot comparing samples T-A and M-A.

An S-plot (Fig. 5) indicates that the most distinguishing markers of both traditional and mushroom coffees were oligosaccharides (e.g., hexaose, *m/z* 989.321, Fig. 6). However, the oligosaccharide isomers in mushroom coffee elute later, as shown by hexaose XICs (Fig. 7). Oligosaccharides in mushrooms are believed to confer a range of health benefits, including anticancer and antibiotic properties and as prebiotics supporting the gut microbiome.[3,4]

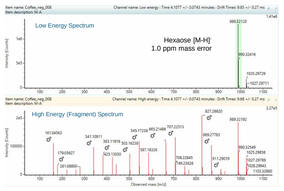

Figure 6. RT- and drift-aligned mass spectra (low and high energy) for the hexaose peak at 4.1 min.

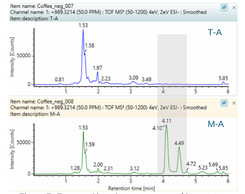

Figure 7. Extracted ion chromatograms of hexaose isomers (m/z 989.321) for traditional coffee (T-A, top) and mushroom coffee (M-A, bottom)

RESULTS & DISCUSSION

Comparison Between Mushroom Coffee Products

The markers that most distinguished M-B from M-A were identified as trehalose adducts (by comparison to an authentic standard). Trehalose is a disaccharide commonly found in mushroom species.[5] The peak response of trehalose varied significantly across the mushroom coffees (Fig. 9), with little correlation to the specific mushroom species present (Table 1). In contrast, trehalose content was comparable across the cordyceps, chaga, and lion's mane capsules (Fig. 9).

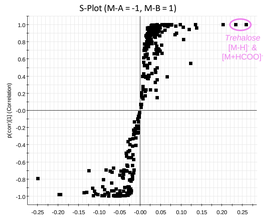

Figure 8. S-Plot comparing samples M-A and M-B; markers corresponding to two adducts of trehalose are indicated.

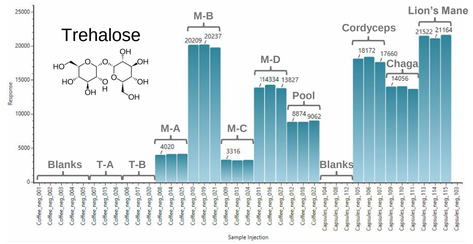

Figure 9. Summary of trehalose (a disaccharide) peak response across all tested samples.

CONCLUSION

- **A series of oligosaccharides were observed in mushroom coffees and cordyceps capsules, but not chaga or lion's mane capsules, nor in traditional coffee. These oligosaccharides may contribute to enhanced health benefits of mushroom coffee.**
- **Trehalose disaccharide displayed variability among the mushroom coffees, but was relatively consistent across the cordyceps, chaga, and lion's mane capsules.**
- **Taken together, these observations suggest that the extraction process (and/or the parts of the mushrooms extracted) governs the oligosaccharide content more than the specific mushroom species included in a product, though further testing is needed.**

References

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2. Ba, D.M., et al. Mushroom intake and cognitive performance among US older adults: the National Health and Nutrition Examination Survey, 2011-2014, British J. of Nutrition (2022), 128, pg 2241
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