Using Carbon Isotope Ratios to Detect Adulteration in Red Yeast Rice Supplements

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Abstract

Red yeast rice (RYR) is marketed as a dietary supplement as it contains monacolin K, a natural cholesterol-lowering statin. To enhance health claims, there is concern that some RYR supplements could be adulterated with the pharmaceutical drug, lovastatin, an HMG CoA reductase inhibitor (statin). Testing for adulteration in RYR supplements is challenging because monacolin K and lovastatin are chemically identical but are derived from different sources. Natural abundances of monacolin K in RYR also vary depending on rice type and fermentation conditions. Lovastatin is fermented from the fungus *Aspergillus terreus* using mostly C_4 plant sources (e.g. corn), whereas monacolin K is derived from the mold *Monascus purpureus* grown on rice, a C_3 plant. These two plant groups have unique stable carbon isotope ratio ($\delta^{13}C$) distributions that are reflected in monacolin K/lovastatin. This study seeks to optimize the preparation procedures to isolate monacolin K/lovastatin from a complex RYR supplement matrix and use stable carbon isotope ratio analysis to differentiate between monacolin K and lovastatin. Samples were screened for monacolin K/lovastatin using liquid chromatography with mass spectrometric detection (LC-MS). Positive samples were fraction collected using high performance liquid chromatography with ultraviolet detection (HPLC-UV) and residual pigments were removed by solid phase extraction using graphitized carbon black. Samples were then analyzed on an Elemental Analyzer-Isotope Ratio Mass Spectrometer (EA-IRMS) for carbon isotopes. To ensure the preparation process does not induce isotope fractionation (a $\delta^{13}C$ value shift), two previously analyzed lovastatin standards with known $\delta^{13}C$ values were added to two RYR supplements without monacolin K to mimic adulterated RYR supplements. No isotope fractionation was detected. Monacolin K and monacolin K acid were also measured in RYR supplements and their ratios were compared to their respective $\delta^{13}C$ values. Previous studies suggest monacolin K and monacolin K acid have a fixed ratio in natural RYR products and large deviations may indicate the addition of lovastatin.

Introduction

Red Yeast Rice

Lovastatin/Monacolin K

Background: Red yeast rice (RYR) is marketed as a dietary supplement because it contains **monacolin K**, a natural cholesterol-lowering statin. To enhance health claims, there is concern that some RYR supplements could be **adulterated** with the pharmaceutical drug, **lovastatin**, an HMG CoA reductase inhibitor (statin).

Problem: Differentiating monacolin K and lovastatin is chllallenging because they are structurally and chemically identical.

Project: Develop a framework to **extract and purify** monacolin K/lovastatin for carbon isotope analysis.

1. Using stable carbon isotopes as tracers

Lovastatin is fermented from the fungus *Aspergillus terreus* using mostly **C_4 plant** sources (e.g. corn).

Monacolin K is derived from the mold *Monascus purpureus* grown on rice, a **C_3 plant**.

These two plant groups have unique stable carbon isotope ratios ($\delta^{13}C$) distributions that are inherited in monacolin K/lovastatin.

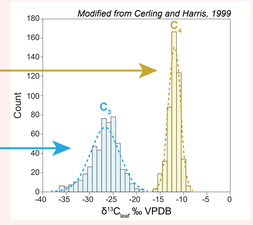
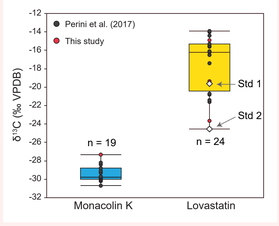


Figure 1: Carbon isotope distrubtion of C_3 and C_4 plants.

2. Lovastatin and Monacolin K have distinct $\delta^{13}C$ values



Pure lovastatin standards and one monacolin K standard were analyzed "neat" on the EA-IRMS. and compared to previously reported values. These distinct, non-overlapping $\delta^{13}C$ values carbon isotope values can be used to differentiate between their natural versus synthetic sources.

Figure 2: The $\delta^{13}C$ values of monacolin K (blue) and lovastatin standards (yellow).

Materials and Methods

- 15 RYR supplements were purchased from local retail stores and screened for monacolin K/lovastatin using LC-MS (see Figure 7).
- RYR-5 and RYR-14 had monacolin K/lovastatin levels < LOD and were designated as "blank" samples (see Section 5).

3. Pigments are coeluting with monacolin K/lovastatin

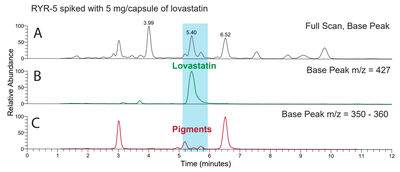
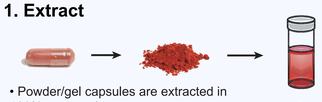


Figure 3: (A) LC-MS data showing full spectra scan of a highly pigmented RYR sample with (B) lovastatin coeluting with (C) pigments, highlighted by the blue shaded region

- EA-IRMS analysis yields bulk $\delta^{13}C$ values of material analyzed, therefore monacolin K/lovastatin must be to be free of other carbon containing components that could bias their $\delta^{13}C$ values.
- Various solvent ratios, gradients, buffers, and column types were explored, but full resolution of lovastatin and all pigments could not be achieved. The addition of solid phase extraction (SPE) step improved the purity.

4. Workflow to extract and purify monacolin K/lovastatin for carbon isotope analysis on the EA-IRMS

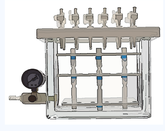
1. Extract



• Powder/gel capsules are extracted in 100% methanol

2. Fraction Collection on the HPLC-UV

Instrument: Agilent 1200 HPLC System
Column: Phenomenex Luna 5 µm C18(2) 100 Å, 150 x 10 mm
Flow Rate: 3.5 mL/min
Injection Volume: 275 µL
Column Temp: 25 °C
Run Time: 9 minutes
Detection: 237 nm, 470 nm
Mobile Phase: Isocratic, 85:15 ACN:Water



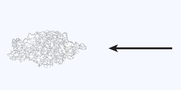
3. Solid Phase Extraction (SPE) using a vacuum manifold

Cartridge: 500 mg/6 mL graphitized carbon black (GCB)
Condition: 5 mL ACN
Equilibrate: 5 mL Water
Load: 2 mL of fraction collected sample
Elute: 15 mL 90% MeOH

• Pigments stay in the GCB and monacolin K/lovastatin is eluted

4. Carbon isotope analysis

• Bulk extracts are measured on the EA-IRMS



• Evaporate to dryness

Results and Discussion

5. Method validation shows no significant isotope fractionation

- Two lovastatin standards with known $\delta^{13}C$ values (●) were added to RYR-5 and RYR-14 to create 'adulterated' samples at varying concentrations. Fraction collected samples (●) show isotope fractionation due to coelution with an unknown red pigment. Fraction collection plus SPE (●) removes both the red pigment and isotope fractionation (0.09 ± 0.4‰ for Std-1 and 0.04 ± 0.1‰ for Std-2).

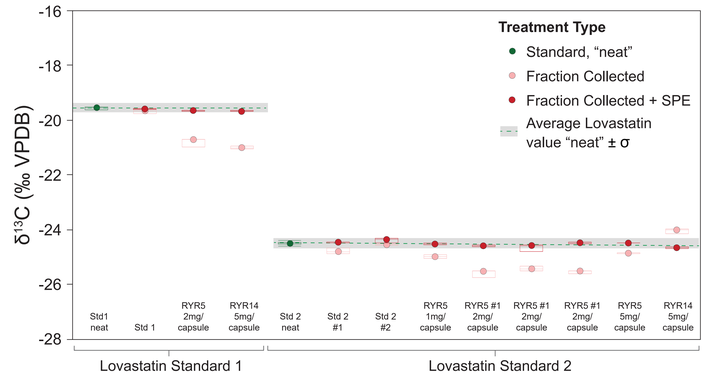


Figure 4: $\delta^{13}C$ values of standards (green circles), 'adulterated' samples after fraction collection (pink circles), and 'adulterated' samples after fraction collection plus SPE (red circles).

6. $\delta^{13}C$ analysis reveals adulterated RYR supplements

- Based on the δ13C values of lovastatin and monacolin K standards from Perini et. al., and our study, values more positive than -27‰ were considered to be lovastatin (synthetic).
- 4 RYR samples with monacolin K/lovastatin > 1 mg/capsule were selected for isotope analyses, revealing two authentic and two adulterated RYR supplements.

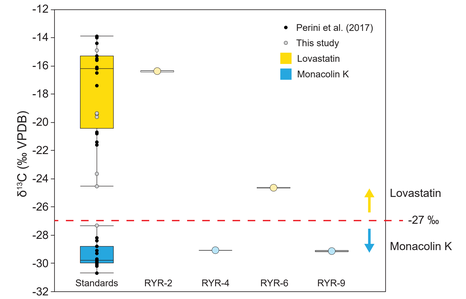
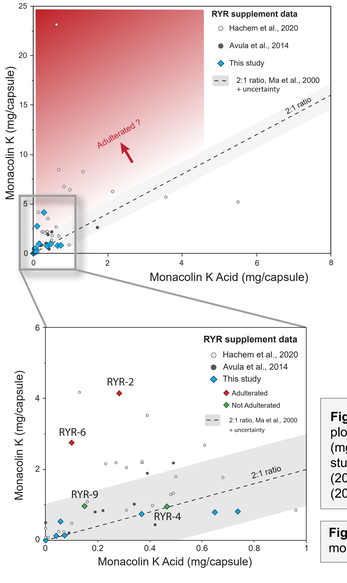


Figure 5: $\delta^{13}C$ values of monacolin K and lovastatin standards (right) and a subset of RYR samples with concentrations above 1 mg/capsule (left).

7. Using MK:MKA Ratios as a qualitative indicator of RYR adulteration



- Studies have suggested that monacolin K (MK) and monacolin K acid (MKA) have a fixed ratio and monacolin K exceeding a certain ratio may be an indicator of adulteration.
- $\delta^{13}C$ values from Figure 5 confirm that the two samples that plot farthest above the 2:1 line (red diamonds) are adulterated and the two samples that plot on/near the 2:1 line (green diamonds) are authentic. The MK:MKA ratio may be a useful screening tool, but more work is needed to correlate the $\delta^{13}C$ values to this ratio.

Figure 6: Monacolin K (mg/capsule) plotted against monacolin K acid (mg/capsule). Data is compiled from this study (blue diamonds), Hachem et al., (2020) (light gray circles), and Avula et al. (2014) (dark gray circles).

Figure 7: Zoomed in plot of monacolin K to monacolin K acid data.

Conclusion

- Carbon isotope analysis can be used to definitively detect adulteration in RYR supplements
- A small portion of the RYR supplements purchased as part of the preliminary market basket survey were adulterated
- Fraction collection followed by SPE using GCB is a robust method for extracting and purifying monacolin K/lovastatin from RYR supplements for carbon isotope analysis without causing significant isotope fractionation
- The MK:MKA could possibly be a useful qualitative approach to rapidly screen for adulteration, but may become less reliable as monacolin K/lovastatin nears 1 mg/capsule and should be verified using carbon isotopes analysis.

Future Work

- Analyze more samples including RYR supplements and RYR powders used for supplements.
- Validate the method with at least one additional laboratory.
- Utilize LC-IRMS and/or GC-IRMS for a more direct analysis approach.

References

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