Mushroom β-Glucan Authentication and Quantification Using NMR Spectroscopy

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01 Introduction

Among several health benefits of functional mushrooms and their extracts, immunomodulatory effects are associated with the presence of polysaccharides having β -glucan with β(1,3)/β(1,6) linkages. Acid and enzymatic digestion methods employed to determine beta glucan and other carbohydrates' concentrations can be unreliable if not done correctly. Also, precise structural information is lacking since these methods are tailored solely to deliver β-glucan quantification and in some cases α-glucan. Consequently, other chemical attributes of ingredients and products cannot be probed with digestion methods alone. A novel NMR spectroscopy-based β-glucan authentication and quantification approach has been developed, which renders accurate quantitation of β-glucan concentration in extracts and products of several commercially important fungal species. β-glucan structural authenticity is rendered by ^{13}C NMR spectroscopy of the polysaccharide extracted in its native structural form, and quantification is achieved using ^{1}H NMR spectroscopy. These samples are further authenticated for the species and product matrices using NMR metabolomics and multivariate statistical analysis. A consolidated NMR spectroscopy-based approach to quantify β-glucan in mushroom products, authenticate mushroom species, verify product matrix and support supply chain for complete material traceability is presented.

02 Materials and Methods

Mushroom extracts of different species were supplied by Nammex® and were extracted using proprietary Purity-IQ (PIQ) methods. Their ^{1}H NMR spectral data were applied against the PIQ Global Spectral Registry for species authentication, rendered through multivariate statistical analysis. β-glucan was extracted in its native structural form and quantified using newly-developed methods. The β-glucan content was cross-validated using Megazyme® beta-glucan assay kit from Nammex. The ^{1}H and ^{13}C NMR spectra were acquired on a Bruker 400 MHz NMR spectrometer at PIQ.

03 Species and Product Matrix Authentication

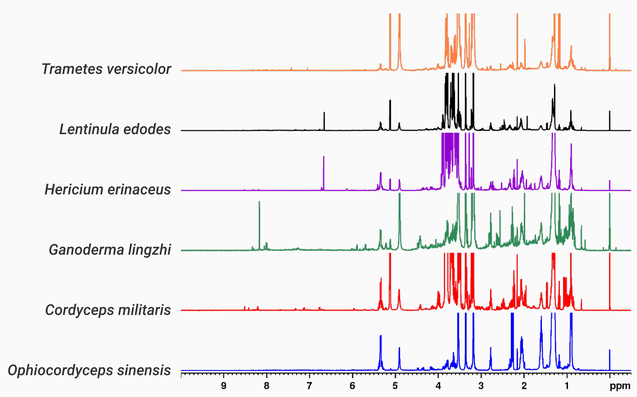


Figure 1. ^{1}H NMR spectra of different mushroom vouchers

- ^{1}H NMR spectral profiles are unique to each fungal species (**Figure 1**), and function as chemical fingerprints.
- NMR spectra are highly reproducible, identical among authentic samples of same matrix type, and match the chemical fingerprint of respective species (**Figure 2**).
- Species authenticity of a product in question is derived by assessing the similarity of the test spectrum sample to the chemical fingerprint of that species and through statistical analysis which employs the PIQ spectral library (PIQ Global Registry).
- The species and product format information available in the PIQ Global Registry is used to authenticate the products for these aspects simultaneously.

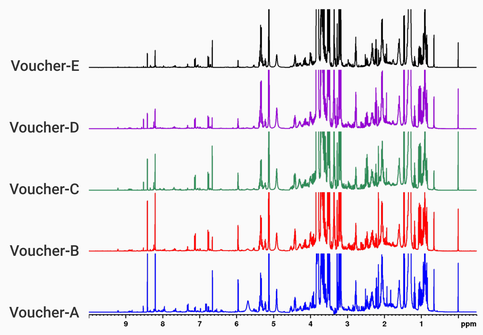
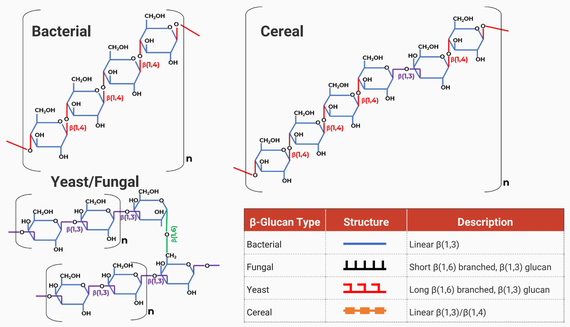


Figure 2. ^{1}H NMR spectra of five *Cordyceps militaris* vouchers

NMR spectroscopy simultaneously probes different product aspects, and a typical workflow with respect to the β-glucan analysis involves:

Species and product matrix validation
↓
β-glucan extraction and authentication
↓
β-glucan quantification

04 β-Glucan Extraction for Authentication



β-Glucan Type	Structure	Description
Bacterial		Linear β(1,3)
Fungal		Short β(1,6) branched, β(1,3) glucan
Yeast		Long β(1,6) branched, β(1,3) glucan
Cereal		Linear β(1,3)/β(1,4)

Figure 3. Structures of β-glucan from different origins

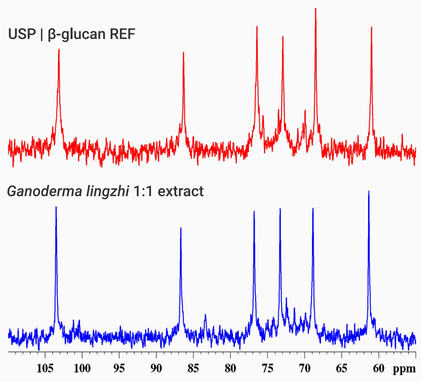


Figure 4. ^{13}C NMR spectra of USP β-glucan reference (top) and *Ganoderma lingzhi* 1:1 extract (bottom)

- β-glucan from different origins have different structures and linkages (**Figure 3**), hence can be differentiated by NMR.
- The ^{13}C NMR spectral profile of β-glucan extracted from a *G. lingzhi* extract aligns with the reference standard (**Figure 4**) confirming its fungal origin therefore authenticating the product for the presence of fungal β-glucan in the product.
- This method can be used to probe products adulterated with β-glucan of different origin.
- The ^{13}C NMR spectroscopy can be used to:
  - Verify presence of β-glucan in the product.
  - Authenticate the β-glucan for both structure and origin.

05 β-Glucan Quantification

Product	β-Glucan (wt%) NMR	β-glucan (wt%) Megazyme (Acid)
Hericium erinaceus 1:1 extract	28.2	30.4 (H_2SO_4)
Hericium erinaceus 1:1 extract	28.6	30.7 (H_2SO_4)
Trametes versicolor 1:1 extract	52.6	51.1 (HCl)
Cordyceps militaris 1:1 extract - A	9.8	10.5 (H_2SO_4)
Cordyceps militaris 1:1 extract - B	12.6	11.6 (H_2SO_4)

06 β-glucan Quantification in Diluted Products

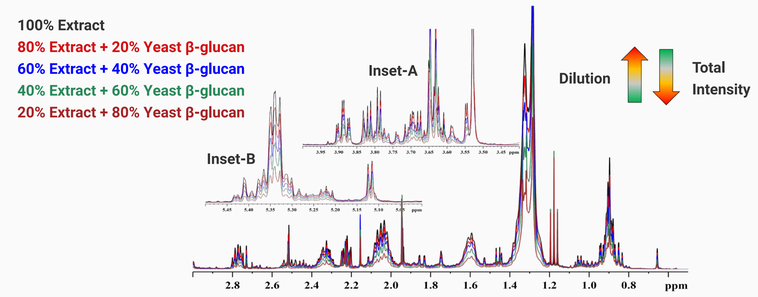
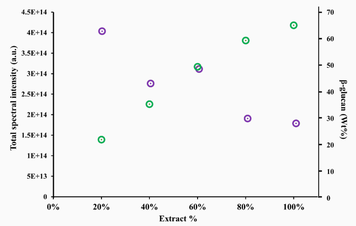


Figure 5. ^{1}H NMR spectra (0.5–3 ppm) of the *Hericium erinaceus* extract and extact+yeast β-glucan blends; Inset-A: Sugar region; Inset-B: [−CH=CH−] groups of fatty acids.



- Dilution of mushroom extracts with carriers can be probed using changes in total spectral intensity vis-à-vis a pure product (**Figure 5**).
- Intentional adulteration of mushroom products with yeast β-glucan to increase β-glucan content can be identified by benchmarking total intensities and β-glucan content in pure products.
- Intensity and β-glucan content plots are inversely proportional in adulterated products (**Figure 6**), therefore allowing for identifying such adulteration.

Figure 6. Total spectral intensity counts (**green circles**) and β-glucan concentrations (**purple circles**) in pure and adulterated products as a function of the extract content.

07 Conclusions

- NMR spectroscopic methods accurately authenticate fungal products for both species and product matrix.
- The origin of β-glucan can be verified using ^{13}C NMR spectroscopy, a strong analytical trait that can cull adulteration practices prevalent in the market
- NMR β-glucan quantification methods are on par with the commonly used acid digestion methods (Megazyme®).
- The results presented here confirm the analytical capabilities of NMR spectroscopy within the fungal products sector in enhancing supply chain traceability and ensuring product authenticity.