

Refining Enzymatic Approaches for β-Glucan Testing in Functional Mushrooms and Derived Products

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INTRODUCTION

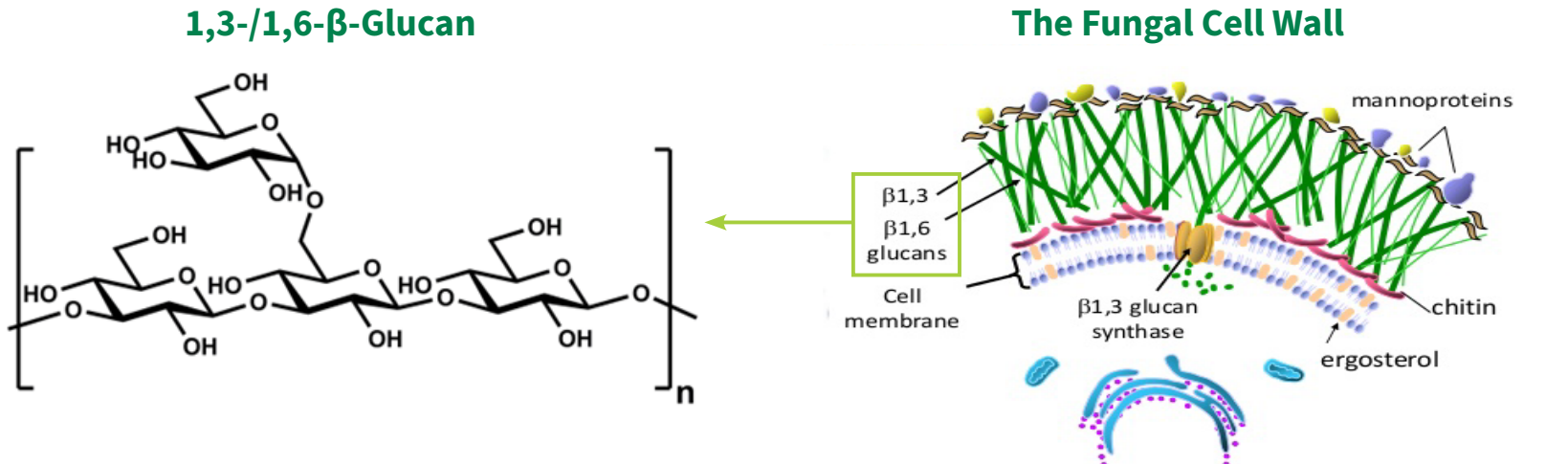
Background

Increased interest in health and wellness is driving rapid growth in the functional mushroom market. Key to their health benefits are 1,3-/1,6-β-glucans, bioactive polysaccharides found in functional mushrooms and derived products.¹

Challenge:

The mushroom and yeast β-glucan testing landscape is evolving. β-Glucan supplements are now available in both solid and liquid forms with emerging ingredients to meet modern demands. β-Glucans from different sources, such as algae, are also gaining industry adoption.

Figure 1. General β-Glucan structure within the fungal cell wall



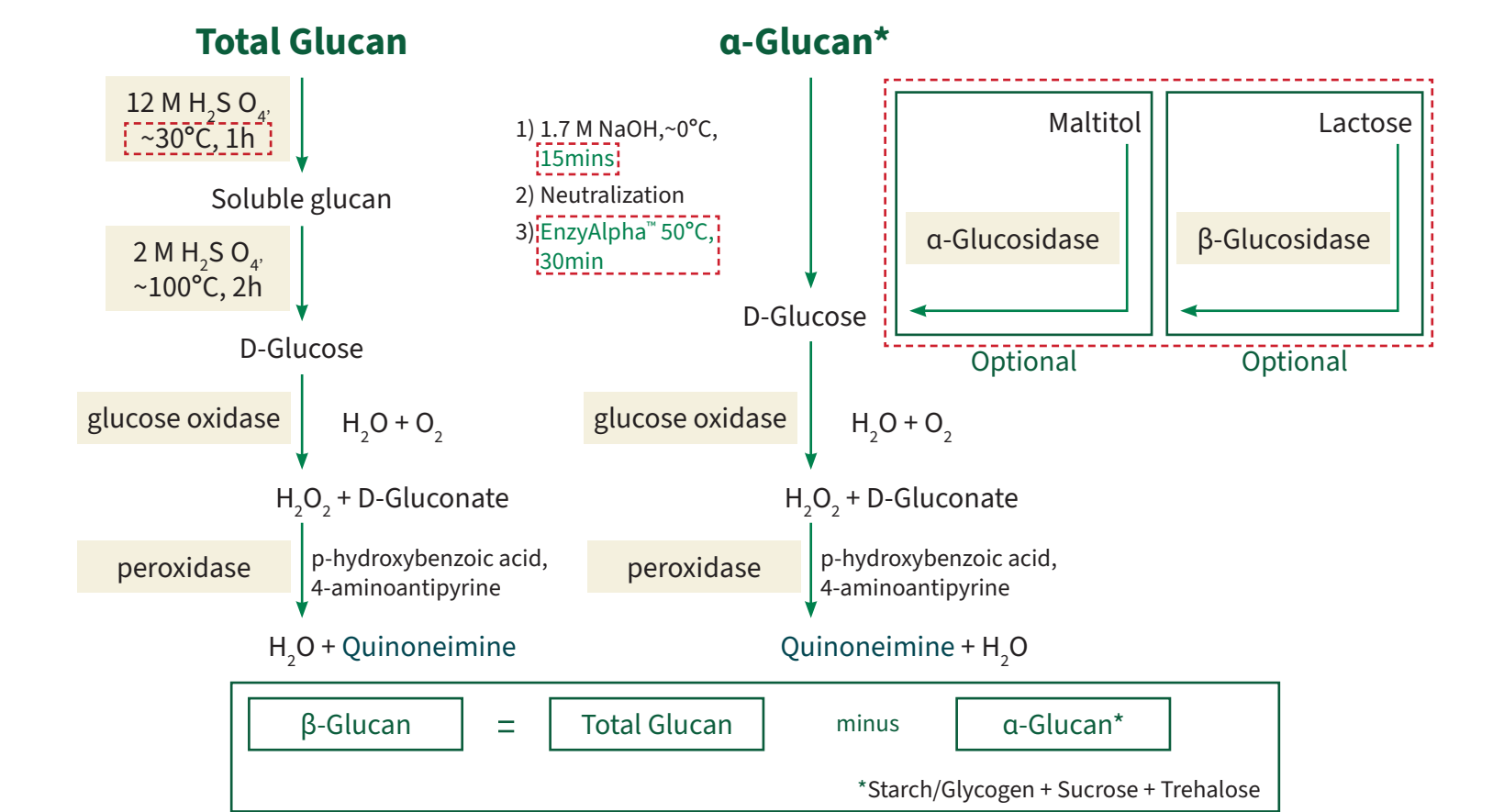
Approach

Building on the work of McCleary et al.², this study refines the indirect enzymatic method for β-glucan analysis. In the absence of globally accepted reference standards for measuring mushroom β-glucans, this enhanced method offers a step towards greater analytical accuracy and standardization, supporting quality assurance and consumer confidence in the rapidly expanding mushroom industry.

MATERIALS AND METHOD

The original enzymatic method (K-YBGL, SKU 70004358), hereafter referred to as YBGL 2023, was re-engineered through systematic evaluation of sample preparation, incubation conditions, enzyme specificity, hydrolysis conditions, and matrix interference testing.

Figure 2. Refined enzymatic methodology. Red highlights indicate updates to the method. These updates have enabled the removal of certain steps present in previous protocols, such as the polishing enzyme step in the total glucan method.



The newly developed procedure demonstrates robust performance across both liquid and solid sample types, enabling β-glucan quantification in a wide range of complex functional mushroom products. Performance has been validated in diverse matrices, including:

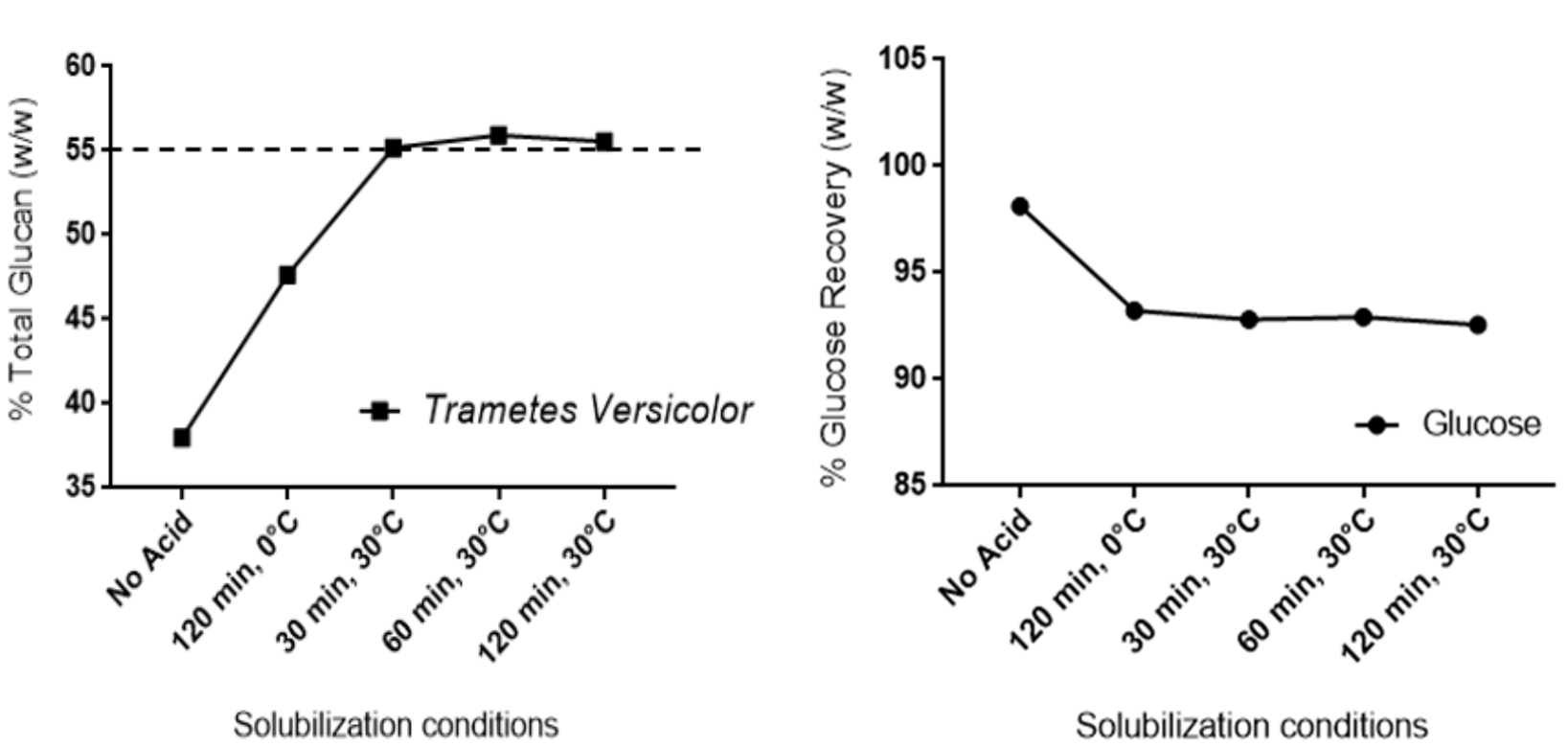
- Mushroom gummies containing maltitol
- Products containing high levels of sugars and starch
- Liquid tinctures

RESULTS

Refined Acid Hydrolysis:

Enhanced solubilization conditions for improved glucose yield and inclusion of Hydrolysis Correction Factor (HCF) to adjust for glucose degradation.

Figure 3. Effect of acid solubilization conditions on Total glucan content of Trametes versicolor and glucose degradation



Total Hydrolysis method comparison:

Total glucan concentrations were quantified using a reference method, ASTM E1758 Standard Test Method for Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography, that employs harsh acidic hydrolysis conditions through an autoclave cycle followed by monosaccharide quantification using HPLC. Results were compared across the refined 2025 method and the 2023 protocol.

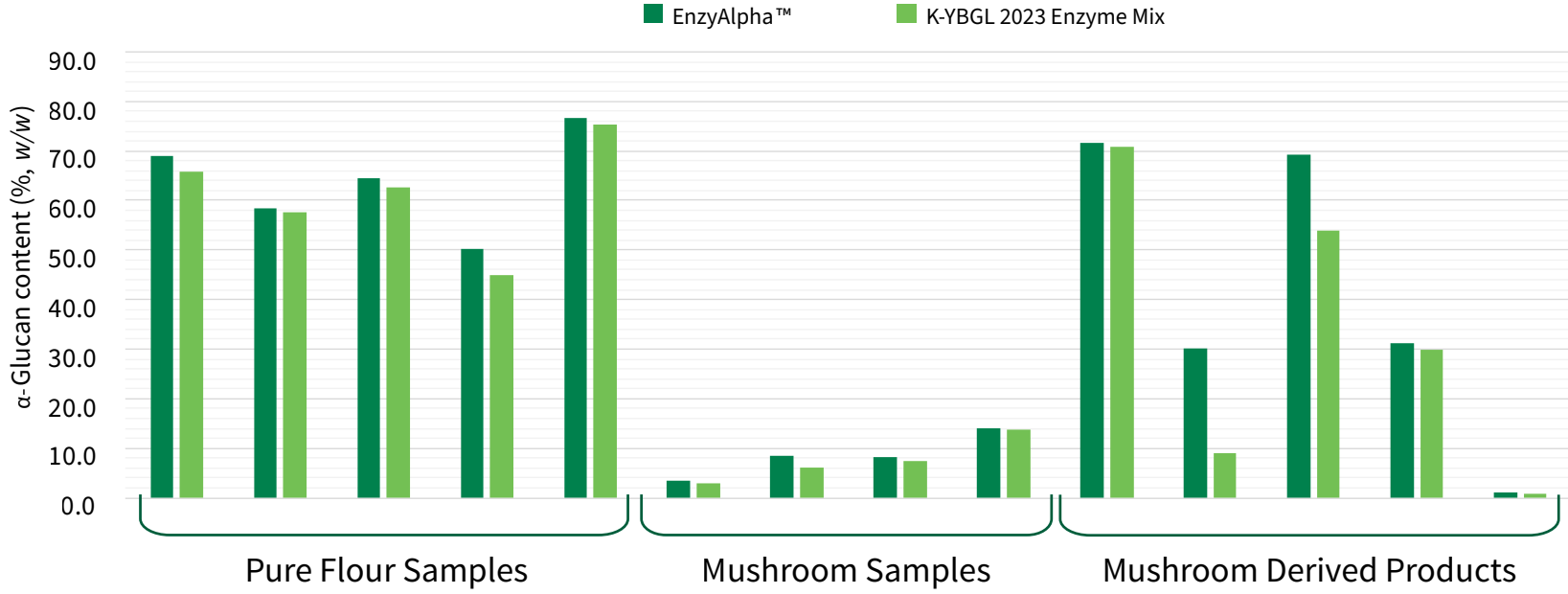
Table 1. Total glucan analysis in mushroom samples using the optimized method, benchmarked against a reference method, ASTM E1758.

Sample	ASTM 1758 Total Glucan (% w/w); [0.93 %]	Recovery vs ASTM 1758 Total Glucan (%)	
		Total Glucan YBGL (2023)	Total Glucan YBGL (2025)
Lion's Mane – <i>Hericium erinaceus</i>	48.7; [0.93 %]	94.4	97.7
Reishi – <i>Ganoderma lucidum</i>	54.7; [0.35 %]	90.7	98.4
Cordyceps – <i>Cordyceps militaris</i>	37.8; [0.10 %]	92.6	95.0
Chaga – <i>Inonotus obliquus</i>	17.6; [0.89 %]	76.2	98.5
Turkey Tail – <i>Trametes versicolor</i>	58.8; [0.32 %]	91.1	99.7
Extract 8:1 - <i>Cordyceps militaris</i>	29.9; [0.51 %]	89.6	96.8
Mushroom Capsule - Chaga	75.5; [0.77 %]	82.9	96.0
Mushroom Tincture	75.0; [2.4 %]	75.0	96.4
Mushroom Gummy	46.6; [1.2 %]	91.2	96.5

EnzyAlpha™ Improved Efficiency:

Improves hydrolysis efficiency in flours, pure mushrooms and mushroom derived products

Figure 4. EnzyAlpha™ showed improved hydrolysis efficiency of 2% - 10% α-glucan in tested samples.



A method for emerging ingredients and products:

Maltitol is commonly utilised as a sweetener in many functional mushroom supplements. Prior to this method revision, maltitol was incorrectly included within the Total Glucan test, resulting in over-estimation of β-glucan in product containing maltitol. The new methodology employs an α-glucosidase (E-TSAGS, SKU 700004243) in a pre-treatment step, quantitatively hydrolysing maltitol for measurement in the α-glucan determination and allowing for subtraction of maltitol interference, thereby affording the correct β-glucan result.

Figure 5a. Extracted mushroom gummy product – HPLC TSK Chromatogram

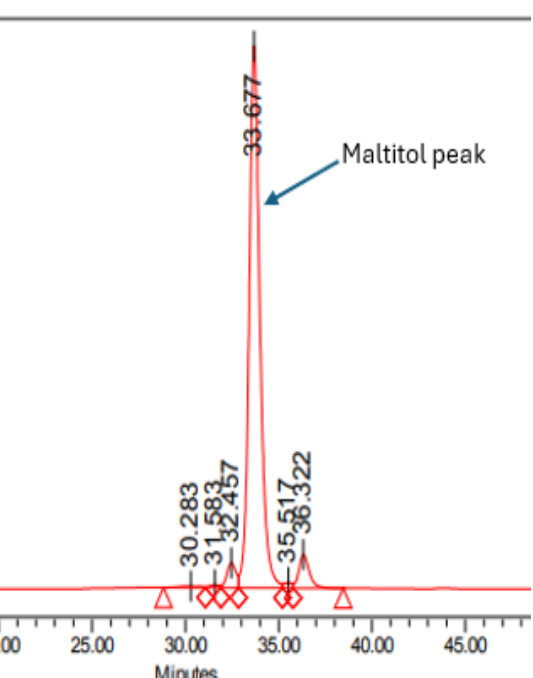
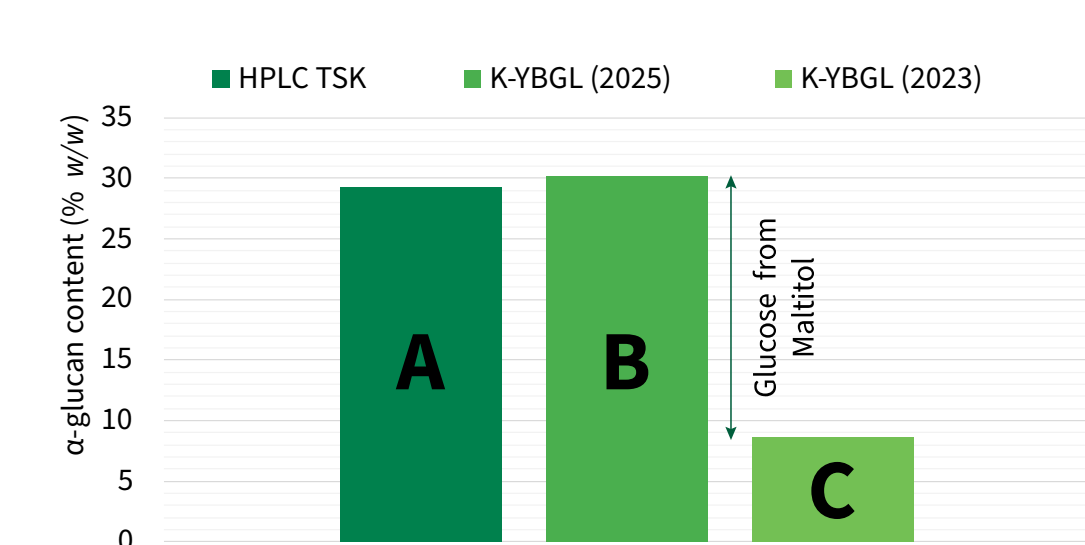


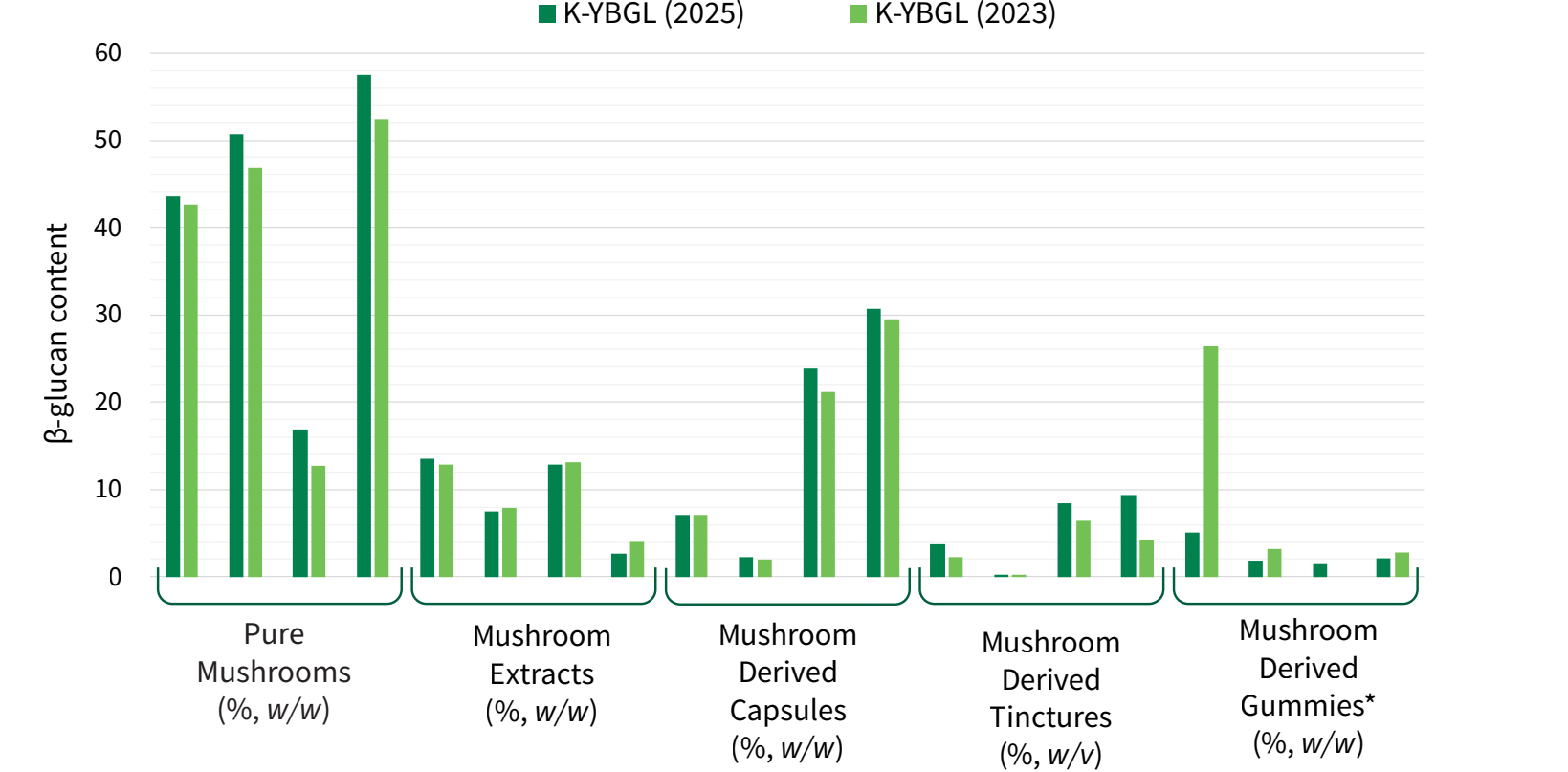
Figure 5b. Comparison of α-glucan results for a gummy product depicting A) the predicted value accounting for maltitol content measured by HPLC, B) the newly developed YBGL 2025 procedure including the α-glucosidase treatment, and C) the legacy YBGL 2023 procedure with no α-glucosidase treatment.



β-Glucan : Method Comparison:

An overall increase in β-glucan levels was observed, except in more complex mushrooms and mushroom-derived products containing maltitol, which showed a greater relative increase in α-glucan content.

Figure 6. Method comparison between legacy mushroom β-glucan method (K-YBGL 2023) and newly developed method (K-YBGL 2025) applied to pure mushrooms, mushroom derived products, including optional methods to capture emerging ingredients. *Samples treated with α-glucosidase for removal of maltitol interference.



CONCLUSIONS

The study describes the only enzymatic method currently available for the quantification of β-glucans in mushrooms and derived products.

- Updated methodology improves accuracy, often yielding higher β-glucan results in mushroom samples, while maintaining assay integrity and reliability.
- Optimized α-glucan degradation cocktail, now enhanced with α-amylase designed to improve performance in complex matrices.
- Condensed run time from 7 to 4.5 hours improves lab efficiency and throughput.
- Compatible with both liquid and solid mushroom products. Optional methods developed to capture emerging ingredients such as maltitol and lactose.
- Fewer pipetting steps reduce hands-on time and lower the risk of manual error, enhancing overall ease of use.
- AOAC PTM planned study with NAMMEX as co-sponsors

REFERENCES

- Driscoll M. et al. (2019) *Cancer Biol. Ther.* 8:218–225.
- McCleary, B. v & Draga, A. Measurement of β-Glucan in Mushrooms and Mycelial Products. *J AOAC Int* **99**, 364–373 (2016).