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Application Note
Version 2

Authentication and Quality Assessment of Yeast Beta-Glucan Products

Using ^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy

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Introduction

Beta glucan (BG), a type of polysaccharide constituting the cell walls of bacteria, yeast, fungi, algae, lichens, and plants such as oats and barley [1,2], is under the limelight owing to its many potential health benefits. These include immune system stimulation and immunomodulation, inflammation reduction, management of cholesterol levels, regulation of blood sugar levels, antimicrobial antitumour effects and stimulation of cellular regeneration, among many others [1–5]. Attributed to these qualities, favourable rheological and other properties, BG has found applications in food & beverage, personal care, pharmaceutical, dietary supplements, and animal feed sectors, resulting in a high demand for the product and tremendous market growth in the recent years. While the estimated value of the BG market in 2023 is USD 501M, it is expected to grow to USD 734M by 2028, with a cumulative annual growth rate of 7.9% [6]. Although all BGs comprise glucopyranosyl units interconnected with glycosidic bonds, the molecular weight of the macromolecule and its three-dimensional conformation, driven by the nature of connectivity between individual monomers, changes between different sources (**Figure 1**). Briefly, plant-derived and bacterial BG have a linear structure with β(1,3) and β(1,4)-D-glucopyranosyl linkages, and ββ(1,3)-alone linear structure, respectively. Yeast and fungal BG have a linear β(1,3)-D-glucopyranosyl backbone with side chains connected through β(1,6) linkages, where the sidechain length is shorter in the latter. These connectivities dictate the therapeutic property and other applications of the BG, therefore, in-depth structural characterization of beta glucan products is warranted. Moreover, there is an absolute need for a sophisticated and reliable analytical tool to assess a product's quality and authenticity, BG origin, associated health claims and evaluate manufacturing/processing practices. Yeast-derived BG is known for immunomodulation effects [7], attributed to its β(1,3)-D-glucopyranosyl backbone. Research suggests that these glycosidic bonds are essential for the BG to function as an immune booster as the mechanism involves detection and binding of the molecule to the Dectin-1 receptor [8] of white blood cells. The receptor has limited affinity for non-linked glucans and plant glucans which have a linear β(1,3) - β(1,4)-D-glucopyranosyl backbone. Furthermore, the length of the β(1,3)-linked chain and the branching ratio (ideally, 0.2-0.33) affects the functionality of the BG [7,9,10]. A detailed account of BG structure-function-relationship is documented elsewhere [7,9–13]. Considering the importance of the structure and sources of BG, it is prudent in the health sector to thoroughly characterize beta glucan products, authenticate them, and determine their quality, for which Nuclear Magnetic Resonance (NMR) Spectroscopy is a well-suited analytical technique [14–18]. This note focuses on the utility of ^{1}H and ^{13}C NMR spectroscopy in assessing the quality of yeast beta glucan and highlights the advantages offered by NMR testing for product characterization. Yeast BG authentication, extraction/manufacturing process monitoring, new formulation implementation, and determination of branching ratio in compliance with the USP method [19] are demonstrated in this note.

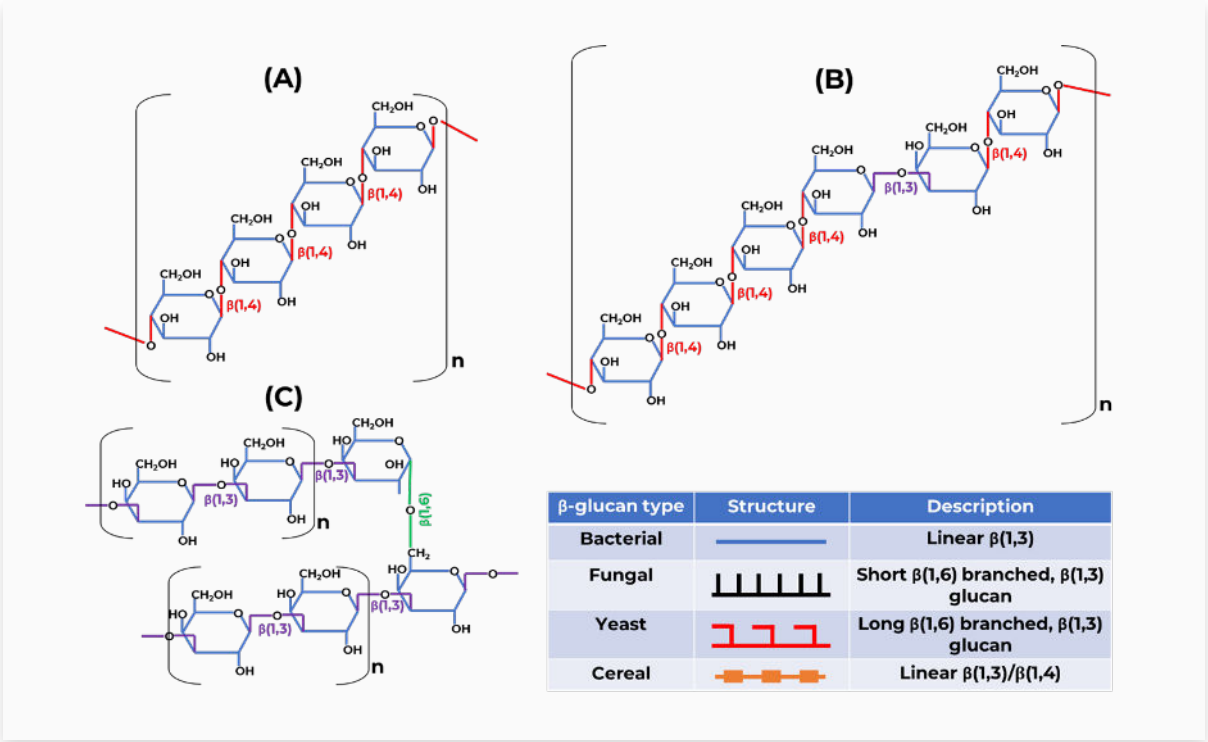

Figure 1: Structures of beta glucan originating from different sources: [A] Bacterial; [B] Cereal; [C] Yeast and Fungi. Adapted from ref [2].

Experimental

Yeast beta glucan reference and intermediates (Intermediates A-C and final product) from different stages of processing were obtained from a research collaborator. Three vbeta glucan samples were sourced commercially from two different vendors (products A-C). The samples were extracted using deuterated dimethyl sulfoxide (DMSO-d6) as per the USP method [19] through several cycles of heating and homogenization, which were optimized for each sample. These samples were further extracted using a different solvent to aid in the product characterization through ^{1}H NMR spectroscopy. ^{1}H and ^{13}C NMR spectra were acquired on an Avance III HD Bruker® NMR spectrometer operating at 9.4 T (400 MHz). High-temperature (80 °C) ^{1}H NMR spectra with water presaturation and room-temperature ^{1}H NOESY spectra with gradient pulses for off-resonance solvent suppression were acquired using DMSO as a solvent, and for extracts of a different solvent, respectively. ^{13}C NMR spectra of the DMSO extracts were acquired with power-gated decoupling and optimized acquisition parameters.

Results and Discussion

^{13}C NMR spectral analysis is an efficient way to authenticate BG products as the chemical shifts and relative intensities of the resonances are sensitive to the BG conformation, that is, the type of glycosidic linkages and the backbone and sidechain lengths. Product purity can also be ascertained as the ^{13}C spectra capture other organic excipients/constituents in the sample. The ^{13}C NMR spectra plotted in **Figure 2** clearly demonstrate this attribute where the spectral profile and resonance positions claim the identity of the macromolecule and the purity of the product. The analysis reveals that the product-B spectrum consists characteristic BG resonances (vis-à-vis reference) albeit with additional resonances appearing from other constituents present in the product. Whereas the spectral profiles of product-A and -C mismatch with the reference to a great degree and resemble that of dextran or a similar type.

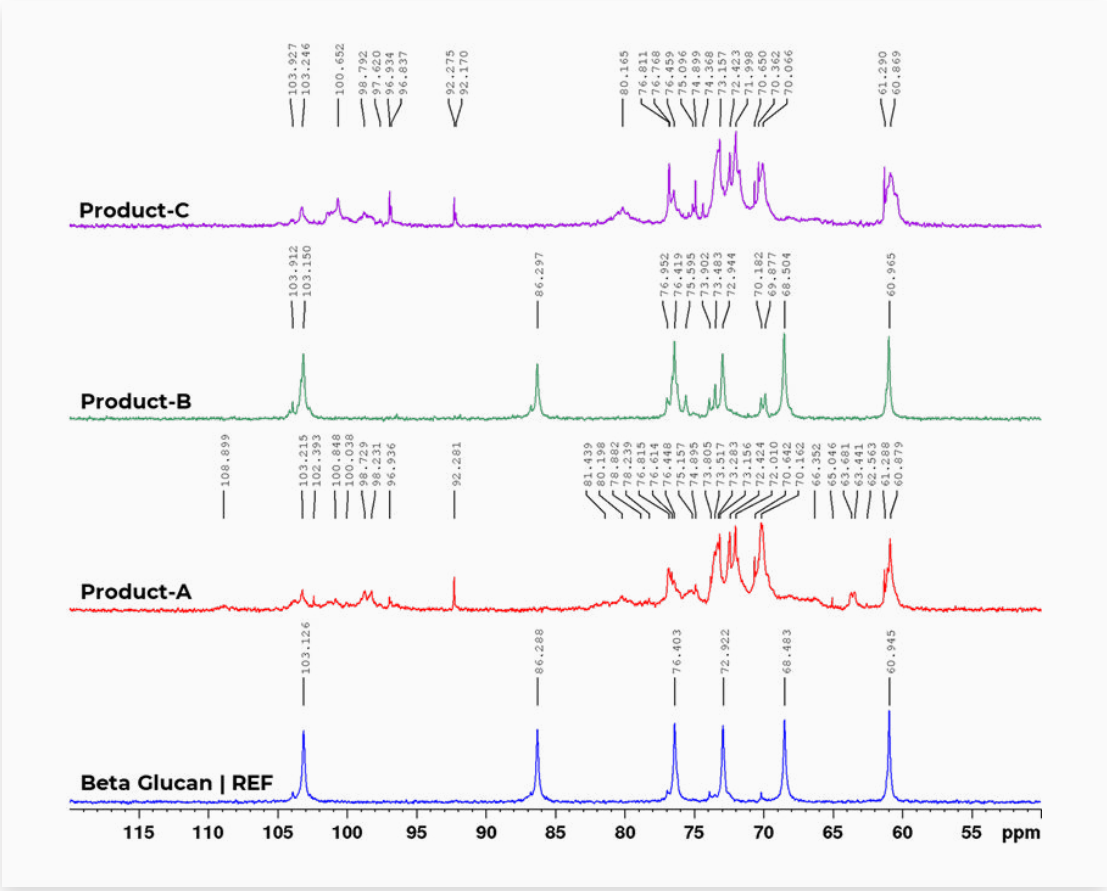

Figure 2: ^{13}C NMR spectra of reference and commercially procured beta glucan products.

Extraction of BG from yeast involves multiple critical and stringent stages and therefore warrants a robust analytical method to assess the efficiency of the overall process. ^{13}C NMR offers a unique way to address this concern wherein the products from intermediate step can be analyzed to gain insight into the procedural steps, which might help in fine-tuning the extraction/manufacturing parameters. An example is demonstrated in **Figure 3**, where the ^{13}C NMR spectral profile reveals the gradual transformation of the initial batch into the final product over a series of processing stages. While the resonances highlighted in blue correspond to the yeast BG, the rest (highlighted in yellow) represent additional constituents from the raw material present in the batch such as saturated and unsaturated fats, and others. These spectra capture the efficiency of extraction over several stages, attesting to the versatility and robustness of ^{13}C NMR as an analytical tool to screen the production processes and practices. Additionally, this approach can be used to test new formulations and novel processing methodologies.

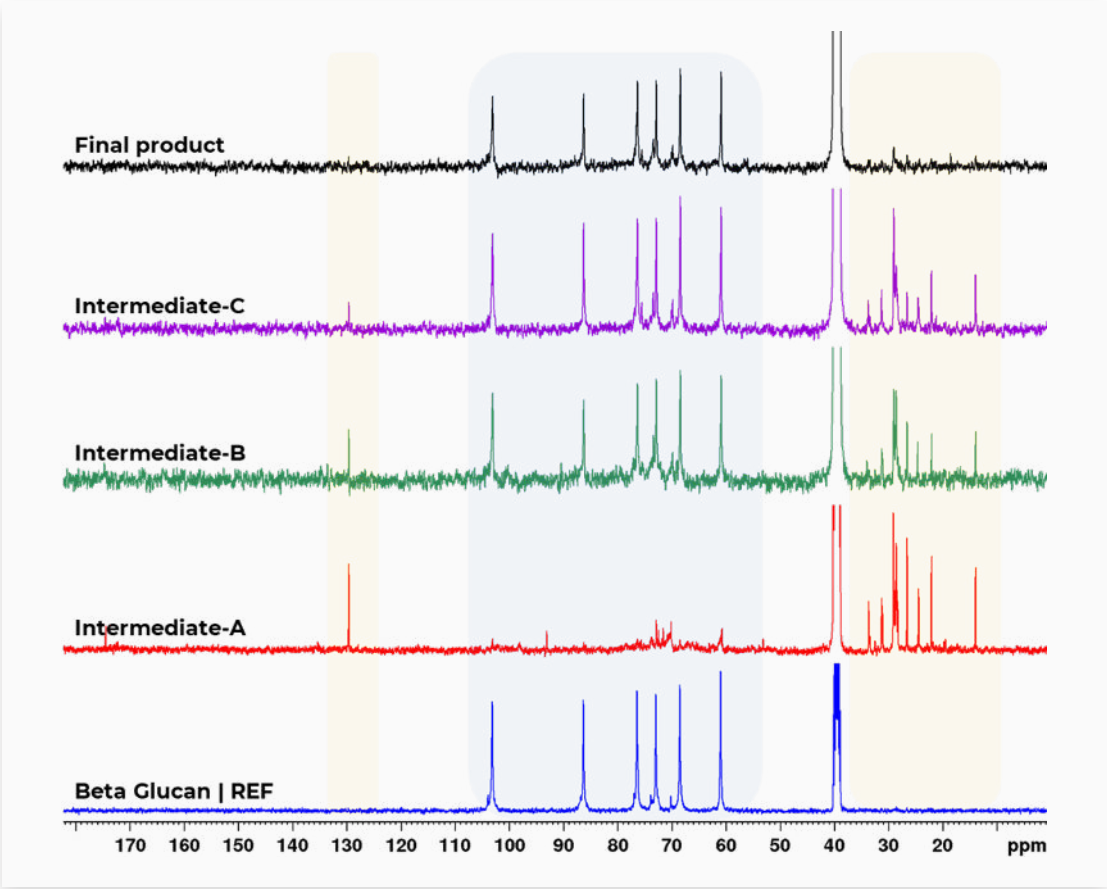

Figure 3: ^{13}C NMR spectra of intermediate and final BG products from different stages of extraction/manufacturing.

One of the favourable attributes of NMR is its ability to probe products through heteronuclear experiments, notably protons (hydrogen, ^{1}H). These are ubiquitous in all organic molecules and the ^{1}H NMR spectroscopy proves to be an easy and reliable method to quickly screen products and assess compositional similarities between them. The ^{1}H spectra shown in **Figure 4** demonstrates this capability where the spectra consist of resonances from fat, sugars, organic acids, and other constituents of the product. Their comparison can be used to study product chemistry, assess batch-to-batch consistency, evaluate formulations, screen processing/ manufacturing stages and other aspects.

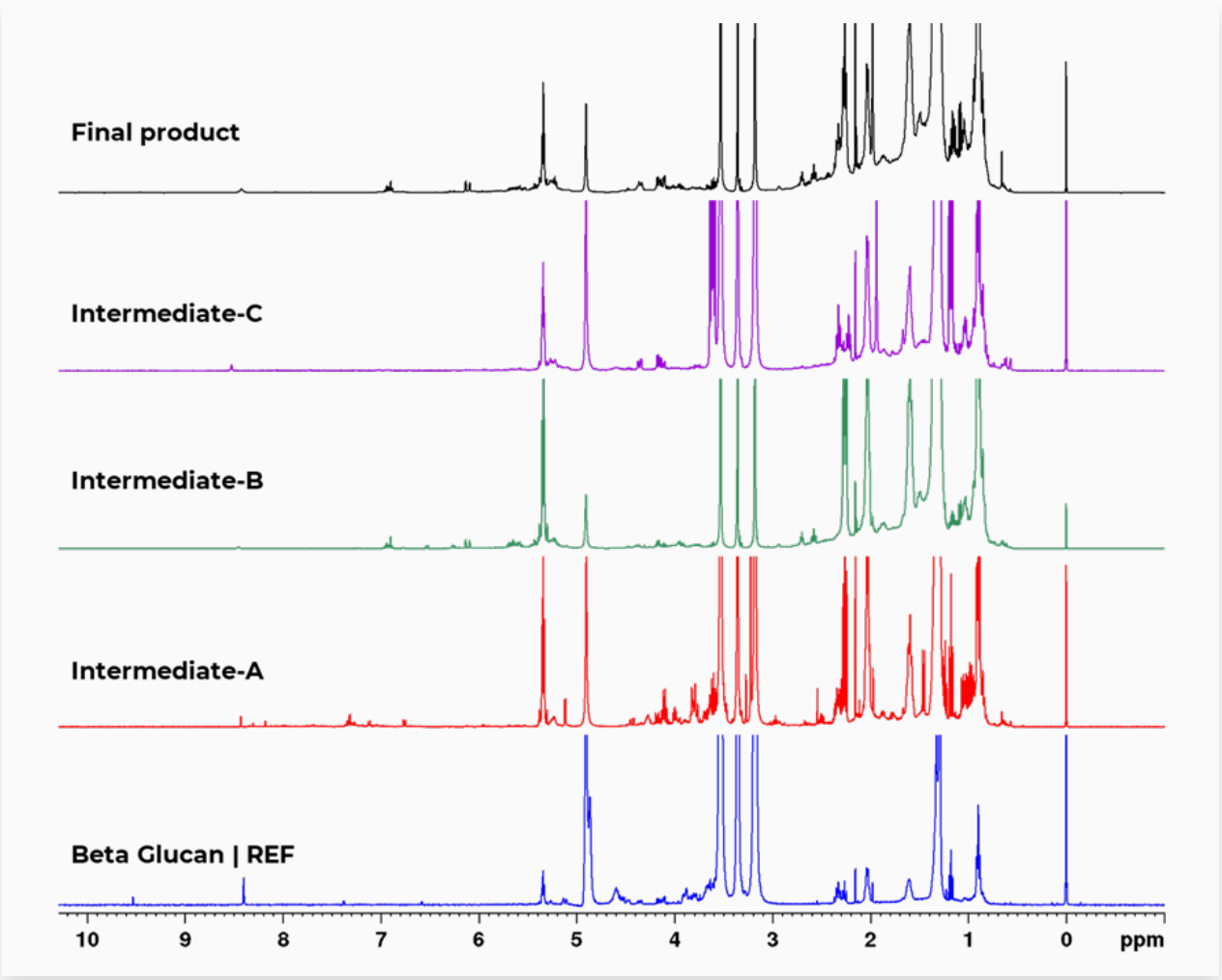

Figure 4: ^{1}H NMR spectra of intermediates and final BG products from different stages of extraction/manufacturing.

The ratio of β(1,3) and β(1,6) linkages govern the applications and utilitity of beta glucan products for different health applications, and the following analysis in accordance with the standard USP methodology outlines the capability to assess this. High-temperature ^{1}H NMR spectra of two products were acquired (**Figure 5**) and the peaks characteristic to β(1,3) and β(1,6) linkages (**Table 1**) are integrated independently at least five times and the relative percentage of β(1,6) linked glucan is determined. With the ratio ideally expected to be 0.2 -0.33, product-1 is expected to be of high quality vis-à-vis product-2, as the ratio is 28% (0.28). Overall, the ^{1}H and ^{13}C NMR examples presented here and other multidimensional NMR experiments in combination can be tailored to address the analytical needs of the BG industry.

^{1}H major signals	USP Beta glucan RS	Peak identity
H-1 (1,3)-glucan	4.52 ppm, d, J = 7.5 Hz, 1H	B
H-2, 4, and 5 (1,3-)	3.27—3.33 ppm, m, 3H	—
H-3 and 6b (1,3-)	3.45—3.48 ppm, m, 2H	—
H-6a (1,3-)	3.71 ppm, d, J = 11 Hz, 1H	—
H-1 (1,6)-glucan	4.27 ppm, d, J = 7.7 Hz, 1H	A

[a]USP monograph [19]

Table 1: Major resonances associated with beta glucan[a]

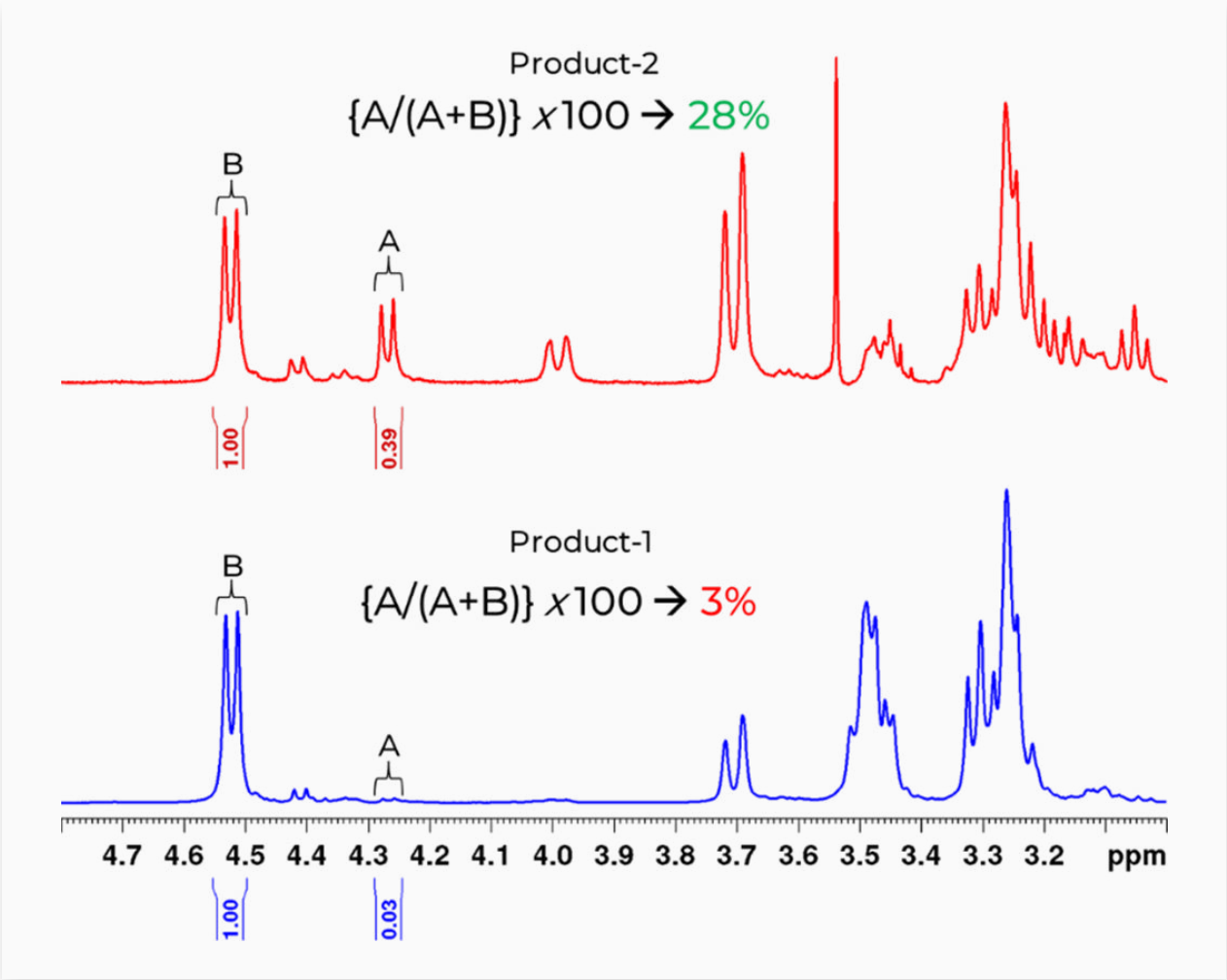

Figure 5: High-temperature ^{1}H NMR spectra of two products with peaks corresponding to β(1,6) and β(1,3) linkages identified as A, and B, respectively.

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