Efficient generation of a fermentable hydrolysate is a primary requirement in the utilization of fibrous plant biomass as feedstocks in bioethanol processes. The first biomass conversion step usually involves a hydrothermal pretreatment before enzymatic hydrolysis. The purpose of the pretreatment step is to increase the responsivity of the substrate to enzymatic attack and the type of pretreatment affects the enzymatic conversion efficiency. Destarched corn bran is a fibrous, heteroxylan-rich side-stream from the starch industry which may be used as a feedstock for bioethanol production or as a source of xylose for other purposes. In the present study we demonstrate the use of diffuse reflectance near infrared spectroscopy (NIR) as a rapid and non-destructive analytical tool for evaluation of pretreatment effects on destarched corn bran. NIR was used to achieve classification between 43 differently pretreated corn bran samples using principal component analysis (PCA) and hierarchal clustering algorithms. Quantification of the enzymatically released monosaccharides by HPLC was used to design multivariate calibration models (biPLS) on the NIR spectra. The models could predict the enzymatic release of different levels of arabinose, xylose and glucose from all the differently pretreated destarched corn bran samples. The present study also demonstrates a generic, non-destructive solution to determine the enzymatic monosaccharide release from polymers in biomass side-streams, thereby potentially replacing the cumbersome HPLC analysis.