Optimization of 2nd generation bioethanol production from wheat straw requires comprehensive knowledge of plant intake feedstock composition. Near Infrared Spectroscopy is evaluated as a potential method for instantaneous quantification of the salient fermentation wheat straw components: cellulose (glucan), hemicelluloses (xylan, arabinan), and lignin. Aiming at chemometric multivariate calibration, 44 pre-selected samples were subjected to spectroscopy and reference analysis. For glucan and xylan prediction accuracies (slope: 0.89, 0.94) and precisions (r²: 0.87) were obtained, corresponding to error of prediction levels at 8–9%. Models for arabinan and lignin were marginally less good, and especially for lignin a further expansion of the feasibility dataset was deemed necessary. The results are related to significant influences from sub-sampling/mass reduction errors in the laboratory regimen. A relative high proportion of outliers excluded from the present models (10–20%) may indicate that comminution sample preparation is most likely always needed. Different solutions to these issues are suggested.