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RESEARCH ARTICLE

HSQC-NMR-based profiling approaches for raffinose family oligosaccharides in pulses

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Abstract

Background and Objectives: Due to insufficient resolution, ^{1}H nuclear magnetic resonance (NMR) spectroscopy-based methods are limited to quantify carbohydrates. In the past, heteronuclear single quantum coherence (HSQC)-based methods were demonstrated to be superior as the second dimension greatly improves resolution. However, whether these experiments are also suitable to determine structurally similar oligosaccharides such as raffinose family oligosaccharides (RFO) still needs to be demonstrated.

Findings: By optimizing NMR parameters, well resolved signals for the analysis of glucose, fructose, galactose, sucrose and the RFO raffinose, stachyose, and verbascose were identified. Application of fast HSQC methods in combination with nonuniform sampling enables analyses of sucrose and RFO in pulses (blue lupin seeds, red lentils, kidney beans) within 24 min. If the analytes are present at levels greater than 0.5 g/100 g, HSQC-based methods provide data equivalent to an anion-exchange chromatography-based reference method.

Conclusions: High resolution fast HSQC-based approaches are suitable tools to analyze complex carbohydrate mixtures as demonstrated for RFO in different pulses.

Significance and Novelty: Fast HSQC experiments were applied for the first time to analyze structurally similar oligosaccharides. In the future, this approach will be a most valuable tool to analyze complex mixtures of carbohydrates in food products.

KEYWORDS

ASAP-HSQC, legumes, lowCost-ASAP-HSQC, NMR, nonuniform sampling, raffinose family oligosaccharides

1 | INTRODUCTION

Due to their high protein and fiber contents pulses, the edible seeds from legume plants, are essential components of vegetarian and vegan diets, with peas, beans, lentils, and lupins being most important. In addition, specific health benefits were demonstrated or are suggested for a diet rich in legumes. For example, consumption of legumes may have cholesterol- and blood pressure-lowering effects (Ha et al., 2014; Jayalath et al., 2014), and legume dietary

fiber has prebiotic properties by promoting the growth of probiotic microorganisms in the gut such as bifidobacteria (Holscher, 2017; Kadyan et al., 2022; Micioni Di Bonaventura et al., 2017). In addition to raffinose family oligosaccharides (RFO), many other indigestible carbohydrates such as pectins or hemicelluloses contribute to legume dietary fiber (Brummer et al., 2015). RFO are structurally based on sucrose. Addition of a galactose unit to the glucose unit of sucrose via an α-1,6-linkage results in the trisaccharide raffinose. Starting from raffinose, additional galactose units are attached, and the oligosaccharides stachyose, verbascose (Figure 1), and ajugose are formed. High amounts of RFO were demonstrated in, for example, lupins, lentils, peas, and beans (Kotha et al., 2020; Xiaoli et al., 2008). They serve as storage carbohydrates in the seed and are used as an energy source, especially during germination (Peterbauer & Richter, 2001). Also, raffinose was suggested to contribute to the tolerance of plants against abiotic stress such as drought stress tolerance (Li et al., 2020). Contents of the individual representatives of RFO differ among legume species. Raffinose and stachyose are normally present in larger contents of up to 40 mg/g in legumes. Verbascose may be present at up to 15 mg/g, while ajugose (hexamer) is usually found only in trace amounts (Kotha et al., 2020; Xiaoli et al., 2008). RFO cannot be metabolized in the small intestine and reach the terminal ileum. Thus, it can be assumed that they enter the colon where they are metabolized (Shimaya et al., 2009). Therefore, they belong to the category of dietary fiber, which—according to Art. 2 (4) in conjunction with Annex I VO (EU) No. 1169/2011—is defined as carbohydrate polymers with three or more monomer units that are not digested in the small intestine. RFO are fermented by some bacterial strains with considerable gas formation, potentially causing flatulence (Myhara et al., 1988). Their flatulent effect is highly individual and can be reduced by soaking. In this process, up to one-tenth of RFO are dissolved out of the source material (Brummer et al., 2015).

High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) has been widely established for the analysis of RFO, because this method can be used to quantify both the individual representatives of RFO as well as additional mono- and disaccharides in food products (Kotha et al., 2020). However, one run takes up to 90 min, so nuclear magnetic resonance (NMR)-spectroscopic methods combined with time-optimized concepts (as described below) were applied to determine the RFO in the matrices in shorter measurement times. In a previous study, mono- and disaccharides have been successfully quantified by using heteronuclear single quantum coherence (HSQC)-based methods (Fels & Bunzel, 2022). But, the resolution generated in these methods is not sufficient to adequately resolve the closely spaced signals of the RFO members. Thus, the resolution in the indirect dimension needs to be improved, which significantly increases experiment times. A measure to reduce experiment time is the application of nonuniform sampling (NUS). Here, only a certain fraction of the data points is recorded and missing data points are added via reconstruction after the actual data acquisition (Hyberts et al., 2012). We already demonstrated that the quantification of mono- and disaccharides is not negatively affected by the application of NUS (Fels & Bunzel, 2022). In addition to NUS, time-optimized pulse sequences such as the acceleration by sharing adjacent polarization (ASAP)-HSQC pulse sequence can also be used to determine sugars (Fels & Bunzel, 2022). In addition to the ASAP-HSQC pulse sequence, a more advanced pulse program can be used, the so-called lowCost-ASAP-HSQC pulse program (Cost: cooling overall temperature), which is characterized by improved resolution in the ^{13}C dimension (Schulze-Sünninghausen, 2016).

Here, we demonstrate the application of time-optimized HSQC methods as well as ASAP-HSQC methods to analyze RFO, and the optimized methods are applied to different types of legumes.

2 | MATERIALS AND METHODS

2.1 | General chemicals, reference materials, and food samples

General laboratory chemicals and solvents were obtained from Sigma-Aldrich, Carl Roth, or VWR. Deuterium oxide (99.9%) was from Deutero. D-Glucose (≥99.5%), D-galactose (≥99%), and D-fructose (≥99%) were purchased from Sigma-Aldrich, sucrose (≥99.5%) from Carl Roth, raffinose pentahydrate (99.0%) from Alfa Aesar, stachyose (98.0%) from Carbosynth, and verbascose (>95%) from Megazyme. Blue lupin seeds (*Lupinus angustifolius*), variety Sonet, were from the Kammerleithner Organic Farm. Red lentils (*Lens culinaris* Medik.) and kidney beans (*Phaseolus vulgaris* L.) were purchased at a local grocery store. All samples were ground, dried, and defatted.

2.2 | Methods

2.2.1 | HPAEC-PAD

D-Glucose, D-galactose, D-fructose, and sucrose were analyzed on an ICS-5000 system (Thermo Fisher Scientific Dionex; detector CS-5000 DC) equipped with

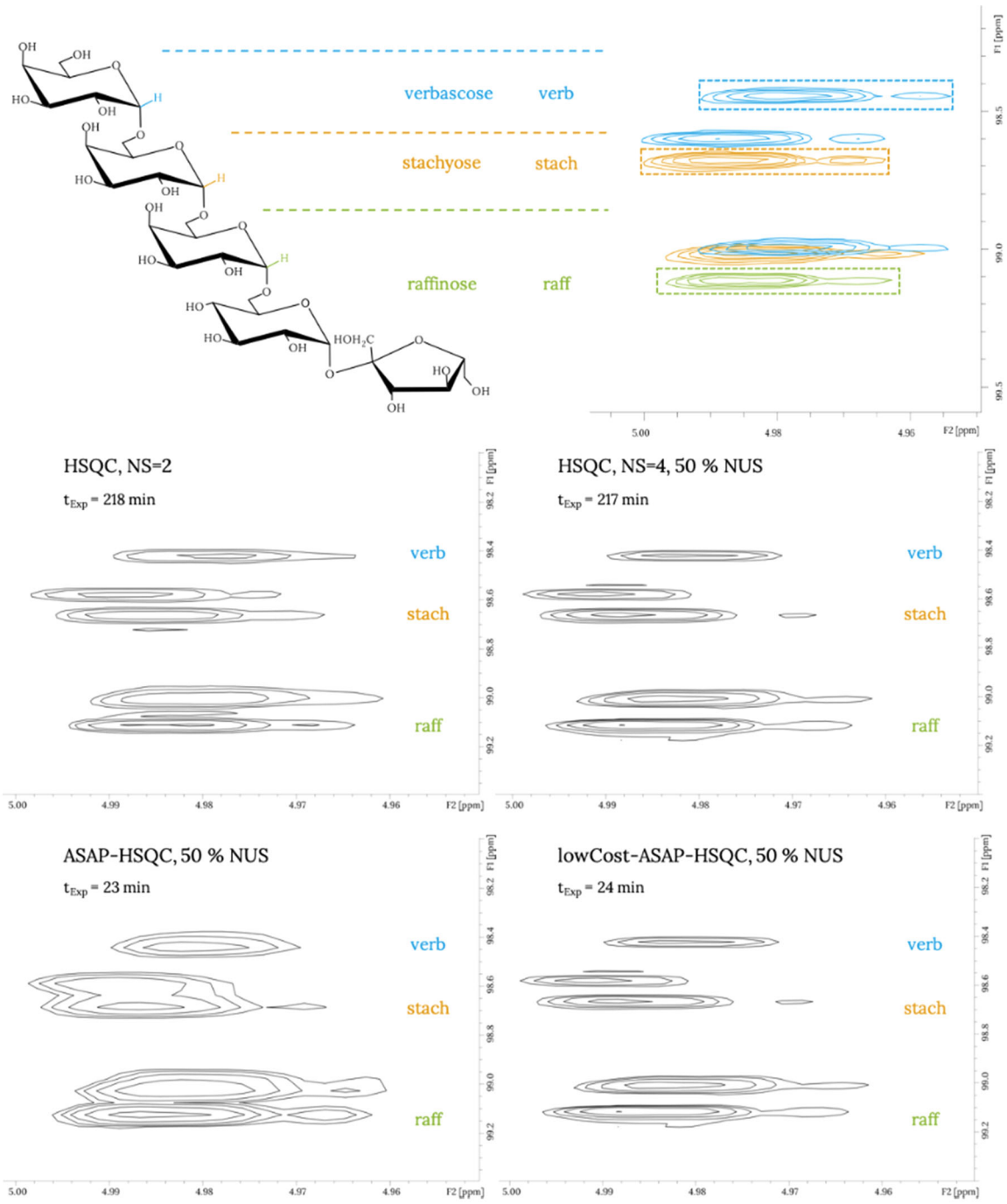


FIGURE 1 Sections of heteronuclear single quantum coherence (HSQC) and acceleration by sharing adjacent polarization (ASAP)-HSQC spectra showing signals of the analytes raffinose (raff), stachyose (stach), and verbascose (verb) according to selection as described in 2.2.2. The upper spectrum shows an overlay of the individual spectra. Experiments (pulse sequences "hsqcetgp" [HSQC], "asap_hsqc_sp_bruker" [ASAP-HSQC], and "asap_hsqc_sp_bruker_12cinz_5" [lowCost-ASAP-HSQC]) were performed with two scans (with the exception of the second HSQC experiment, four scans), and 1024 data points in F_2 and 4096 data points in F_1 were recorded. Spectra were processed using linear prediction and zero filling (F_2: 2048 data points; F_1: 8192 data points). Experiment times (t_{Exp}) are given. The measured sample included all mono-, di,- and oligosaccharides analyzed here in a concentration of 4.0 g/L per analyte, dissolved in deuterium oxide. Cost, cooling overall spin temperature; NS, number of scans; NUS, nonuniform sampling. [Color figure can be viewed at wileyonlinelibrary.com]

a CarboPacPA20 column (150 x 3 mm i.e., 6 μm particle size; Thermo Fisher Scientific Dionex). A gradient of (A) bidistilled water, (B) 0.1 M sodium hydroxide, and (C) 0.1 M sodium hydroxide with 0.2 M sodium acetate was used with a flow rate of 0.4 mL/min at 25°C. The column was rinsed before every run with 100% B for 10 min, followed by 96.5% A and 3.5% B for another 10 min. After injection, the following gradient was applied:

0.0–0.5 min linear to 98.0% A and 2.0% B, isocratic for 23.5 min, 24–28 min linear to 60% A and 40% B, holding for 2 min, 30–37 min linear to 100% B, isocratic for 3 min, 40–42 min linear to 100% C and holding for 11 min.

Raffinose, stachyose, and verbascose were analyzed on a CarboPacPA200 column (250 x 3 mm i.e., 6 µm particle size; Thermo Fisher Scientific Dionex). The following gradient composed of (A) bidistilled water, (B) 0.1 M sodium hydroxide, and (C) 0.1 M sodium hydroxide with 0.5 M sodium acetate was used: rinsing with 100% B for 10 min, followed by 90% A and 10% B for 10 min. After injection, the following gradient was applied: 0.1–1.5 min, linear to 97% A and 3% B, isocratic for 22.5 min, 24.0–28.0 min, linear to 60% A and 40% B, holding for 2 min, 30–37 min, linear to 100% B, isocratic for 3 min, 40.0–42.0 min, linear to 60% B and 40% C, holding for 5.0 min followed by rinsing the column with 100% C for 10 min.

A five-point calibration was performed using mixtures of the analytes D-glucose, D-galactose, D-fructose, sucrose, raffinose, stachyose, and verbascose in the following concentrations: 1.0, 2.5, 5.0, 7.5, and 10.0 mg/L.

2.2.2 | HSQC experiments

NMR spectroscopy was carried out on an Ascend 500 MHz spectrometer (Bruker) equipped with a Prodigy cryoprobe at 25°C. The standard Bruker HSQC pulse program "hsqctgp" was used. ASAP-HSQC pulse programs ("asap_hsqc_sp_bruker" and "asap_hsqc_sp_bruker_12cinz_5") were provided by the working group of Prof. B. Luy (Karlsruhe Institute of Technology, KIT). Using the ASAP-HSQC experiment, the D_1-delay could be reduced to 0.05 s. A spectral width of 4.5006 ppm at ^{1}H dimension and 100.0005 ppm at ^{13}C dimension was used for all measurements. Two scans were monitored. The spectral width in the ^{1}H dimension was 4.50 ppm acquiring 1024 data points (acquisition time [AQ] = 0.227 s) and 100.00 ppm in the ^{13}C dimension using 4096 data points (AQ = 0.163 s). HSQC experiments were performed with an interscan delay of 1.5 s. All ASAP-HSQC experiments were acquired by applying NUS. In addition, a four-scan HSQC experiment with NUS was performed to test the possibility of increasing the number of scans to quantify small concentrations. When NUS was applied, a 50% NUS level and unweighted sampling was used. The following processing parameters were applied: Linear prediction and zero filling (two times the recorded time dimension, i.e., 2048 data points in the direct and 8196 data points in the indirect dimension) were performed for all spectra. A cosine bell apodization was used as a weighting function. All spectra were subjected to manual phase correction and automatic baseline correction. The NUS reconstruction algorithm IST was used as compressed sensing method, and Hilbert transformation was performed in the indirect dimension to allow phase adjustment. All processing was performed in Topspin 4.0.2 (Bruker Biospin). Longitudinal relaxation times were acquired by the standard Bruker pulse program "t1ir" (inversion recovery experiment), using 16 scans, a spectral width of 19.99 ppm, and an AQ of 0.819 s. Solutions that contain individual analytes in a concentration of 5.0 g/L were used.

The following correlation signals were selected for quantitative purposes: sucrose ^{1}H $\delta = 3.83$ ppm, ^{13}C $\delta = 73.01$ ppm; raffinose ^{1}H $\delta = 4.98$ ppm, ^{13}C $\delta = 99.10$ ppm; stachyose ^{1}H $\delta = 4.99$ ppm, ^{13}C $\delta = 98.41$ ppm; verbascose ^{1}H $\delta = 4.98$ ppm, ^{13}C $\delta = 98.65$ ppm. The following signals are also adequately resolved, but were not detected in the samples tested (presumably due to low concentrations): D-glucose ^{1}H $\delta = 5.22$ ppm, ^{13}C $\delta = 92.72$ ppm; D-galactose ^{1}H $\delta = 3.69$ ppm, ^{13}C $\delta = 64.51$ ppm; D-fructose ^{1}H $\delta = 3.54$ ppm, ^{13}C $\delta = 64.51$ ppm and ^{1}H $\delta = 3.69$ ppm, ^{13}C $\delta = 64.51$ ppm. A manual integration of the 2D volume integrals was performed in Topspin 4.0.2. Five-point-calibrations were performed using aqueous mixtures of the analytes D-glucose, D-galactose, D-fructose, sucrose, raffinose, stachyose, and verbascose in the following concentrations 1.0, 2.5, 5.0, 7.5, and 10.0 g/L. Solutions were mixed with 10% deuterium oxide and 0.5 µL of acetone for referencing (^{1}H $\delta = 2.22$ ppm, ^{13}C $\delta = 30.89$ ppm; [Gottlieb et al., 1997]). The working range of the lowCost-ASAP-HSQC method was set between 2.5 and 7.5 g/L. Calibration as well as measurement of the samples should be done on the same day.

Limits of quantification (LOQ) were determined according to DIN 32645 using the calibration line method (DIN 32645, 2008). A calibration line was measured in the range of the LOQ and the uncertainty of the blank value was inferred.

2.2.3 | Sample preparation

Blue lupine seeds (0.5 g), red lentils (1.5 g), and kidney beans (2.5 g) were extracted with 50% aqueous ethanol (addition of 5 mL of 50% ethanol to the solid, ground samples) for 30 min at 50°C (shaking), followed by centrifugation (5000 rpm) (Xiaoli et al., 2008). The supernatant was removed, and the treatment was repeated twice. All supernatants were combined, the solvent was evaporated, and the residue was redissolved in 2 mL of deuterium oxide.

2.2.4 | Recovery experiments

The spiking experiments were performed with half the sample weight. Aqueous solutions of the analytes were added to the samples so that the concentrations correspond to twice the sample weight. The sample preparation was performed as described in 2.2.3.

2.2.5 | Statistics

For the statistical evaluation of data generated by the different methods, a one-way analysis of variance (one-way ANOVA) was performed in combination with a post hoc Tukey test in Origin 2019. Samples were analyzed in triplicate. Recovery rates were analyzed either in triplicate (lupine seeds) or in duplicate (red lentils, kidney beans).

3 | RESULTS AND DISCUSSION

NMR-based profiling approaches for oligosaccharides that show similar structural characteristics are hard to establish because diagnostic and well resolved signals are required for each oligosaccharide. Due to structural similarities of oligosaccharides such as RFO the chemical shifts of the monomer units hardly differ and high resolution is required. This can nicely be demonstrated by using the example of raffinose, stachyose, and verbascose (Figure 1). These oligosaccharides only differ by additional α-1,6-linked galactopyranosyl units. Both the ^{1}H and ^{13}C chemical shifts of the anomeric proton/carbon signals of these galactopyranosyl units are very comparable (Figure 1), thus requiring an optimization of the acquisition parameters to achieve sufficient resolution.

3.1 | Optimization of acquisition parameters

In general, a number of 4096 data points in the indirect dimension are necessary to sufficiently resolve the signals of raffinose, stachyose, and verbascose (Figure 1). In previous studies we demonstrated that application of the pulse sequence hsqcetgp with an interscan delay of 1.5 s is well suited to quantify monosaccharides and disaccharides (Fels & Bunzel, 2022). Since the longitudinal relaxation times of oligosaccharides are in the same order of magnitude as the longitudinal relaxation times of the monosaccharides and disaccharides studied so far (Supporting Information: Table S1), it can be assumed that an interscan delay of 1.5 s is also sufficient for the quantification of oligosaccharides. We tested (a) the HSQC experiment with two scans and (b) the HSQC experiment with four scans and 50% NUS, which takes about the same time (217 vs. 218 min). Resolution of the two scan HSQC experiment appears to be insufficient, whereas the signals of the HSQC experiment with four scans and 50% NUS are sufficiently separated (Figure 1). Because sensitivity was logically also improved by using these acquisition parameters, this experiment was preferred to analyze RFOs.

Time-optimized HSQC pulse sequences coupled with 50% NUS require much less experiment time if compared to the common HSQC experiment (ASAP-HSQC 23 min, lowCost-ASAP-HSQC 24 min, two scans each). However, application of the ASAP-HSQC pulse sequence resulted in considerably worse separated signals as compared to application of the lowCost-ASAP-HSQC pulse sequence (Figure 1). When the number of data points in the indirect dimension is increased to 4096, the homonuclear coupling formed during the incrementation time and the resulting reduction in the size of the magnetization reservoir appear to affect the spectral resolution in the ^{13}C-dimension. By using the lowCost-ASAP-HSQC pulse sequence, this drawback of the ASAP-HSQC experiments can be circumvented. The developers of the pulse sequence reported, however, a potential reduction in signal intensities (Schulze-Sünninghausen, 2016), depending on the actual signal. Due to the application of an external calibration strategy, this does not appear to affect quantification, but may affect sensitivity: The methods used show clearly different LOQ (Table 1). As expected, the HSQC-based methods are much less sensitive as compared to the HPAEC-PAD reference method. But, HSQC-based methods can still detect analytes in the range of a few µg/100 mL. When the lowCost-ASAP-HSQC method is used, the sensitivity is reduced by a factor of about 10.

3.2 | Application of the HSQC-based methods to edible seeds of legumes

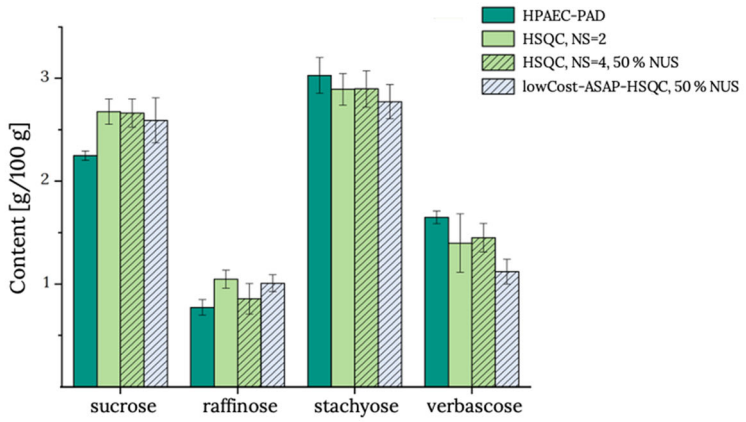
Both HSQC methods as well as the lowCost ASAP-HSQC method were applied to analyze RFO and mono- and disaccharides in pulses, and data were compared to those of the HPAEC-PAD reference method. Generally, monosaccharides were not detected; even with the very sensitive HPAEC-PAD method, the monosaccharides D-glucose, D-fructose, and D-galactose were not consistently detected in the analyzed pulses.

The contents of sucrose, raffinose, stachyose, and verbascose in blue lupin seeds as determined by the optimized 2D NMR spectroscopic methods show no significant deviations (based on one-way ANOVA) from

TABLE 1 Limits of determination of the selected methods

	HPAEC-PAD [μg/100 mL]	HSQC, NS = 2 [μg/100 mL]	HSQC, NS = 4, 50% NUS [μg/100 mL]	lowCost-ASAP-HSQC [μg/100 mL]
D-glucose	0.01	6.6	10.7	70.0
D-fructose	43.5×10^{-3}	19.3	7.2	61.3
D-galactose	6.5×10^{-3}	12.2	6.9	64.4
Sucrose	35.6×10^{-3}	18.9	66.5	182.3
Raffinose	29.1×10^{-3}	24.4	17.7	42.0
Stachyose	0.9×10^{-3}	5.0	2.8	25.5
Verbascose	9.5×10^{-3}	24.6	12.5	163.0

Abbreviations: ASAP, acceleration by sharing adjacent polarization; Cost, cooling overall spin temperature; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; HSQC, heteronuclear single quantum coherence; NS, number of scans; NUS, nonuniform sampling.

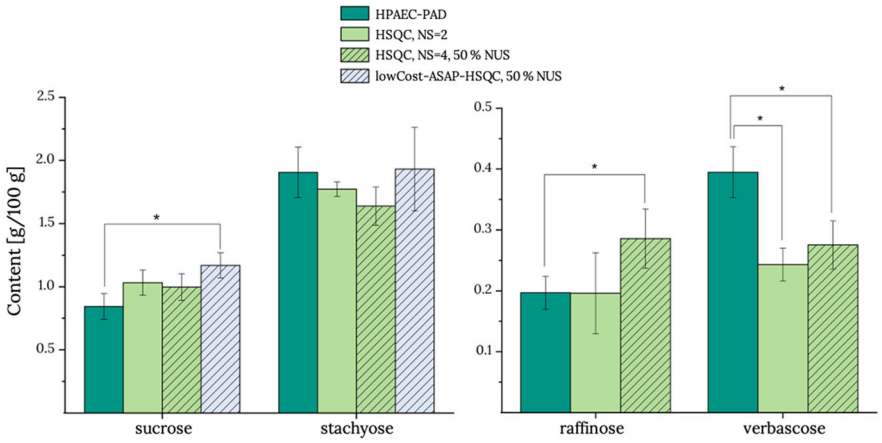


	sucrose	raffinose	stachyose	verbascose
HPAEC-PAD	94.5 ± 12.4	89.0 ± 3.9	95.9 ± 3.2	43.4 ± 3.1
HSQC, NS=2	89.4 ± 15.9	84.6 ± 18.0	82.7 ± 8.2	84.2 ± 4.7
HSQC, NS=4, 50 % NUS	84.4 ± 15.0	107.5 ± 3.7	80.7 ± 10.1	72.6 ± 12.0
lowCost-ASAP-HSQC, 50 % NUS	103.7 ± 24.4	95.3 ± 16.0	113.9 ± 14.0	127.5 ± 29.5

FIGURE 2 Top: Contents of sucrose, raffinose, stachyose, and verbascose in blue lupin seeds. Bottom: Recovery rates ± standard deviation of triplicate analyses of sucrose, raffinose, stachyose, and verbascose in blue lupin seeds. * indicates mean values that are statistically different (one-factor analysis of variance, $\alpha = .05$). ASAP, acceleration by sharing adjacent polarization; Cost, cooling overall spin temperature; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; HSQC, heteronuclear single quantum coherence; NS, number of scans; NUS, nonuniform sampling. [Color figure can be viewed at wileyonlinelibrary.com]

each other and from the values as determined by HPAEC-PAD (Figure 2, top). However, the precision of the HSQC-based data appears to be somewhat lower as compared to HPAEC-PAD-based data. Especially the verbascose content as determined by the HSQC method without NUS coupling shows a large standard deviation. The recoveries determined after spiking (Figure 2, bottom) are in a similar range to data of the HPAEC-PAD method for all NMR-spectroscopic methods. But, a moderate precision of this data is observed, too, and recoveries are generally not fully satisfactory.

In red lentils, which contain much lower levels of RFO than blue lupin seeds, it was only possible to quantify sucrose and stachyose by using the

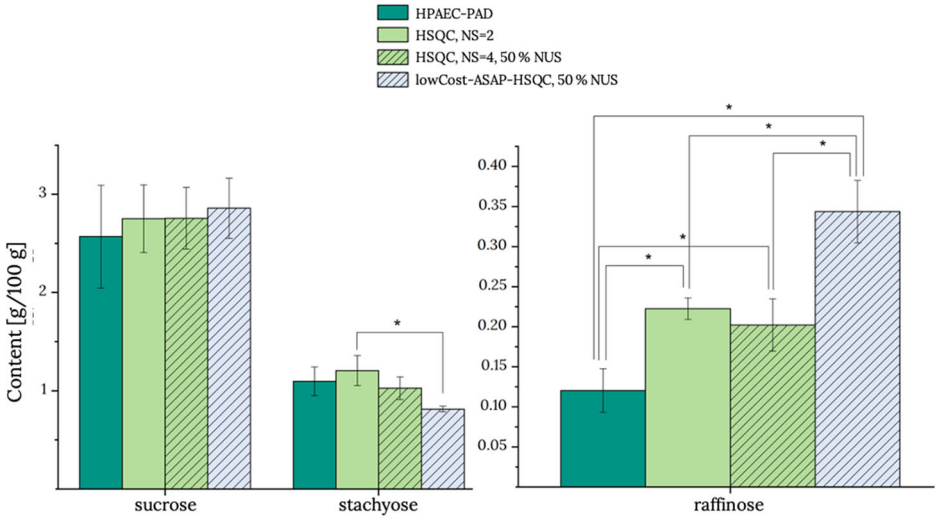


	sucrose	stachyose	raffinose	verbascose
HPAEC-PAD	77.6 ± 14.0	118.9 ± 10.3	108.1 ± 11.9	64.2 ± 4.1
HSQC, NS=2	93.8 ± 17.4	72.8 ± 6.6	105.9 ± 10.8	60.1 ± 0.8
HSQC, NS=4, 50 % NUS	92.0 ± 19.8	101.6 ± 1.6	117.7 ± 4.3	69.1 ± 5.6
lowCost-ASAP-HSQC, 50 % NUS	90.4 ± 17.5	85.9 ± 8.7	-	-

FIGURE 3 Top: Contents of sucrose and stachyose in red lentil seeds. Bottom: Recovery rates ± half range of duplicate analyses of sucrose, and stachyose in red lentil seeds. * indicates statistically different mean values (one-factor analysis of variance, $\alpha = .05$, post hoc Tukey test). ASAP, acceleration by sharing adjacent polarization; Cost, cooling overall spin temperature; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; HSQC, heteronuclear single quantum coherence; NS, number of scans; NUS, nonuniform sampling. [Color figure can be viewed at wileyonlinelibrary.com]

lowCost-ASAP-HSQC method (Figure 3, top). All other carbohydrates including raffinose and verbascose were below the limit of quantification for this approach. Another limiting factor of the lowCost-ASAP approach appears to be that sucrose values were quantified significantly different from the reference value (HPAEC-PAD). Stachyose contents are statistically equivalent independent of the method used, although the standard deviation of the value determined by using the lowCost-ASAP-HSQC method is significantly higher. It was possible to quantify raffinose and verbascose by both HSQC methods, but the analyzed contents do not show sufficient agreement with the HPAEC-PAD contents. Again, recoveries (Figure 3, bottom) are not fully satisfactory for all methods used. The recoveries determined for verbascose are in the same range for all methods, but at a very low level, which means that verbascose is likely to be underestimated. Since this is in the same range for all methods, insufficient extraction is likely, probably due to reduced solubility of verbascose in 50% ethanol. We also observed reduced solubility of verbascose in ethanolic solutions when we studied the dietary fiber composition of lupins: the soluble dietary fiber fraction (which is precipitated out in 80% ethanol) did contain some verbascose (Keller et al., 2022). Thus, future studies need to re-evaluate the extraction conditions and/or the use of a suitable internal standard. Generally, this example demonstrates that the HSQC-based approaches are harder to apply to legume seeds with lower RFO contents. In this case, sample amounts and other factors such as the volume that is used to redissolve the extracted carbohydrates need to be more closely adjusted. Otherwise some RFO cannot be analyzed or only with moderate precision.

In kidney beans, sucrose, stachyose, and raffinose can be quantified by using HSQC-based methods (Figure 4, top). The contents of sucrose and stachyose determined by NMR-spectroscopic methods are not statistically different from the reference values (HPAEC-PAD). As expected, given the low raffinose contents in kidney beans, the raffinose levels determined by each HSQC-based method differ from the levels determined by HPAEC-PAD. Just as seen before, recovery rates are not fully satisfactory (Figure 4, bottom).



	sucrose	stachyose	raffinose
HPAEC-PAD	90.0 ± 8.1	95.6 ± 12.3	89.4 ± 10.2
HSQC, NS=2	77.4 ± 16.6	96.4 ± 7.8	86.8 ± 9.8
HSQC, NS=4, 50 % NUS	78.2 ± 11.0	104.3 ± 4.2	89.2 ± 6.8
lowCost-ASAP-HSQC, 50 % NUS	79.3 ± 11.3	86.6 ± 8.5	75.4 ± 4.6

FIGURE 4 Top: Contents of sucrose, stachyose, and raffinose in kidney beans. Bottom: Recovery rates ± half range of duplicate analyses of sucrose, stachyose, and raffinose in kidney beans. * indicates statistically different mean values (one-factor analysis of variance, $\alpha = .05$, post hoc Tukey test). ASAP, acceleration by sharing adjacent polarization; Cost, cooling overall spin temperature; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; HSQC, heteronuclear single quantum coherence; NS, number of scans; NUS, nonuniform sampling. [Color figure can be viewed at wileyonlinelibrary.com]

The applications demonstrate that it is generally possible to use both conventional and time-optimized HSQC methods to quantify sucrose, raffinose, stachyose, and verbascose in pulses, although sensitivity issues may require adjusted sample preparation procedures (sample amounts, solvent volumes etc.) if low contents of RFO are suspected. Low concentrations cannot be reliably detected using the HSQC methods by applying the general procedure used here. Although the solutions measured were above the determined LOQ, the quality of the data was sometimes not sufficient for an exact quantification of the analytes. In this case, the approach can only be regarded as semiquantitative that, however, may fulfill the requirements of quick profiling approaches. More precisely, if the analytes are present at levels greater than 0.5 g/100 g, both HSQC methods with and without NUS as well as the lowCost-ASAP-HSQC method provide data equivalent to HPAEC-PAD data. Although, depending on the actual concentrations, the results of the lowCost-ASAP-HSQC method in particular may be less precise than those of the HPAEC-PAD method. Therefore, for exact quantifications we recommend a sample preparation procedure that is adapted to both the HSQC-method used and the sample material. In this regard, the extraction procedure also needs to be re-evaluated, especially if verbascose analysis is of interest because verbascose appears to be less soluble in ethanolic solutions. In this respect, incorporation of internal standard compounds that reflect, for example, sucrose (dp 2) and verbascose (dp 5) should be considered.

4 | CONCLUSION

2D HSQC methods are suitable tools to perform NMR-based profiling approaches for oligosaccharides of similar structures as demonstrated for RFO. However, depending on the concentration of the oligosaccharides of interest, sample preparation procedures and potentially the number of scans

need to be adjusted. As demonstrated here, larger concentrations of RFO can be measured within 24 min if the lowCost-ASAP-HSQC experiment is applied. But, precision data of the HSQC-based profiling approaches appear to be somewhat worse if compared to HPAEC-PAD. In future studies, HSQC-based approaches will be applied to monitor even more complex oligosaccharide mixtures in food products or hydrolysates of polysaccharides.

AUTHOR CONTRIBUTIONS

Lea Fels: conceptualization, methodology, investigation, laboratory work, writing, and editing. **Mirko Bunzel**: conceptualization, methodology, writing and editing, supervision.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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