INTRODUCTION
The supercontinuum laser combines the broad spectral range of a lamp with the collimated beam of a laser. These properties might prove to be an advantage when measuring on small samples, gases or remote sensing. Single seed analysis, is one area that could benefit from the high brightness of the laser. So far, single seed transmission measurements have mainly been performed in the wavelength region below 1100 nm because of the increased absorbance at longer wavelengths. In some situations it could be beneficial to use longer wavelengths because of the possibility to get information from the first overtone region (1400-1800 nm) or the combination tone region (1900-2500 nm). One example is the measurement of β-glucan in barley seeds, whereas long-wavelength near-infrared (NIR) measurements from 2260-2380 nm in reflectance mode have shown information on the level of β-glucan. The content of β-glucan is important in beer production because a high content can result in clogging of filters and the generation of “grandma’s cough” precipitation in the final beer. β-glucans have also received attention because of their health promoting properties such as their stabilizing effect on the blood glucose level and the lowering of serum cholesterol.

The aim of the present study was to investigate the potential of a supercontinuum laser applied to barley seeds in transmission mode in the region 2260-2380 nm and to explore the chemical information obtained. One mm slices from 350 barley seeds from five barley genotypes were measured through the endosperm. To add chemical interpretation, oils from the same five barley genotypes were measured from 2003-2497 nm. The quality of the obtained spectra showed a good signal-to-noise ratio after applying a pulse-to-pulse normalization of the supercontinuum light. The spectra showed information on C-H vibrations from starch and β-glucan.

KEYWORDS: Near-infrared transmission spectroscopy; NIT; Supercontinuum laser; Food; Barley.

MATERIALS AND METHODS
The NIR instrumental setup is depicted in Figure 1. It consisted in the supercontinuum source (NKT Photonics), a scanning-grating monochromator and a PbSe detector. The supercontinuum source is a pulsed source, and these pulses have intensity variations across the spectrum. It was therefore necessary to perform a pulse-to-pulse normalization with the signal from a second detector which only measured pulse variations without the sample. The size of the laser beam reaching the sample was 0.1 mm x 0.5 mm. A rotating sample holder was...
produced which could automatically present 36 barley slices in one run. The five barley oils together with a nujol mineral oil and a sunflower oil was measured in a quartz cuvette with a 1 mm path-length. Each spectrum for a barley slice or oil measurement took 60 seconds.

![Figure 1.](image1.png)

**Figure 1.** The supercontinuum light exits a fiber and is guided by off-axis parabolic mirrors (PM) and silver mirrors (M) into the scanning-grating monochromator. The light that exits the monochromator is then focused by a plano-convex CaF2 lens (L1) onto the sample. PbSe detectors are placed behind the sample (D1) and the back-reflection of the L1 lens (D2). The second detector was used for pulse-to-pulse normalization.

A custom-made barley slicer shown in Figure 2a was produced in order to make precise and fast one mm slices from 350 grains (70 of each of five barley genotypes). As seen from Figure 2b the cut was made from the center of the grain which contains the endosperm. Four out of the five barley genotypes were mutants with low starch and high β-glucan content (lys5.f, lys5.g, lys16 and lys95) and the last genotype was a normal barley (Bomi).

![Figure 2.](image2.png)

**Figure 2.** (a) The barley slicer. (b) The knives of the barley slicer were tilted to position the angle of the bevel perpendicular on the seed. This was done to increase the precision of the slice thickness and ease the collection of the slices.

The barley oils were extracted with 100 % hexane by Pressurized Solvent Extractor (PSE), ASE200® from Dionex (Sunny-vale, CA).

Reference measurements on moisture, β-glucan, starch, protein and lipid content were performed for each barley genotype by measuring on flour prepared from bulk samples.

The absorbance was calculated with air as the background for the barley slices and an empty cuvette for the oil measurements. The absorbance spectra were preprocessed as second derivative spectra (Savitzky Golay, 2. degree polynomial, window size 7).
RESULTS AND DISCUSSION

Figure 3 shows the content of moisture, β-glucan, starch, protein and lipid for each barley genotype. The highest variation was found in the content of starch and β-glucan which corresponded to findings in previous studies.\(^5\)

![Figure 3](image)

**Figure 3.** The content of moisture, β-glucan, starch, protein and lipid in the five barley genotypes.

The second derivative of the spectra of barley endosperm in Figure 4a showed absorbance bands (depicted as valleys) at 2287 nm, 2311 nm, 2325 nm and 2349 nm. The covarygram shown in Figure 4b shows the correlation between the absorbance at each wavelength and the content of moisture, β-glucan, starch, protein and lipid. Since an increased absorbance is shown as a valley in the second derivative spectrum then a Pearson correlation coefficient of -1 will in this case mean that the absorbance and reference measure are positively correlated. It can therefore be seen that the starch and moisture content has a positive correlation with the wavelengths at 2287 nm and 2325 nm. Whereas the content of lipid, protein and β-glucan is positively correlated with the absorbance bands at 2311 nm and 2349 nm.

![Figure 4](image)

**Figure 4.** (a) Average second derivative spectrum for each barley genotype. (b) The second derivative spectrum shown together with the Pearson correlation coefficient calculated between each wavelength and the content of moisture, β-glucan, starch, protein and lipid.

The spectra of barley endosperm were compared with the oil spectra in order to investigate the influence from lipids. In Figure 5 the absorbance bands of barley and sunflower oil have their valley minimum at slightly lower wavelengths compared to the mineral oil. So the barley and sunflower oils have absorbance bands at 2312 nm and 2351 nm, and the mineral oil has absorbance bands at 2313 nm and 2352 nm. Since the absorbance bands
of the oils are placed very close to the absorbance bands in the spectra of barley endosperm it is possible that C-H vibrations from either lipids, proteins or β-glucans can absorb in this region. However, since the endosperm contains more starch and β-glucan, and less lipid and protein compared to the average content in the whole seed then it is more likely that the endosperm spectra contain information of starch at 2287 nm and 2325 nm and information on β-glucan at 2311 nm and 2349 nm.6,7 This interpretation is in agreement with the assignment in the literature.8–10

Figure 5. (a) Raw averaged oil spectra. (b) Second derivative oil spectra.

CONCLUSION

It was possible to apply a supercontinuum laser in NIR transmission measurements in the region from 2260-2380 nm of one mm barley endosperm. This proof-of-concept opens the possibility to go further and measure on whole seeds. This technology could potentially be used in fast and non-destructive measurements of β-glucans and perhaps even more inferior compounds in intact seeds with regard to quality sorting or in plant breeding.

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References