Monitoring process and purity in the flavours and fragrance industry using NIR

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Introduction

The flavour and fragrance industry typically produces or supplies high volumes of well-characterised materials from natural sources for a wide variety of end-use products. Thus, as with other industries, quality testing of incoming material is a necessary requirement. Also, concentration levels of multiple components and purity need to be verified for products that may consist of complex matrices. Finally, implementation of new analytical methods must be straightforward and rapid, in order to be of economic value. In this presentation, two distinct studies will be presented: (1) the use of near infrared (NIR) to evaluate quality of incoming material, with emphasis on rapid implementation and (2) the evaluation of NIR to determine concentration levels of three sugars in a complex natural product in-process formulation derived from carob tree pods (otherwise known as "St. John's Bread"). Both laboratory, as well as "at-line" fibre-optic probe sampling methods, were evaluated.

Experimental

NIR spectra were collected using Büchi NIRFlex N-400 and NIRLab N-200 FT-NIR spectrometers. All liquid samples measured by the NIRFlex N-400 were scanned using a standard reflectance probe fitted with a transflectance attachment. Spectra collected in the ***Uniformity Testing*** application were acquired with a gap of 2 mm. Spectra collected with a probe in the ***Carob Pod Extract*** sugar analysis at-line study used a gap of 1 mm.

In the case of the laboratory (NIRLab N-200) measurement of carob pod extract, spectra of the samples were collected using a transflectance cover placed over the sample in a glass petri dish. Radiation from the interferometer was directed up through the sampling window, through the bottom of the sample petri dish, and then finally through the sample (0.3 mm), to then strike the bottom of the transflectance cover which returned the light back to the NIRLab through the same path for analysis, resulting in an effective pathlength of 0.6 mm. Measurement times were less than one minute for both spectrometers.

Uniformity testing

Goal

Polarome International, the world's largest supplier of essential oils and aroma chemicals, tests the purity of every material entering and leaving their facility, and so the speed and ease of use of NIR was clearly desirable. However, as their inventory includes over a thousand ingredients, Polarome was concerned over the start-up time associated with typical NIR calibration development for each individual material that would need to be tested.

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In order to accelerate the usefulness of NIR, a three-phase plan was adopted:

Phase1: reduce GC (gas chromatography) testing by using NIR to confirm that all ten to twenty containers in a typical lot are the same (uniformity testing—no calibration model development required)
Phase2: collect quantitative calibration spectra from the database of the "Phase 1" measurements
Phase3: identify, and predict purity of each container by NIR using the spectra from Phase 2 and "standard" NIR calibration methods.

Discussion and results

By using a simple spectral comparison algorithm for testing a group of containers, NIR is used to confirm that all the containers are filled with the same material (or to detect variant containers). Therefore, only one GC measurement needs to be performed instead of 10 or 20 GC runs. Although in Phase 1 the GC measurements are still necessary for determining the actual identity and purity of the sample, the number of GC runs were immediately reduced dramatically, bringing a rapid return of investment on the NIR instrument.

The spectral comparison is a simple subtraction of the first container's NIR spectrum from the spectra of all subsequent containers of the lot (after second derivative math pre-treatment). The spectral subtraction result with amplitude less than an established threshold serves to demonstrate uniformity between the containers. An example of the method demonstrating sensitivity in evaluating a "spiked" sample is shown below in Figures 1 and 2. In this test, two acceptable samples of orange oil were obtained. One was used as a "baseline", acting as the spectrum of the first container. The second sample was measured and considered the "second container". Finally a portion of the second sample was "spiked" with linalool (a naturally occurring compound in orange oil present at about 40%) to an additional 2% and then measured as the "third container". The raw transflectance spectra are shown in Figure 1. After subtraction (in second derivative mode) of the first "baseline" spectrum, the spectra in Figure 2 demonstrate the capability of NIR to quickly detect even slightly higher than ordinary levels of linalool, although the difference between the samples are too slight to be observed in the raw spectra.

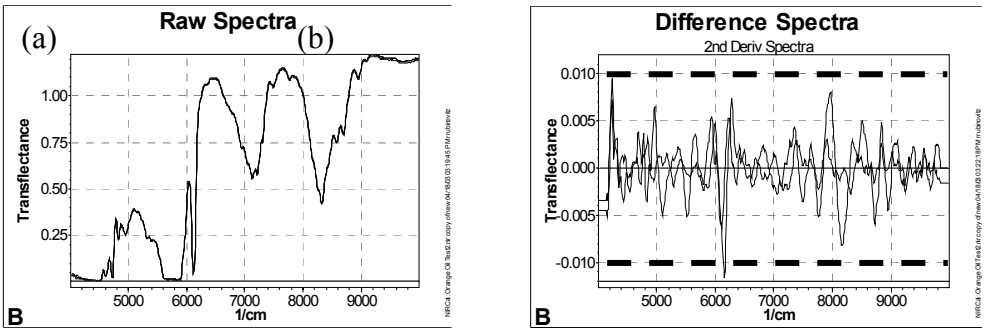

Figure 1. (a) Spectra of all three orange oil samples used in study to detect higher-than normal concentrations of linalool. (b) Spectra of all three samples used in study to detect higher-than normal concentrations of linalool after 2nd derivative pre-treatment and subtraction of baseline spectrum.

As NIR measurements were collected over time, standard NIR quantitative calibrations were developed, allowing for simultaneous prediction of uniformity as well as concentration of particular components by operators via a simple user-interface and printout.

Quantitative sugar prediction in carob pod extract

Goal

Although many different products are produced from carob pod extract, the formulation that was part of this study was one that contained relatively high levels of sucrose as well as fructose and glucose. The goal of this study was to determine the capability of partial least squares (PLS) equations to yield rapid and simple determination of the levels of these three sugars by NIR measurements.

Discussion and results

Twenty-three process samples (very thick, and dark brown in color) were provided with primary analysis concentration values for the three sugars covering the ranges shown in Table 1. These samples also contained propylene glycol in amounts varying between 1–2%. Also shown in Table 1 are the correlations of the individual sugars to each other. Unfortunately, as is common when dealing with natural products, a relatively high amount of correlation exists between the components of interest in the calibration samples (correlation "*r*" values between 0.80 and 0.94). Therefore, in order to be certain that NIR is indeed predicting the individual sugars, the NIR predictions must compare to the primary values with correlations greater than r=0.95.

Table 1. Concentration ranges of carob pod extract process samples and intercorrelation of measured components within the supplied samples.

	Concentration (%)			Correlation		
	Minimum	Maximum	Average	Fructose	Glucose	Sucrose
Fructose	12.20	17.98	15.41	1	0.93402	–0.90848
Glucose	10.09	15.62	13.06	0.93402	1	–0.80429
Sucrose	8.89	27.26	17.59	–0.90848	-0.80429	1

PLS equations were developed and the results are summarised below. Due to the relatively small number of samples available (23), two parallel methods were used in this study to determine the correct number of PLS factors. First, samples were randomly split into 18 calibration/5 validation samples with the same sample sets used for the at-line as well as the laboratory spectra. Then, the number of factors that produced minimal error in predicting the validation set was determined and used for the final predictions (*SEC* and *SEP*). Alternatively, all 23 samples were used for calibration and the number of factors to be used in the final equations were determined by means of selecting the number of factors that produced the smallest standard error of cross-validation (*SECV*) for each sugar type. It was found that these two methods agreed on the selection of the number of factors for each data set. One clear difference between the calibrations developed from the laboratory versus

the at-line presentation is the wavelength range used by each. Because of the relatively large gap needed for the transflectance probe to be used in a practical manner, these spectra had too much absorbance in the region above 5300 cm^{-1} to be useful. Thus, although the probe spectra would be expected to have stronger absorbance due to the sugars of interest, less spectral range was available for method development. Results are shown in the tables and figures below.

Table 2. NIR at-line measurement prediction equation parameters.

Property	Math pretreatment	cm^{-1} range	PLS factors
Fructose	2nd derivative (segment)	5,532-7,608	5
Glucose	2nd derivative (segment)	5,532-7,608	4
Sucrose	none	5,364-9996	7

Table 3. NIR at-line measurement prediction equation results.

Property	*SE* N_{Cal}=18	*SEP* N_{Val}=5	SEC N_{Cal}=23	SECV N_{Cal}=23	R_{Cal} N_{Cal}=18	R_{Val} N_{Val}=5	R_{Cal} N_{Cal}=23
Fructose	0.28	0.34	0.28	0.39	0.9863	0.9670	0.9850
Glucose	0.40	0.38	0.40	0.60	0.9621	0.9565	0.9545
Sucrose	0.36	0.90	0.40	1.61	0.9982	0.9857	0.9975

Table 3. NIR laboratory measurement prediction equation parameters.

Property	Math pretreatment	cm^{-1} range	PLS factors
Fructose	MSC	4593–10000	7
Glucose	Normalisation	4593–10000	7
Sucrose	2nd derivative (segment)	4594–8011	7

Table 4. NIR laboratory measurement prediction equation results.

Property	*SEC* N_{Cal}=18	*SEP* N_{Val}=5	*SEC* N_{Cal}=23	*SECV* N_{Cal}=23	R_{Cal} N_{Cal}=18	R_{Val} N_{Val}=5	R_{Cal} N_{Cal}=23
Fructose	0.29	0.33	0.30	0.63	0.9826	0.9768	0.9797
Glucose	0.29	0.27	0.32	0.58	0.9799	0.9729	0.9711
Sucrose	0.72	1.06	0.81	1.26	0.9932	0.9723	0.9903

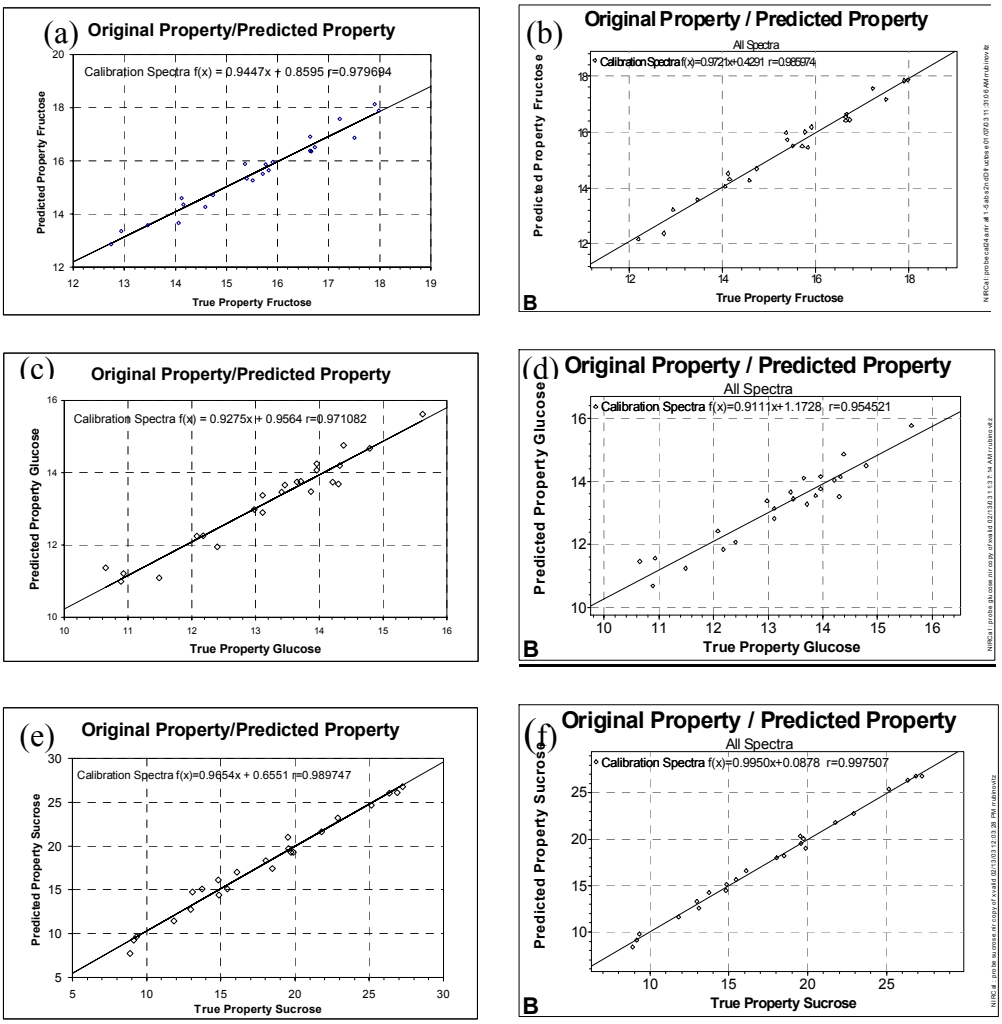

Figure 2. NIR predicted values plotted versus "true" lab values for a) fructose - laboratory sample presentation, b) fructose - at-line sample presentation, and c) glucose - laboratory sample presentation, d) glucose - at-line sample presentation, e) sucrose - laboratory sample presentation, f) sucrose – at-line sample presentation.

Results were within the required accuracy for the measurement, and correlation values greater than 0.93 were observed as desired. Increasing the number of calibration samples with low correlation between fructose and glucose would be desirable, however.

Fructose and glucose gave very similar quality of predictions. Sucrose predictions were somewhat less accurate (although it had the highest correlation to primary values of all the sugars), but it is likely that improvement would be seen here with additional samples.

The at-line (probe) spectra equations with less spectral range than the laboratory spectra, produced similarly accurate results to the laboratory method, with fewer PLS factors. This was probably due to the higher sensitivity (due to the longer pathlength) in the 1st overtone C–H region.

Conclusions

NIR has been shown to be effective for rapid, simple and accurate analysis for a number of measurements critical for the flavours and fragrance industry. In this report, "Uniformity" methods were successfully developed that allowed NIR to reduce testing time and costs almost immediately, while simultaneously collecting data for methods that would allow multiple component analysis for concentration and purity. NIR also demonstrated its ability for quantitative predictions of even complex natural products in either a laboratory or at-line sampling mode.