Quantitative determination of vitamin C at sub-percent level in infant cereals by NIR spectroscopy

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Introduction

Infant cereals are foods based on cereals, processed to a low moisture content.[1] Vitamins are added to the product in the form of a specific vitamin premix to meet all legal requirements and the nutritional needs of the consumer. Due to its relatively higher concentration vitamin C is used as a tracer for ensuring the correct dosing of the other vitamins into the product. Therefore, as part of the quality control procedures, vitamin C is regularly analysed during the production of infant cereals.

There are numerous spectroscopic or non-spectroscopic methods for the quantitative determination of vitamin C.[2,3] However, all these methods are time-consuming, require skilled operators and tedious sample preparation. The introduction of a fast and accurate method for the quantification of vitamin C would be beneficial to improve process control efficiency.

Near infrared (NIR) spectroscopy has already been widely used for the quantitative determination of various physico–chemical properties of food products.[4,5] NIR is for example already used for the quantitative determination of moisture and fat content in infant cereals. The development of a NIR calibration for the quantitative determination of vitamin C would therefore allow the fast and simultaneous analysis of the three major parameters used to monitor the process and guarantee consistent product quality.

It has already been reported that NIR techniques can be applied for the quantification of vitamin C at concentration levels higher than 1% in binary mixtures or solutions,[6] in pharmaceutical preparations[7,8] or in vitamin premixes.[9,10] The new challenge was to develop a NIR calibration for the vitamin C determination at concentration levels of 30–200 mg 100 g^{-1}. Although Norris showed that NIR can accurately predict specific constituents at levels below 100 ppm in simple matrices,[11] the suitability of NIR to analyse vitamin C determination at sub-percent level had to be carefully investigated.

Materials and methods

Eighty-five samples of infant cereals covering 21 different recipes were collected from two manufacturing plants, with the objective of including into the calibration most of the possible matrix variability, but also to a lesser extend to limit the risk of collinearity between the constituents.

The samples were analysed for their vitamin C content by the reference titrimetric method (AOAC 976.21, § 5.1.14). They covered a concentration range between 33.8 and 191.1 mg 100 g^{-1}. The accuracy of the reference method can be estimated by its standard deviation of reproducibility obtained during internal inter-laboratory tests: $SD_R = 6.2$ mg 100 g^{-1}.

In order to obtain more reproducible (lower RMS) and representative spectra all samples were ground with a mortar and pestle to a particle size <1mm. They were then scanned in triplicate in a

natural sample cup (IH-0395) with a Foss-NIRSystems 6500 (Foss-NIRSystems, Silver Spring MD, USA) equipped with a transport module. All spectra were recorded from 400 to 2500 nm at 2 nm intervals and saved as the average of 50 scans. A 4-point Fourier smoothing was applied during data collection. The replicate reflectance spectra were averaged prior to any further processing. The averaging aimed at reducing the random noise of the instrument and the sampling error.[11]

Data analysis was conducted with WinISI v. 1.5 (Infrasoft International Inc, Port Matilda PA, USA). The raw log $1/R$ data were corrected for the scatter effect using standard normal variate (SNV) and detrend, and then transformed into second derivative using a 12-point gap and a 12-point smoothing function. The spectra were then trimmed to the wavelength range 1100–2500 nm and split into a calibration set (55 samples) and a validation set (30 samples).

Results and discussion

Data collection and visual examination of the spectra

The additive effect of grinding the infant cereals sample, scanning 50 times a large sample portion and averaging three replicate spectra brought the RMS down to 5.3×10^{-4}. RMS was 3.7×10^{-3} when the ungrounded samples were recorded as the average of 25 scans in a small ring cup.

The NIR spectra of pure vitamin C and that of a typical infant cereals sample are presented in *Figure 1*. Vitamin C has very distinctive absorption bands in the NIR region, at 1456, 1754 and 2250 nm. The first one is in a region where water absorbs, the two others at wavelengths where infant cereals do not display a strong absorbance.
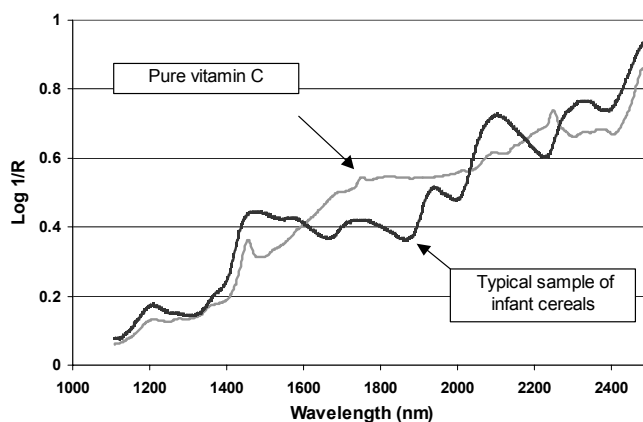

Figure 1. Raw log(1/R) spectra of pure vitamin C and of a typical sample of infant cereals.
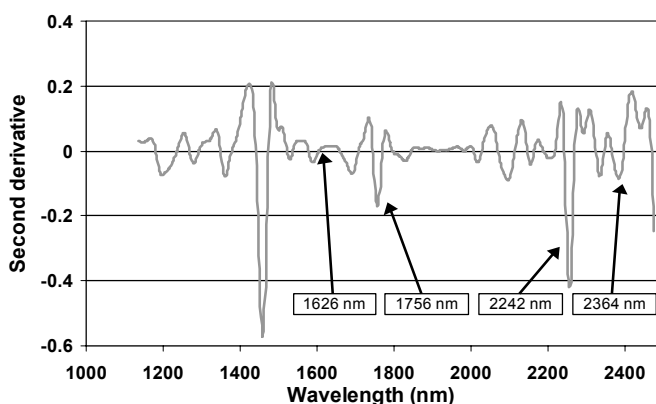
Calibration

A stepwise MLR calibration was developed based on 4 wavelengths, without removing any sample from the calibration set. The selected wavelengths and their respective F values are listed in Table 1. All selected wavelengths are highly significant (p<0.001).

Table 1. Selected wavelengths of the MLR calibration for vitamin C determination in infant cereals and their respective F-values.

Wavelength (nm)	F-value
1626	22.14
1756	26.04
2242	34.02
2364	39.06

Three of the selected wavelengths correspond to specific absorption peaks of pure vitamin C (1756, 2242 and 2364 nm) visible in the spectrum presented in Figure 1 or in its second derivative (Figure 2). Two of these wavelengths have been characterised earlier [6]: 1756 nm (5731 cm^{-1}, first overtone of C-H stretching) and 2242 nm (4459 cm^{-1}, combination of C-H stretching and bending). The fourth wavelength (1626 nm) may be a correction wavelength.

Figure 2. Second derivative of a NIR spectrum of pure vitamin C with the MLR wavelengths.

The performance characteristics of the calibration are listed in Table 2. The calibration was acceptable and did not show any sign of overfitting: the coefficient of determination of the cross-validation (1-*VR*) was higher than that of the calibration (R^2) and standard error of cross-validation (*SECV*) was lower than the standard error of calibration (*SEC*).

Table 2. Performance characteristics of the NIR calibration for the analysis of vitamin C in infant cereals.

R^2	0.704
SEC	21.2 mg 100 g^{-1}
1-*VR*	0.815
SECV	16.7 mg 100 g^{-1}

Validation

The scatter plot of the validation data is presented in Figure 3. The statistical evaluation did not show any significant bias nor any significant proportional error. Standard error of prediction (*SEP*) was 17.0mg/100g. SEP was lower than SEC and the ratio SEP/SEC met the requirement expressed in the recommended performance target of the AACC[12] (*SEP*/*SEC*<1.2).
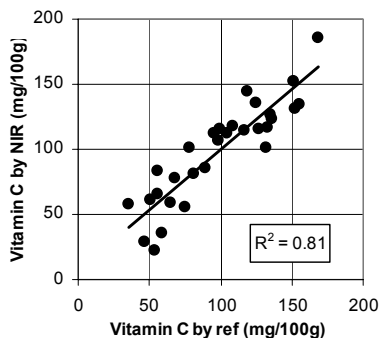
Figure 3. Scatter plot of the reference values versus the NIR predicted values for the analysis of vitamin C in infant cereals.

Conclusions

This study demonstrated that NIR spectroscopy could be used for the analysis of vitamin C at sub-percent level in complex food matrices such as infant cereals. Reduction of the sampling error achieved by optimisation of the sample presentation and measurement as well as adequate mathematical treatment of the spectra allows widening the field of potential application of NIR spectroscopy.

The performance of the NIR calibration developed was $SEP = 17$ mg 100 g^{-1} and the calculated ratio of data range to SEP (R/SEP) was 9.24. This value is just below the minimum recommended target of the AACC[12] for a good calibration for quality control (target: R/SEP>10).

Further development of the method should lead to an improvement of SEP, which could ultimately become closer to the accuracy of the reference method given by its standard deviation of reproducibility ($SD_R = 6.2$ mg 100 g^{-1}).

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