Rapid determination of vitamins A and C in fortified milk powders by NIR

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Introduction

Wet chemical methods for determining the levels of vitamins A and C in fortified milk powders are time consuming and expensive. A recent review of potential NIR applications within Fonterra suggested that there is the potential to use near infrared (NIR) spectroscopy to quickly determine the levels of these vitamins in a process environment. With an NIR calibration for vitamins in fortified products, considerable savings are then possible because the test could be performed simultaneously with the NIR analyses for fat, protein, moisture and bulk density that are routinely carried out. This study aimed to use a series of fortified whole milk powders (WMPs) to look specifically at the determination of vitamins A and C by NIR.

Methods

Fortified WMP samples from one manufacturing site was analysed for vitamin A (80 samples) or vitamin C (132 samples). Reference analyses for the calibration sets were:

- Vitamin A - Reverse phase high-pressure liquid chromatography (HPLC).
- Vitamin C - Indophenol titration.

These samples were then analysed by the Foss NIRSystems 6500 using the powder cell and sample transport module. The powder cell was cleaned with a horsehair brush and compressed air between samples. Winisi II acquisition and chemometric software (Version 1.50, Foss NIRSystems) was used for calibration and chemometric processing. Various data pretreatments and maths treatments were used, typically MSC or SNV and 1, 4, 4, 1 or 2, 8, 6, 1. The wavelength range 1120-2500nm was used. The region 400-1100nm was found to be too noisy to allow useful correlation with the reference data.

The scores option was used to divide the data set up into a training set (good data) and a rejection set (outliers) by comparison with the distance from the average spectrum. The outliers were not used in any further calculations.

Two approaches were used to generate calibrations.

A generic approach with all samples across the entire vitamin range included in the calibration.

A more selective approach where specific ranges of vitamin concentrations pertaining to commonly produced specifications were used to produce calibrations.

Predicting the vitamin content of samples that were not part of any of the original calibration sets then validated these calibrations.

Results

Tables 1 and 2 summarise the results of the validation exercise. Table 1 shows that effective calibrations for vitamin A were possible when the validation set range exceeded 400 retinol equivalents per 100 g. When the validation set range was smaller than this, the standard error of prediction (SEP) observed precluded useful accuracy. Effective vitamin C calibrations (table 2) were less dependent upon sample range as the SEP values were proportionately less compared to the

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sample range. The SEP of the vitamin C calibrations compares favourably to the standard error of the reference method (5.8 mg/100g). This is in part due to the uniformity of the sample matrix. A greater SEP could reasonably be expected if the samples were sourced from a number of manufacturing sites.

Table 1 Vitamin A validation statistics

No. samples in validation set	Validation set range (retinol equivalents per 100 g)	R^2	No. PLS factors	SEP
65	283–2797	0.996	3	45.64
52	283–1490	0.975	5	46.36
44	283–864	0.889	5	46.38
34	680–864	0.880	6	44.61

Table 2 Vitamin C validation statistics

No. samples in validation set	Validation set range (mg/100g)	R^2	No. PLS factors	SEP
88	38–220	0.995	7	2.61
68	38–124	0.983	6	5.08

An investigation was undertaken to see if the use of selected wavelengths would improve prediction accuracy. There is the potential for reducing the influence of spectral noise from regions that are not directly correlated with the vitamin content. To this end NIR spectra of the ingredient vitamins A and C were collected under the same conditions as the routine WMP spectra. Examination of the loading plots for both vitamin A and C calibrations did not demonstrate the expected areas of high correlation in NIR regions of high infrared absorbance of the ingredient vitamins. Presumably the calibration is based upon changes to the matrix of the WMP brought about by and in proportion to the addition of the vitamins. Further investigation of this and the influence of changes in the nature of the ingredient vitamins is in progress.

In summary, NIR of WMP provides an effective rapid screening tool for assessing the correct wet dosing of vitamins A and C in a production environment.