Detection of buckwheat (*Fagopyrum esculentum*) adulterant in black pepper (*Piper nigrum* L.) by NIR reflectance spectroscopy and imaging

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Introduction

Black pepper (*Piper nigrum* L.) is considered to be the most widely-used spice due to its diverse utilisation.[1] It is used in human diets, as preservative, in perfumery and for medicinal purposes. Black pepper plays an important role in the global spice trade, fetching the highest return among all spices.[1,2] This encourages adulteration with inferior materials. Utilisation of adulterants in food is usually intentional to maximize revenues or to increase, for example, the overall appearance of a food product.[3] Incidental adulteration also occurs due to ignorance, negligence or lack of proper facilities.

Adulteration with inexpensive or inferior material has been reported in black pepper, with buckwheat (*Fagopyrum esculentum*), other cereals (for example, millets), pepper shells, nutshells or any organic material that can be reduced to a powder.[2,4] Established analytical techniques used for analyses of spices include microscopic examination and spectrophotometric (UV/vis) and chromatographic methods (LC-MS and TLC).[5] These methods provide high sensitivity, but are costly, time-consuming and require specialised personnel.

Near infrared (NIR) reflectance spectroscopy in combination with chemometrics is being applied successfully in the food industry. Its use in confirming food authenticity has also been proven.[6,7] This is due to the rapidity, ease of use and non-destructiveness of the technique, combined with mathematical reduction of captured data for in depth chemical exploration of the analysed sample. NIR hyperspectral imaging (HSI) is a technique that combines NIR spectroscopy and digital imaging to attain both spatial and spectral information from a sample.[8] It thus gives rise to the possibility to extract chemical information from an image. NIR HSI is increasingly being used to investigate food products.

In the work presented, NIR spectroscopy and HSI were evaluated for the quantification and detection of buckwheat and millet flours as adulterants in ground black pepper.

Materials and methods

Samples

Seven batches of ground black pepper, kindly made available by four suppliers, were adulterated with buckwheat and millet flour in evenly spread intervals between 2 and 50% (w/w). All samples were dried at 100°C for one hour, cooled in a desiccator and transferred to clear Sepcap vials (15 × 45 mm). The seven sample sets each comprised 11 samples which included one control and 10 adulteration levels. NIR spectra were collected from 1000–2500 nm with a Perkin Elmer Spectrum IdentiCheck FT-NIR system (Wellesley, MA, USA) with 16 spectral collections at 16 cm^{-1} resolution. Multiplicative scatter correction (MSC) and mean-centering were applied to the raw spectral data set (85 samples × 701 variables). Exploratory principal component analysis (PCA) was performed. Partial least squares (PLS) regression with full cross-validation was applied after pre-processing of the raw data with Savitzky–Golay 2^{nd} derivative (15 points, 3^{rd} polynomial order).

For the NIR HSI, the adulterated black pepper samples, buckwheat and millet flour as well as unadulterated ground black pepper were packed into micro-plates (50 × 100 mm; 50 holes) in a

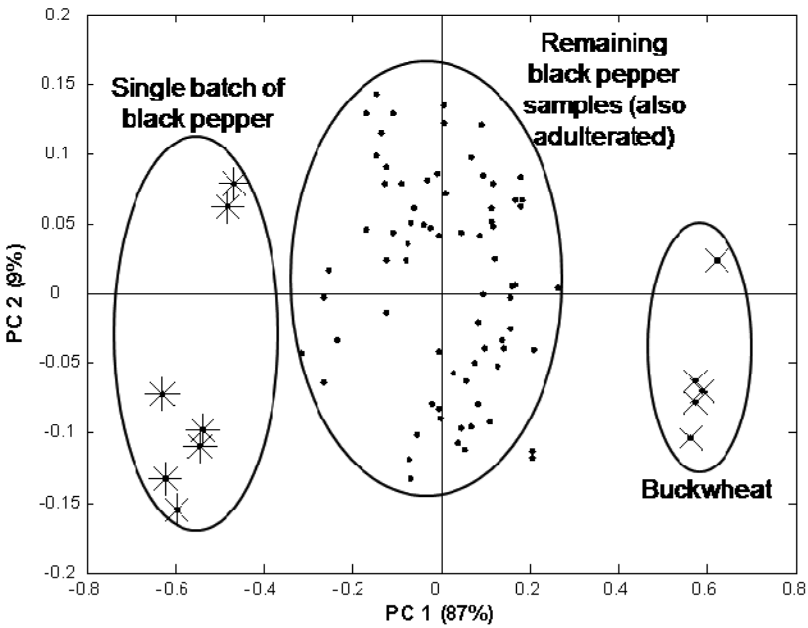

Figure 1. PCA score plot (PC1 vs PC2) with three discerned clusters.

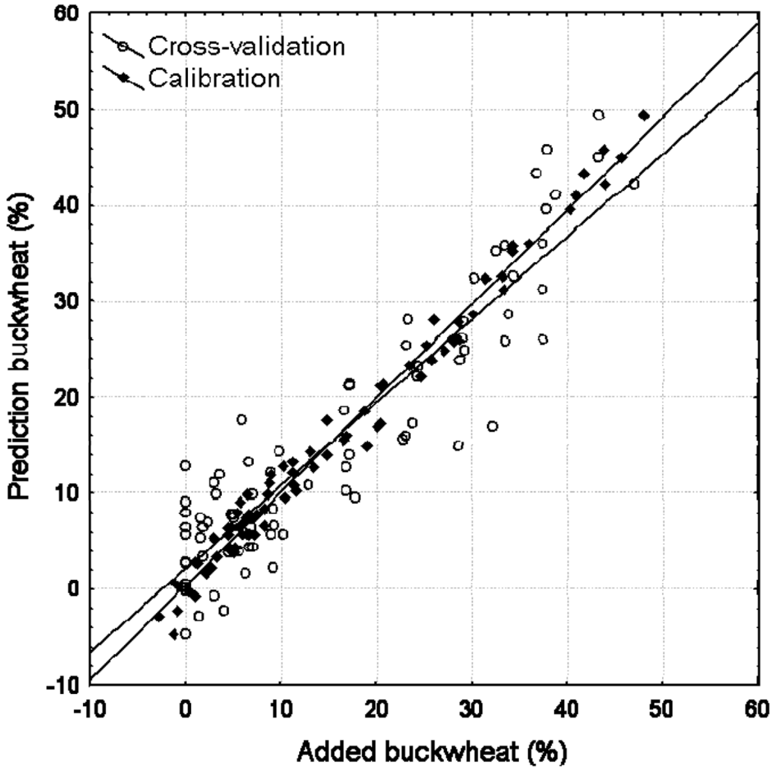

Figure 2. Calibration and cross-validation plots for added buckwheat (%) and predicted buckwheat (%) in ground black pepper.

randomised order. Images were acquired with a sisuChema SWIR imaging system (Specim, Oulu, Finland) from 1000–2498 nm with 6–7 nm intervals in a field of view of 50 × 100 mm, producing images of 320 × 364 pixels × 239 wavelengths. The sisuChema consists of an imaging spectrograph coupled to a 2-D array HgCdTe detector. Dark current and white standard scans were used for image calibration and converting reflectance counts to pseudo-absorbance.

Detection and removal of bad pixels and background in the NIR hyperspectral image was done by delineating clusters in the PCA score plot with 3 principal components (PCs) and using the brushing technique between the score plot and associated score image. Standard normal variate (SNV) and mean-centering were applied and the PCA re-calculated with 6 PCs.

Results and discussion

PCA performed on the pre-processed NIR spectral data set resulted in PC1 and PC2 explaining 87.2% and 8.8% of the variation (% sum of squares) (Figure 1).

In the score plot, three clusters could be discerned in the direction described by PC1, i.e. pure buckwheat, a single batch of black pepper and all the remaining black pepper samples; irrespective

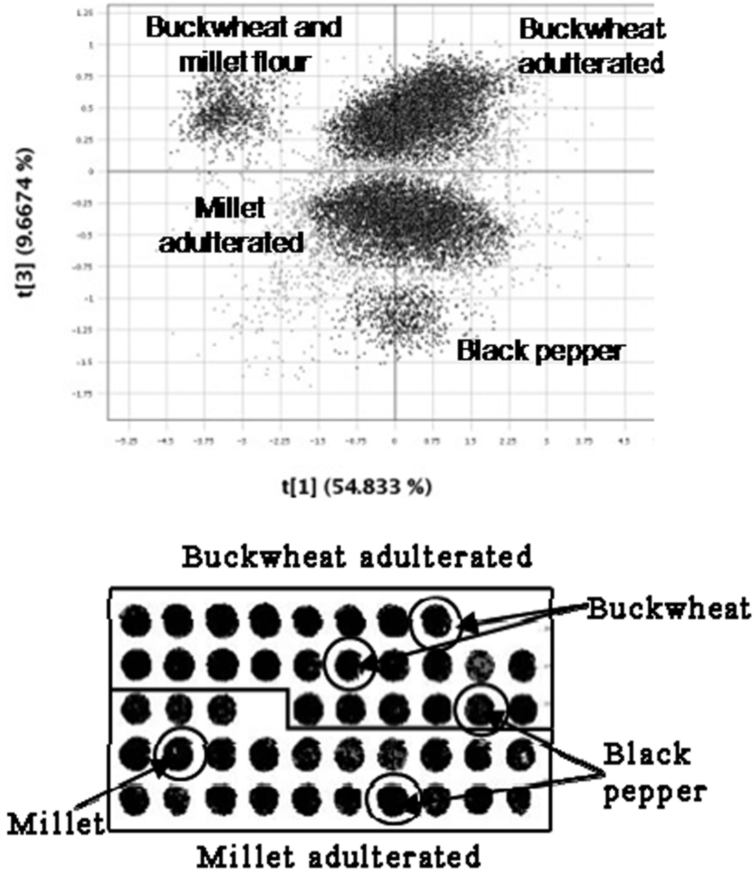

Figure 3. Score plot of (a) PC1 (55%) vs PC3 (10%) and (b) associated score image (PC3).

of level of adulteration. Although it still needs to be validated, it appeared that NIR spectroscopy could be used for detecting adulterated batches of black pepper. PLS regression with full cross-validation, applied to the pre-processed NIR spectroscopy data, gave a root mean standard error of cross-validation (*RMSECV)* of 5.53% and R^2 of 0.85 with five PLS factors (Figure 2).

The effect of particle size, because the samples were not totally homogenous, still needs to be investigated.

The first four PCs of the NIR hyperspectral image explained 54.8, 22.6, 9.7 and 3.9% of the variation [Figure 3(a)].

Clear clusters could be seen in the score plot (PC1 vs PC3) and, using the brushing technique, also clear intensity differences between the unadulterated and adulterated samples in the score image (Figure 3b).

Unadulterated black pepper could be distinguished from black pepper adulterated with millet and buckwheat flour, respectively, in the direction described by PC 3 whereas the millet and buckwheat flour were separated from the adulterated and unadulterated black pepper in the direction described by PC1.

Conclusion

Bulk NIR spectral data, together with PCA has the potential to detect adulterated batches of ground black pepper. A combination of bulk NIR spectra and PLS modelling after pre-processing was able to predict the level of buckwheat in ground black pepper. In contrast to bulk NIR spectroscopy, hyperspectral imaging in combination with multivariate data analysis has the potential to discriminate between unadulterated black pepper and black pepper adulterated with either buckwheat or millet flour. Both applications need to be confirmed, taking sample preparation and difference in particle size into consideration.

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