Application of NIR Analysis to Verify Cocoa Powder Authenticity

A. TRILČOVÁ[1]*, J. ČOPÍKOVÁ[1], M. A. COIMBRA[2], A. BARROS[2], L. EGERT[1], A. SYNYTSYA[1] and H. KŘÍSTKOVÁ[1]

[1]*Department of Carbohydrate Chemistry and Technology, Institute of Chemical Technology, Prague, Czech Republic, *E-mail: anezka.trilcova@vscht.cz; [2]Department of Chemistry, University of Aveiro, Aveiro, Portugal*

Abstract: At the present time the use of reliable control methods to ensure the labeled quality of food is task for organizations that have to limit or eliminate the falsification. The presentation describes the results of common analytical methods, NIR spectrometry and FT-IR spectrometry applied to cocoa powder. The two spectrometric techniques are statistically processed that allows discrimination of authentic and fraudulent cocoa powder samples.

Keywords: cocoa powder; NIR; FT-IR; discriminant analysis; principal component analysis

INTRODUCTION

Near infra-red spectroscopy (NIRS) has been widely used for the last four decades as a fast and accurate method for qualitative and quantitative analysis of biological and non-biological materials in the agriculture, food, textile, petrochemical and pharmaceutical fields [1].

Cocoa powder has gained a significant amount of attention in the industry because of the increase in the consumption of cocoa powder in non-confectionery industry applications (i.e., dairy and bakery segments). Because of this grow in use it is important to be able determine authentic and fraudulent cocoa powder. Cocoa powder is defined as the food prepared by pulverizing the material remaining after part of the fat has been removed from ground cocoa nibs [2].

The spectral data produced by an NIR instrument represents the total chemical and physical properties of sample. Although this spectral data can be obtained in a matter of seconds and is highly repeatable, it is only useful when interpreted in the form of current reference methods.

A good method for reducing the spectral data to independent sources of information is principal component analysis (PCA), and good mathematical technique for grouping samples is Mahalanobis distance or the "H" statistics. Calculating "H" on the PCA scores produces a global "H" for a file. This calculation established the boundary for the population. The spectra of new samples not used in the calibration could be evaluated to their distance from the spectral mean. The "H" values were standardized by dividing them by average "H" value for the calibration file. This standardization feature removes individual file dependency so the same limits could be used regardless of the type of file being used. If the new spectra were more then 3.0 standardized units from the mean of the calibration file, the sample is defined as a global "H" outlier and may not have accurate predictions. The global "H" was also used to discriminate or determine which calibration equation of a series of equations would be the best predict an unknown spectra. A second and even more important use of "H" calculation was neighborhood "H". The neighborhood "H" was used to find the closest neighbors for the samples in the product calibration or library file. This neighborhood of sample with associated laboratory reference values can be used to find the best samples for a local calibration [3].

For certification the authenticity of soy sauce, mid- and near-IR spectra of 27 commercial soy sauce produced from whole soybeans and 30 from defatted soybeans were measured. Factor analysis applied to MIR and NIR spectra individually indicated the existence of some difference between two soy sauces. Then, SIMCA, conventional step-wise linear discriminant analysis (LDA) and LDA using genetic algorithms (GALDA) were applied to their spectra for classifying soy sauce samples according to types of soybeans. In step-wise LDA, 94.7% of samples were correctly assigned based on six wavenumbers in mid-IR spectra but 100% correct classification was obtained by six wavelengths in NIR spectra. Cooman's plots in SIMCA applied to mid- or near-IR spectra indicated that differentiating two types of soy sauce samples was difficult due to high similarity in their constituents. Perfectly correct classification was attained by five to eight mid-IR wavenumbers and four to five near-IR wavelengths selected by GALDA. In general, the differentiation using NIR spectra was more reliable than that using MIR spectra [4].

The reference method for the measurement of the percentage of sugar in sugar beets is polarimetry. Based on polarimetric reference values and infrared spectra, regression techniques, such as partial least square regression (PLS), was used to develop mathematical models to predict the sugar content of beets. The spectra acquired in the two domains were combined by calculating the outer product matrix of the two vector signals of each sample. It was then possible to perform statistical analyses, such as principal components analysis or partial least square regression, on the resulting data set of matrices in order to highlight relations between spectral features in the two domains. This could facilitate the attribution of NIR bands based on their relation to MIR peaks. 2D correlation spectroscopy was another method that was used to allow bands in the NIR spectrum to be resolved and assigned to characteristic absorbencies in the MIR spectrum. The principle of this method was to detect regions in both spectra (NIR and MIR) that change simultaneously as sugar content varies [5].

EXPERIMENTAL

NIR spectroscopy. Near Infrared Spectrometry was performed on NIR Systems 6500 (Perstorp Analytical Company, USA) with software NIR3 version 3.10 Infrasoft International (ISI). Statisti-

cal evaluation was by software Winisi II. Spectra were measured in region from 400 to 2500 nm at a resolution of 2 nm. Number of reference scans to average before sample was 12, number of sample scans to average was 36 and number of reference scans to average after sample was 3. Samples were measured in reflectance mode in the small ring cup.

Determination of moisture. Samples were weighted into dried-up weighing bottles with glass sticks and sand. Samples were dried in oven at 105 ± 2°C for 3 hours, and then they were put in dessicator and after 30 minutes they were weighed. This process was repeated till obtaining constant value [6].

FT-IR spectroscopy. Infrared spectroscopy in mid region was performed on FT-IR spectrometer Bruker IFS-55 (Bruker, Germany) with ATR accessory, beam divider KBr, detector DTGS and apodization: triangular. Spectra were recorded at the absorbance mode from 4000 to 400 cm^{-1} at the resolution 8 cm^{-1}. Five replicate spectra (128 co-added scans) were collected for each sample. The measured spectra were transferred via a JCAMP.DX format into the data analysis software package for PCA and each spectrum within the 1200–800 cm^{-1} region, was auto-scaled.

RESULTS AND DISCUSSION

NIR spectroscopy

Obtained NIR spectra were evaluated by discriminant analysis. This method classifies samples by virtue of Mahalanobis distances (H) referring to certain samples' class. On Figure 1 is 3-dimen-

Figure 1. 3-dimension graf with global "H" values for file of cocoa powders

sion graf with global "H" values for file of cocoa powders. Samples 41 and 11 are out of file.

All the spectra of cocoa powder were calibrated by analytical parameter moisture. Calibration was calculated by MPLS. Calibration was judged according to correlation coefficient (R^2) and by means of calibration coefficient of variation (*CCV*). Dependence with R^2: 0.95–0.99 is very strong, 0.80–0.94 is pretty strong, 0.50–0.79 is middle strong. Calibration with *CCV* values about 10% is good under 5% is very reliable. *CCV* is calculated:

$$CCV = \frac{SEC}{X_P} 100 \qquad (\%)$$

where: *SEC* – standard deviation of calibration (computed by software WINISI II)

      X_P – average value

Value of correlation coefficient (R^2) was 0.949, standard error of calibration (SEC) was 0.235 and calibration coefficient of variation was 6.13%. Dependence of calibration according to *R* was pretty strong and calibration according to *CCV* was good. That means that calibration was successful.

FT-IR spectroscopy

Measured spectra were evaluated by Principal Component Analysis (PCA). The PCA analysis could allow the characterization of the sample relationships (scores plans or axis) and, at the same time, the recover of their sub-spectral profiles (loadings) – Figures 2–3. From Figure 2 is clear,
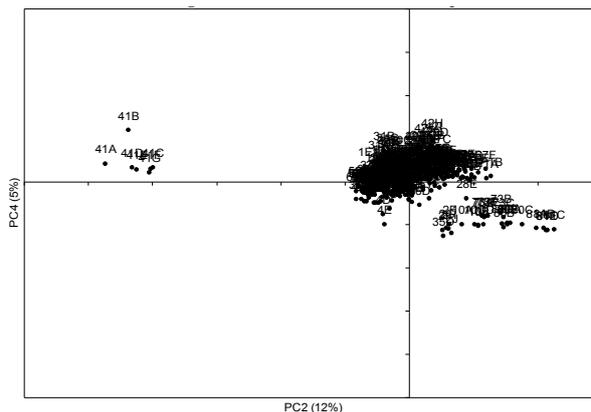

Figure 2. PCA scores scatter plot of the FT-IR spectra

that sample 41 is out of file of others samples (it is also out of NIR spectra file). The reason for sample 41 is likely to contain the higher amount of starch.

CONCLUSION

Spectroscopy joined to statistical evaluation is very powerful technique. NIR spectra of cocoa powders are different, but it is not caused by moisture. MIR spectra were evaluated by PCA and we know that differences are mainly caused by composition of neutral sugars and uronic acids. These results show that spectroscopy can be used as very reliable and quick tool for cocoa powder authentication.
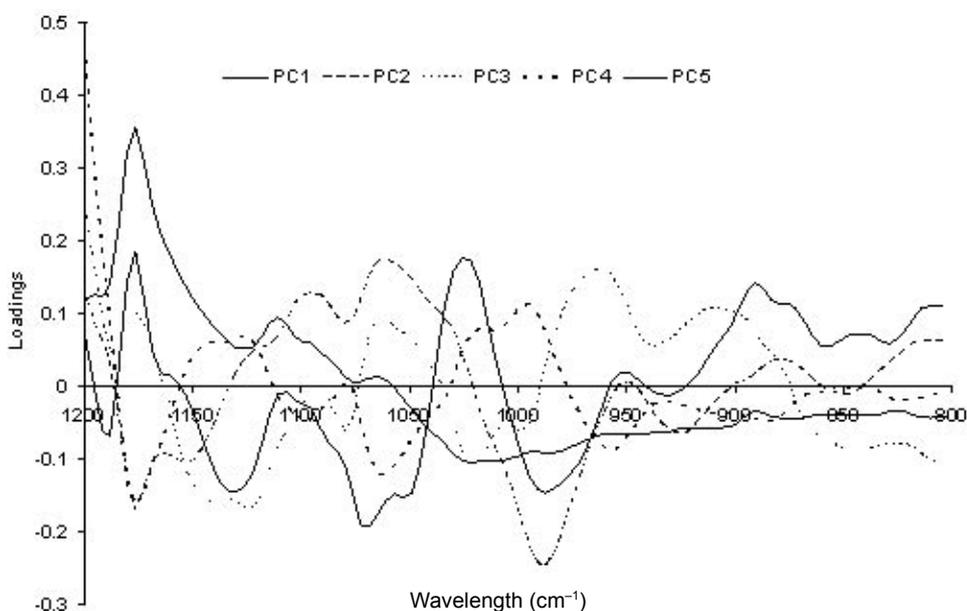

Figure 3. PCA loadings plot in the 1200–800 cm^{-1} wavenumber region

References

[1] Williams P.C., Norris K.H. (1987): Near-Infrared Technology in the Agricultural and Food Industries. American Association of Cereal Chemists Inc., St. Paul.

[2] Dyer B. (2003): Manuf. Confect., **83**: 47.

[3] User manual NIRS 3, 1995.

[4] Iizuka K., Aishima T. (1999): J. Food Comp. Anal., **12**: 97.

[5] Maalouly J., Eveleigh L., Rutledge D.N., Ducauze C.J. (2004): Vibrational Spectroscopy.(in press).

[6] ČSN 56 0146 část 3.

332

Proc. Chemical Reaction in Food V, Prague, 29. 9.–1. 10. 2004