

Near Infrared Spectroscopy As A Competent Method For Qualitative and Quantitative Determination of Detergent Adulteration In Milk

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Abstract - Near Infrared Spectroscopy can act as a feasible tool for detecting the presence of detergent as an adulterant in milk. Present work was carried out to formulate a chemometric model to evaluate the existence of detergent as contaminant in milk, qualitatively as well as quantitatively using NIR Spectroscopy. Total 30 samples were prepared using three different varieties of milk, out of which three samples were free from adulterants and rest were having detergent present in them. Those 27 adulterated milk samples were having detergents at nine disparate concentrations: 0.025%, 0.05%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 2.0% of detergent respectively for each kind of milk. The data collected from NIRS instrument was analyzed using chemometric software (CAMO Unscrambler version X 10.3). The Principal Component Analysis (PCA) was run on the sample set to know the relation between the different samples on the basis of the Near Infrared spectral data. It was noticed that the PCA score plot could classify the samples in three groups on the basis of their adulteration: low, medium and high adulteration. Partial Least Square Regression Analysis (PLS-RA) was deployed to design a statistical model to envisage the percentage detergent content present in the adulterated milk samples by selecting vital wavelengths. It was analyzed that the regression model revealed quite good results for the prediction of detergent adulterated milk samples with coefficient of correlation higher than 0.9 and the root means square error of validation (RMSEV) was 0.013. Thus, it was agglomerated that NIR spectroscopy can bestow dairy industry a lucid, coherent, quick, green and flexible technique for quantification of detergents in milk adulterated samples.

Keywords- Milk, Adulteration, Near Infrared Spectroscopy, Regression, Chemometrics.

I. INTRODUCTION

The value of milk is linked to its protein content, minerals for bone strength, health nourishing vitamins, milk fat and other fatty acids. However, one of the common phenomenon that have been overlooked across the globe is the presence of adulterants, for instance, water, sugar, detergents, whey; melamine etc. that creates threat to the well being of an individual. There are various analytical techniques that can be applied for the detection of milk composition that are quite time-consuming, demands hazardous chemicals, expensive, and are usually destructive and off-line by nature. The edge of NIRS in confirmation of contaminants in milk acts as a green and non-destructive approach in the wavelength region of 700 nm to 2500 nm. Pranita Jaiswal et al have reported the presence of anionic detergent as degraders in milk using FTIR in the range of

1600-996 cm^{-1} and 3040-2851 cm^{-1} . Manjun Tay et al have aimed to investigate the infant milk for the detergent deduction using Liquid Chromatography (LC)-Q trap and LC-hybrid quadrupole ion trap mass spectroscopy (LC-QTOF-MS) with chemometrics. Alessandra Borin et al have revealed the feasibility of NIR along with least square vector machine for assessing some common adulterants like starch, whey, or sucrose in powdered milk samples. Most of the subjective methods for investigating the foul materials in milk are of colorimetric but lacks due to limited range of concentrations and inaccuracy. Many degrading materials are added to revamp the taste of milk while most of them are toxic chemicals used to enhance the physical features and shelf-life of milk. These are mostly harmful and results in fatal ailments to consumers. In this work, we have investigated detergents that are usually added to improve the appearance of milk but leads to harmful diseases.

Previous literature reveals that ample amount of work has been reported that detects the presence of harmful adulterants like urea, melamine, formalin etc. in milk and milk products still very few reports are there that reports satisfactory results of detection of toxic detergents in milk samples. There is a large gap that is needed to be bridged for both quantitative and qualitative determination of fraud chemicals in milk. In this study, we have tried to link the gap between chemical tests and chemometrics by adopting NIR spectroscopic based method along with chemometrics using unscramble X software to ensure the safety of milk consumers in a green, non-destructive and fast way.

II. METHODOLOGY

A. SAMPLE PREPARATION AND INSTRUMENTATION

In this report, three distinct samples of milk were purchased from nearby area of Chandigarh, Punjab region and were investigated which includes:

- Local vendor milk (Collected from local vendor near C.S.I.O market, Chandigarh)
- Amul milk (homogenized Toned Milk), with minimum fat of 3.0% and minimum SNF value of 8.5% with batch no. MKE 3451C and
- Verka milk (with batch no. 1S/ISO9001:2008, IS15000; 1998, having minimum milk fat and milk SNF values of 4.5% and 8.5% respectively).

These milk samples were then adulterated with detergent at nine distinct concentrations: 0.025%, 0.05%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, 1.0% and 2.0% of detergent respectively for each milk. NIR spectra of samples were obtained using NIRS DS 2500 Spectrometer (Metrohm) (@CSIR-Central Scientific Instruments Organisation, Chandigarh) in reflectance mode in the spectral range of 700-2500 nm with a gap of 0.5 nm with 4200 data points. The absorbance of the samples was calculated by employing VISION software. For each sample, spectrometer gave an averaged spectrum of 3 scans. During experimentation and sample collection, all possible precautions were taken to avoid any external contamination. The experimentation and testing work was carried out in CSIO Research laboratory of Chandigarh. Total 27 samples were prepared and analyzed out of which 24 were adulterated with detergents and rests were pure samples.

B. MULTIVARIATE ANALYSIS

The data collected was analyzed by employing chemometric software (CAMO Unscrambler version X 10.3, licensed version @ CSIR-CSIO Chandigarh). This tool promises a facile approach for any sort of multivariate data analysis. This has the benefits of finding variations, co-variations and in stating the key relationships in data matrices. This can also be utilized for developing prediction models for the real time analysis of spectroscopic materials. It was initially developed in 1986 by Harald Martens and later further designed by CAMO software. This provides key features to evaluate the Principal Component Analysis (PCA), Principle Least Square Regression (PLS), Multivariate Curve Resolution and much more. In this study, we have used PCA along with NIPLAS algorithm to verify the correlation between the samples (Scores) and the variables (Loadings). PCA is a mathematical strategy that transforms a number of correlated variables to a number of uncorrelated variables called principal components. It has benefits of reducing ascribe space to a smaller number of factors.

III. RESULTS AND DISCUSSION

A. NIR SPECTRA

NIR spectrum of the samples was carried in reflectance mode and absorbance was calculated by the VISION software of NIRS DS2500 NIR spectrometer as shown in Fig.1. It can be observed

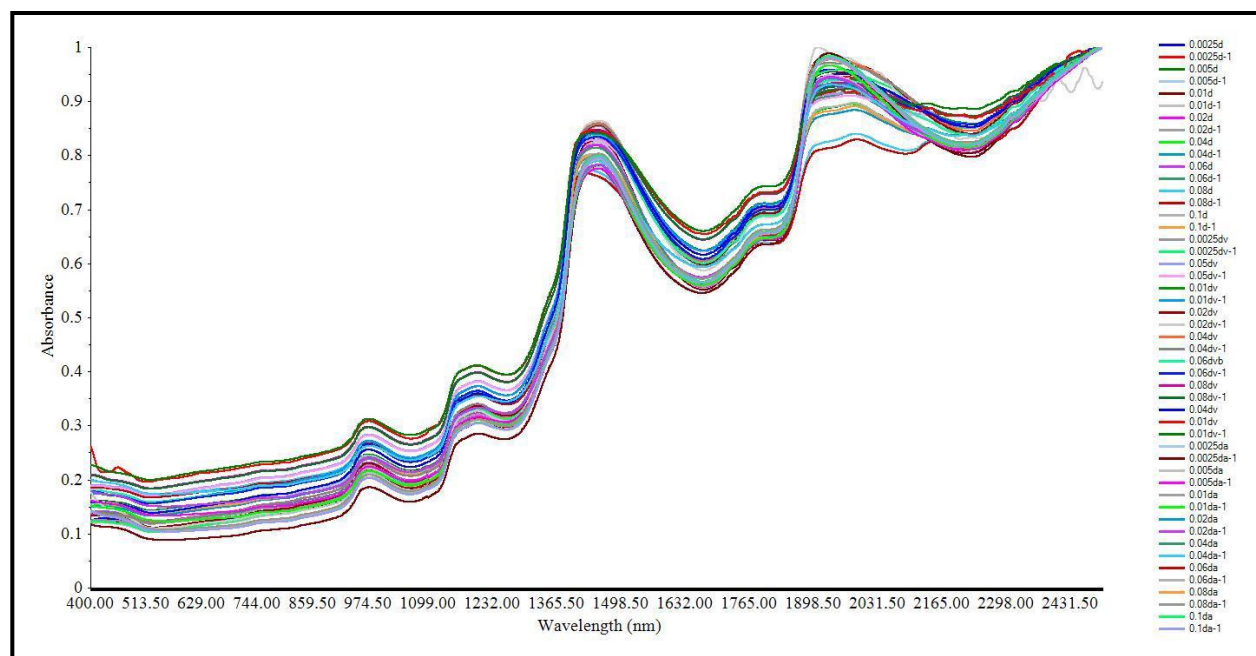


Figure 1VIS/NIR Spectrum of detergent adulterated samples

that absorbance peaks for detergent maximum at (1498.50-1765.00) and minimum at (974.50-1099.00) wavelengths. According to literature these peaks corresponds Aromatic bonds with frequency (1600-1475) cm^{-1} of medium weak intensity, C=C Alkene bonds with frequency (1680-1600) cm^{-1} of medium weak intensity, Ketonic bonds with frequency (1725-1705) cm^{-1} of strong intensity, C=O bonds Aldehyde with frequency (1740-1720) cm^{-1} of strong intensity, COOH bonds with frequency (1760-1700) cm^{-1} of strong intensity, Anhydride bonds with

frequency (1810 -1760) cm^{-1} of strong intensity, N=O, Nitro bonds with frequency (1550-1350) cm^{-1} of strong intensity, CONH_2 (1680-1630) cm^{-1} , COOR (750-1730) cm^{-1} of strong intensity bonds.

B. PRINCIPAL COMPONENT ANALYSIS

The Principal Component Analysis (PCA) was performed for the spectral data to check the

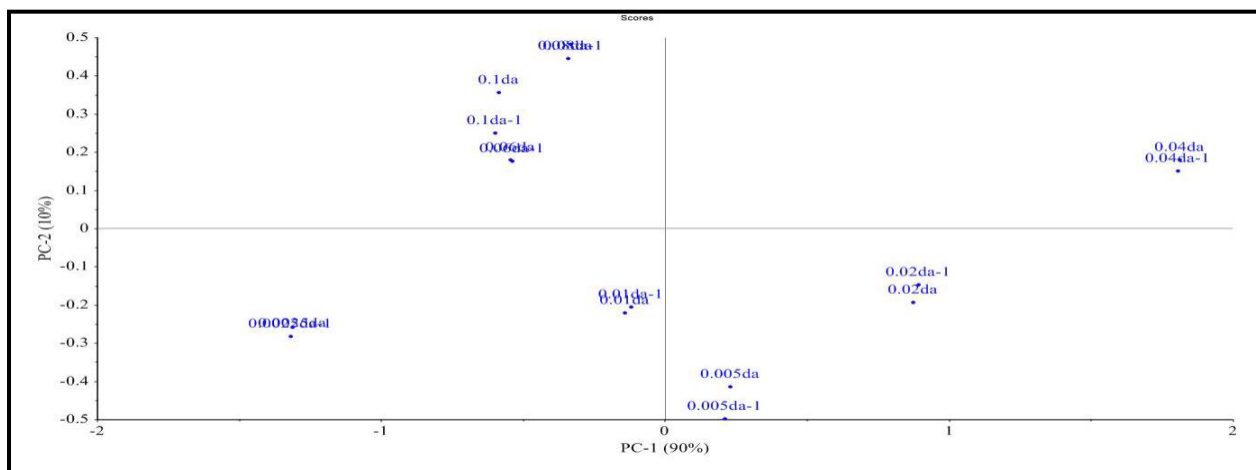


Figure 2 PCA Score plot for adulterated samples

relation between prepared adulterated samples and the absorbance of the wavelengths from 700-2500 nm as shown in Fig 2. It was unfolded that the spectral data alone was sufficient to classify the adulterated samples in groups. It was observed that 100 % data was covered by the first two principal components.

PCA correlation loading plots (Fig. 3) showed that the important wavelengths responsible for the detergent adulteration in milk. Although the spectral data is for the wavelengths from 700-2500 nm However; some wavelengths are not important for a particular molecular bond. Thus, this is

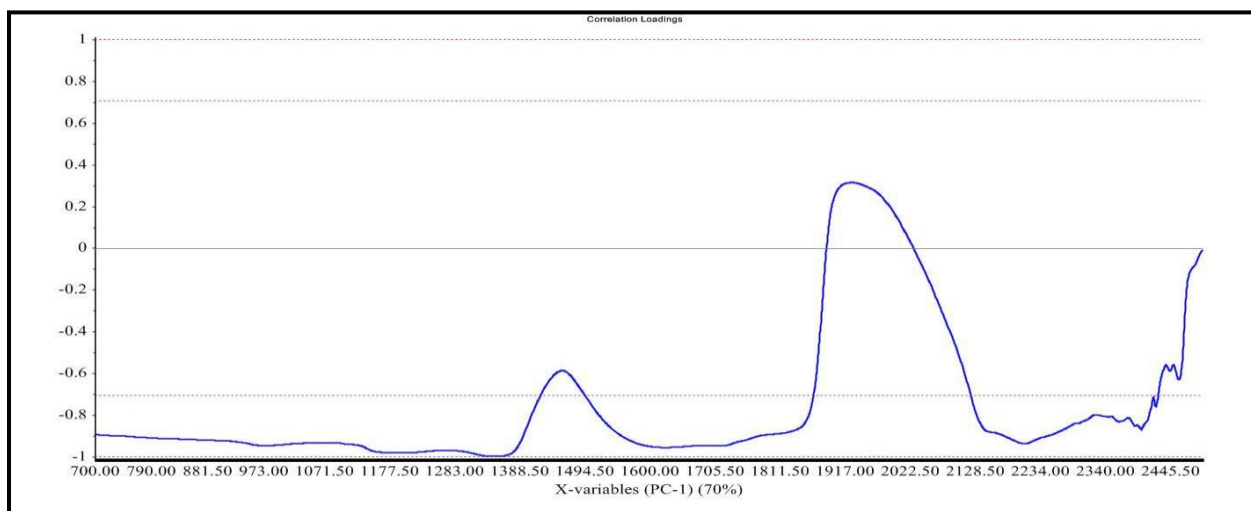


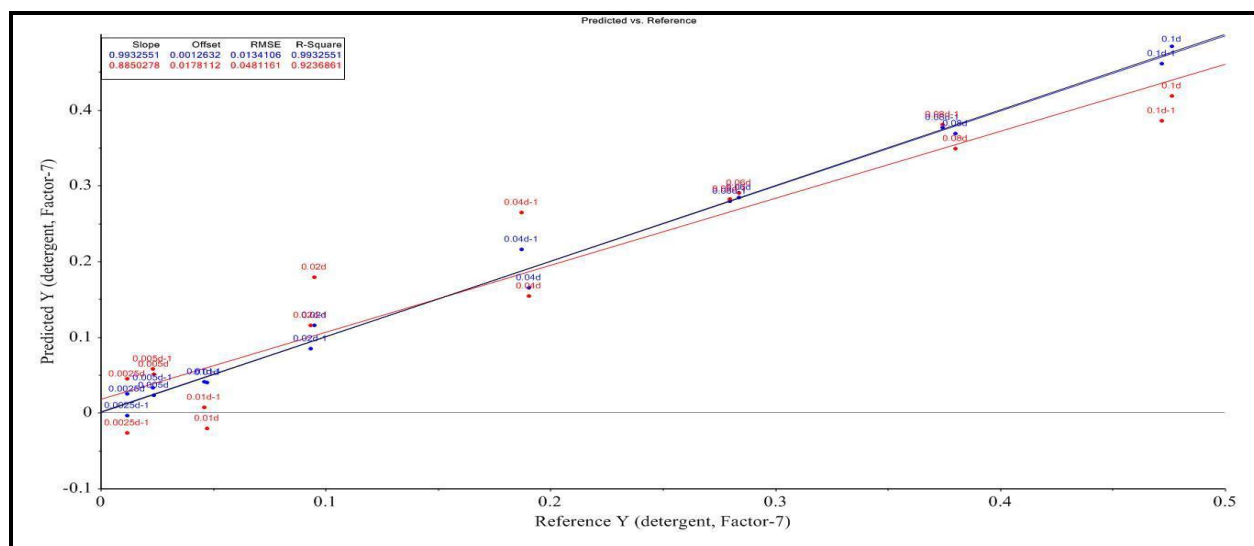
Figure 3 Loading plots for adulterated samples

needed to be eradicated while building a statistical model to predict the adulteration for that molecule.

The correlation loading values varies from -1 to +1 (negative 100% to positive 100 correlations). The variable above 70% correlation (negative or positive) is considered important for the prediction of the respective parameter. It was observed that for detergent adulteration at (1825.50 -2128.00) nm wavelengths were important.

C. PARTIAL LEAST SQUARE REGRESSION MODEL

Partial Least Square regression was used to build the statistical mechanism to predict the adulteration of milk samples qualitatively and quantitatively (Fig. 4). It was observed that coefficient of correlation (r^2) for the percentage of adulteration of prepared samples with the predicted adulteration percentage was more than 0.9 (greater than 90%) for both the set of adulterated samples. The Root Mean Square Error of Validation (RMSEV) was 0.013 for the



respectively. Also, the correlation values of loadings vary from -1 to +1 and the variables above 70% correlation are considered as important for predicted parameters.

Partial Least Square (PLS) regression model was employed to build statistical models for finding the adulterants in milk qualitatively and quantitatively. The result shows that coefficient of correlation (r^2) for predicted as well as for prepared sample was more than 90% and the Root Mean Square Error of Validation (RMSEV) was 0.013 for detergent. Thus, it is CULMINATED that NIR spectroscopy can act as a promising benefic for dairy industry with its various merits, for instance, simple, convenient, rapid, green and non-destructive technique for quantification of adulterated milk samples.

V. ACKNOWLEDGEMENT

Authors of this paper acknowledge Dr. Sunita Mishra, Sr. Principal Scientist and Head of Ubiquitous Analytical Techniques and R & D Division, CSIR-Central Scientific Instruments and Organisation, Chandigarh for the use of NIR DS2500 Near Infrared Spectrometer for the collection of spectral data of the samples and for the use of lincensed version of CAMO Unscrambler for the regression analysis.

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