Near Infrared Spectroscopy as an efficient tool for the Qualitative and Quantitative Determination of Sugar Adulteration in Milk

Uma Kamboj1*, Neha Kaushal2, Shakira Jabeen1
1 Department of Physics, Lovely Professional University, Phagwara, Punjab, India-144411
2 Ubiquitous Analytical Techniques and R&D Division, CSIR-CSIO, Chandigarh, India-160030
Corresponding Address: E-mail: amukam@gmail.com, (Dr. Uma Kamboj), Mobile: 8847056454

Abstract
The present work focuses on detecting the presence of sugar as an adulterant in milk using Near Infrared Spectroscopy. A chemometric model was formulated to evaluate the presence of sugar content in milk, qualitatively as well as quantitatively using multivariate analysis. Total 24 samples were prepared using three different varieties of milk, out of which three samples were pure and the rest were having sugar present in them. Those 21 milk samples were adulterated with sugar at seven different levels: 0.2%, 0.4%, 0.6%, 0.8%, 1.6%, 3.2%, 0.8% and 6.4% of sugar 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 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 observed that the PCA score plot could classify the samples in three different groups on the basis of their adulteration: low, medium and high adulteration. Partial least square (PLS) regression analysis was used to develop a statistical model to predict the percentage of sugar in the adulterated milk samples by selecting vital wavelengths. It was noticed that the regression model revealed quite good results for the prediction of sugar adulterated milk samples with the coefficient of correlation higher than 0.9 and the root means square error of validation (RMSEV) was 0.04. Thus, it was concluded that NIR spectroscopy could provide dairy industry a simple, efficient, quick, green and non-destructive technique for detection and quantification of milk adulteration.

Keywords: Milk, Adulteration, Near Infrared Spectroscopy, Regression, Chemometrics

1. Introduction
Near-infrared (NIR) technology is suitable for the quality determination of food products, because of the inexpensive optical sensors available in the market. Various analytical methods used for detection of milk composition are quite destructive, expensive, time and labour consuming, requires hazardous chemicals and are off-line by nature (Table1).

Table 1 List of common chemical tests to detect the presence of adulterants in milk (Ref. (www.stayyounghealthy.com))
| Name of adulterant       | Aim for addition                                      | Experiment                                                                 | Observation                                                                 |
|--------------------------|-------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Hydrogen Peroxide        | This is added to increase the economic benefits.      | Take about 5 ml of milk in a test tube and add 5 drops of paraphenylenediamine to it and shake well. | Change of milk color to blue confirms the presence of hydrogen peroxide. |
| Starch                   | This rises the SNF content in milk.                   | Boil about 3 ml of milk in a test tube and after cooling add 2-3 drops of 1% Iodine solution to it. | Color changes to blue that confirm the presence of starch.                      |
| Urea                     | This elevates the SNF value of milk.                  | 1.) Take about 5 ml of milk and mix it with equal amount of Para dimethyl amino benzyl aldehyde (16%).
2.) Take 5ml of milk and add 0.2 ml urease to it, shake it and then add 0.1 ml of bromothymol blue solution. | 1.) Solution will turn yellow indicates the presence of urea. 2.) Its presence is confirmed by the appearance of blue coloration after 10-15 minutes. |
| Pulverised Milk          | This is added to earn profit.                         | Take about 10 ml of milk and dilute it by addition of equal amount of hot water to it. Then, addition of 1-2 drops of phenolphthalein is required. | Pink color indicates milk is having soap content present in it. |
| Detergents               | This is added to get economical benefits.             | Take about 5mL of milk and add 0.1ml of bromocresol to it.                   | Violet color indicates the presence of detergents. |
| Buffalo milk in cow milk | In order to get benefit by increasing the              | Hansa Test: Take 1ml of milk and dilute it with 4ml of water to it.          | Precipitation reaction indicates the presence of buffalo milk in |
|                          |            |                                                              |                                                                            |
amount and decreasing the quality. Also treat it with 1ml of antiserum. Cow milk.

Milk constitutes all the vital nutrients i.e. fats, proteins, vitamins, lactose, carbohydrates and minerals that are beneficial for maintaining the health of an individual. India has a remarkable hike in consumption as well as production of milk in the past few decades. According to NDDB, 2018 report, productivity of milk was 55.6 MT in 1991-92 that has reached to 1555.5 MT in 2015-16 and a significant rise in per-capita milk consumption from 178 (g/day) in 1991-92 to 337 (g/day) in 2015-16 has also been recorded (Tiziana and Stephen, 2013). However, recently adulteration of milk has become an ominous issue. According to FSSAI 2014, food adulteration is an act of intentionally moulting the quality of food available for sale either by addition or substitution of low quality substances or by the removal of some vital components. Fraud producers and suppliers are adding a number of adulterants like water, urea, detergents, starch, boric acid, salicyclic acid, hydrogen peroxide, sugar, melamine etc that is causing severe health hazards (Mabood et.al, 2017). Thus, there is a demand of non-destructive, rapid and green method with effective instrumentation for accurate milk analysis.

Near-infrared spectroscopy has been demonstrated as a promising tool in agriculture and food industry for rapid and accurate compositional analysis and for quality control of various products. It offers various benefits, for instance, high speed, simplicity, green and simultaneous non-destructive measurements of a number of constituents. The NIR spectroscopy has been used to measure the contents of various dairy products such as milk, milk powder, whey, and cheese (Tsenkova et.al, 1999). FT-NIRS along with multivariate method has been developed to identify camel milk adulterated with cow milk. NIR spectral data was analysed using PCA, PLS-DA AND PLS statistical methods. Results revealed that this approach is quite fast and non-destructive (Fazal et.al, 2017). Near Infrared region (NIR), was first discovered by Frederick William Herschel. However, the progression of this technology took place in early 1970s, by Phil William, who used this technique to analyze protein and moisture contents as a basis for trading wheat. Although the accuracy level of this method depends upon the reference model used, still it is widely utilized in the field of agriculture, textiles, cosmetics, medical applications, food quality etc (Tsenkova et al, 1999).

Adulteration of natural milk with vegetable oil, urea, detergent powders, sugar, salt, water etc. has also been studied in literature using near infrared spectroscopy. Results disclosed that NIRS is propitious in detecting these adulterants in milk with best range of wavelengths in (926.6 to 939.4, 996.6 to 1021.8, 945.8 to 977.6, 926.6 to 961.7, and 933.0 to 945.8) nm (Jha and Matsuoka, 2004). A recent work has been done on honey (Bázár G, 2016) and results displayed that NIRS in combination with aquaphotomics can be used to find water molecular structures and adulterations in honey. In this work, NIRS along with chemometrics had been applied to detect the occurrence of less expensive adulterant, sugar in milk by examining the spectra in the region of 1100-2500 nm. PLS calibration method SIMCA and DPLS method were employed for classification of adulterants. Results revealed that NIRS is an efficacious method for determination of sugar contents in milk (Sumaporn KASEMSUMRAN, 2007). NIRS and LS-SVM multivariate calibration method had been used to check the presence of sugar whey in milk (Alessandra Borin, 2006). ATR-MIR micro spectroscopy has also been recognized as a rapid detection method to unfold the presence of whey as adulterant in milk. SIMCA and PLSR models developed along with MIR-micro spectroscopy act as a fast and potential method to check the authenticity of milk. Despite of its effectiveness, this method is less in demand due to its high cost of operation (P.M. Santos, 2013).

The aim of this study is to divulge the potential of NIRS for detection of sugar as adulterant in milk. Thus, spectrum was observed for the identification of sugar as adulterant in milk in NIR region from 700 nm to 2500 nm. Chemometrics model was formulated by performing multivariate analysis of obtained data.
using Principal Component Analysis (PCA) and Partial Least Square (PLS) regression methods, it is observed that NIRS act as a fruitful approach for analysis of milk and ultimately to ensure the safety of consumers.

2. Materials and Instruments Used

In this study, three different types of milk samples were purchased from nearby area of Chandigarh, Punjab region and were investigated which includes local vendor milk, Amul milk (homogenized) and Verka milk. NIR spectra of samples were obtained using NIRS DS 2500 Spectrometer (Metrohm) in reflectance mode in the range of 700-2500 nm at a gap of 0.5 nm with 4200 data points. The absorbance of the samples was calculated by VISION software. For each sample, spectrometer gave an averaged spectrum of 3 scans.

2.1 Sample Preparation

Three different types of milk samples were adulterated with sugar at seven different percentage levels: 0.2%, 0.4%, 0.6%, 0.8%, 1.6%, 3.2% and 6.4% respectively for each milk. At the time of collection of samples all possible precautions were taken to avoid external contamination. The experimentation and testing work was done in CSIO Research laboratory of Chandigarh on 9th march 2018 shown in Figure 1. The total number of samples prepared was 24: out of which 3 was without adulteration and 21 was adulterated with sugar. We have done the NIR of all samples and spectra was analyzed using unscramble X.
2.2 Multivariate Analysis Performed

The collected data was analyzed using chemometric software (CAMO Unscrambler version X 10.3). The main strength of the Unscrambler X is to provide tools for analysis of any sort of multivariate data. It calibrates data and also can be used for the prediction of models for the real time analysis of spectroscopic material. Originally, it was developed by Harald Martens and later by CAMO software. The software is used to find the Principal Component Analysis (PCA), Principal Least Square (PLS) regression, multivariate curve resolution and many more. Principal Component Analysis (PCA) was done using this software with NIPLAS algorithm, to find the correlation between the samples (Scores) and the variables: wavelengths (loadings). It transforms a large number of inter-correlated variates to reduced number of variates, along with the reduction of noise from them. It is a data compression method that involves a mathematical procedure in which a number of correlated variables can be transferred into different uncorrelated variables called principal components. It reduces attribute space from a larger number of variables to a smaller number of factors and as such is a "non-dependent" procedure (that is, it does not assume a dependent variable is specified).

3. RESULTS

3.1 NIR Spectroscopic Analysis

NIR spectrum of the samples was carried in reflectance mode and absorbance was calculated by the VISION software of NIRS DS2500 NIR spectrometer. Figure 2 shows the line plot of the sugar adulterated samples. It can be observed that absorbance peaks for sugar can be maximum at (1400-1750) nm and minimum at (968.50-1091.00) nm wavelengths. According to literature these peaks corresponds to 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, ketone 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), COOR (750-1730) cm\(^{-1}\) of strong intensity bonds.

![Figure 2 NIR Spectra of sugar adulteration in milk at different compositions](image-url)
3.2 Principle Component Analysis (PCA)

The Principal component analysis (PCA) was performed for the spectral data to check the relation between prepared adulterated samples and the absorbance of the wavelengths from 700-2500 nm. It was observed that the spectral data alone was sufficient to classify the adulterated samples in groups. Figure 3 shows the PCA score plot for sugar adulterated milk samples. It can be seen that the samples with adulteration from 0.08 – 0.01 mg form group I and those with high adulteration form group II. It was also observed that 100 % data was covered by the first two principal components. The low adulterated samples were grouped in upper part of the score plot and the highly adulterated in lower part. Thus, PCA was successful in grouping the samples on the basis of spectral data alone.

PCA correlation loading plots showed the important wavelengths responsible for the sugar and detergent adulteration in milk. Though the spectral data is for the wavelengths from 700-25000 nm, some wavelengths are not important for a particular molecular bond. Thus, the need is to eradicate
these while building a statistical model to predict the adulteration for that molecule. Figure 4 shows the correlation loading plots for sugar and detergent adulterated samples. The correlation loading values varies from -1 to +1 (negative 100% to positive 100% correlation). The variables above 70% correlation (negative or positive) are considered important for the prediction of the respective parameter. It was observed that for sugar adulterated sample, (881.50-1900.00) nm and (2022.50-2445.00) nm wavelengths were important.

3.3 Partial Least Square regression

Partial Least Square regression was used to build the statistical model to predict the adulteration of milk samples qualitatively and quantitatively. It was observed from Figure 5 that the prediction model revealed good accuracy for the prediction of sugar adulterated samples. 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 the adulterated samples. The root means square error of validation (RMSEV) was observed to be 0.04. The high values of correlation coefficient and low values of RMSEV showed that the PLS regression model can be used for the detection of adulteration in milk quantitatively and qualitatively. The build PLS model can be applied to predict the percentage of adulteration and type of adulterant for any milk samples.

4. Conclusion

NIR act as a bridge between milk safety and people’s daily life. In this study, we explored the application of NIR spectroscopy with principal component analysis (PCA) and partial least (PLS) regression to classify and quantify the adulteration in milk. The study shows that many bonds present in adulterated milk samples can be detected as absorbance peak at (1400-1750) nm, (968-1091) nm, (1498.50-1765) nm and (974-1099) nm respectively. The PCA method was employed to check the prepared adulterated samples in the absorbance wavelength from (780-2500) nm. The result shows that the high adulteration found from group II of PCA and 100% data was covered by first two groups respectively. Thus, PCA plays a very important role in analysis of adulterated milk samples. Also, the correlation values of loadings vary from -1 to +1 and the variable above 70% correlation are considered as important for predicted parameters. Partial least square (PLS) regression model was
used 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 0.04 for sugar. Thus, it is concluded that NIR spectroscopy could provide dairy industry with a simple, efficient, quick, green and non-destructive technique for detection and quantification of milk adulteration.

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