Analysis of Milk Adulteration Using MID-IR Spectroscopy

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Abstract — A straightforward and quick technique for discovery and measurement of milk corruption has been created utilizing mid-infrared (MIR) spectrometers. Milk samples was purchased from local supermarkets and spiked with tap water, hydrogen peroxide, glucose, urea and formaldehyde in different concentrations in milk. Spectral data was collected using mid-infrared (MIR) spectrometers. Partial least-square regression (PLSR) has been used to estimate adulteration level and results showed high coefficients of determination (R^2) and standard error of predication (SEP). The use of Fourier transform infrared (FTIR) spectroscopy coupled with chemo metric techniques to differentiate of milk adulteration. These results proved that FTIR spectroscopy in combination with multivariate calibration can be used for the detection of milk adulteration. The proposed technique is quick, non-dangerous, straightforward and simple to utilize.

Keywords — Adulteration, Multivariate analysis, MIR spectroscopy, PLS, Chemo metric.

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1. INTRODUCTION

Milk is regularly consumed by different age of groups. Additionally, India is the biggest producer and consumer of milk. According to a recent report, India is produce 140.6 million tonnes of milk in 2014 and the demand is set to rise to 150 million tonnes of milk. To take care of the growing demand, milk and its products have been adulterated to decrease the quality and increase the quantity for economic value. The basic adulterants found in milk are urea, starch/blotting paper, glucose/sugar, caustic soda, refined vegetable oil, white paint and common detergent or shampoo. These not just decrease the nutritious value of the refreshment but also pose risk to health.

Milk gives fundamental supplements like (starch, fat, protein, minerals and vitamins) of incredible nutritious importance for people, Generally during childhood. Presently the days event of milk adulteration is a major issue in the dairy industry and has been causing concerns among costumers and food manufacturers. Milk is the seven most basic focuses for defilement by and large refined by the expansion of water, whey, sodium hydroxide and urea.

The use of FT-MIR spectroscopy coupled to chemo metric methods [3], adopting the specificity of the IR signals for identifying chemical components and the minimal sample preparation needed for the measurements. The instrumental design of FT-MIR spectrometers coupled with chemo metrics methods [12,15] have also been described and this enable trace level detection and satisfactory analysis. Fourier Transformed Infrared spectroscopy (FT-MIR) has been connected to focus compositional contrasts between tests on the premise of vibration of different compound gatherings at particular wavelengths in the mid infrared district of the range from 400 to 4000 cm^{-1}. Chemo metric methods, especially partial least squares regression (PLS) is among the most commonly used multivariate calibration [5]. The ultimate aim of this paper was to investigate the potential of PLS method coupled with FT-MIR spectroscopy technique [29], as an alternative logical apparatus for non-dangerous and quick quantitative determination of included glucose, formaldehyde, hydrogen peroxide and urea in milk.

2. MATERIALS AND METHODS

The functional block diagram of the proposed work shown in Fig.1. It consists of light source, interferometer, sample and detector. The sample was placed between interferometer and detector. Ceramic is utilized to produce infrared light source which fall on the sample, produces relating interferogram in the detector. This interferogram obtained from the spectroscopy was Fourier transformed and the resultant spectrum was analyzed through chemo metric Technique.
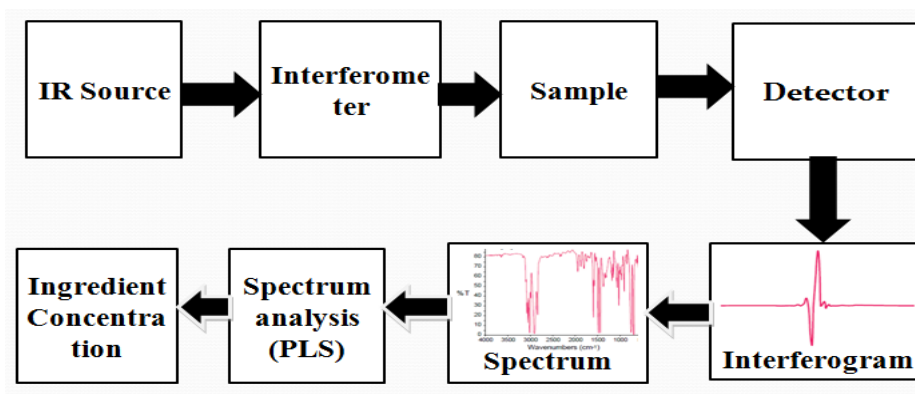

Fig.1 Block Diagram of Proposed Work

2.1 Milk Samples

Milk tests were acquired at a nearby general store. Tests were corrupted by the expansion of faucet water, glucose, formaldehyde, urea, and hydrogen peroxide. All milk tests were spiked with focused on adulterant arrangements and took after the same weakening procedure going from 3 to 50% v/v. The convergence of adulterant spiked into milk tests was ascertained and extended from 0.0087 to 30 g/L for glucose, from 0.78 to 12.5 g/L for urea, from 0.05 to 0.8 g/L for formaldehyde, and from 0.009 to 0.15 g/L for hydrogen peroxide.

2.2 FTIR Instrumental Analysis

Utilizing a Pasteur pipette, samples obtained from the extraction strategy were set in direct contact with attenuated total reflectance (ATR) crystal on a multi bounce plate at controlled ambient temperature (25°C). An Agilent Cary 600 Series FTIR spectrometer equipped with a detector of deuterated triglycine sulphate (DTGS), a beam splitter of KBr/Germanium, and associated to software of the OMNIC operating system (Version 7.0 Thermo Nicolet), were utilized during FTIR data collection. To minimize water vapour interference, the instrument was kept up with dehumidifier of silica gel. FTIR spectra were recorded from 32 scans at a resolution of 4 cm^{-1} at mid infrared region (4000–650 cm^{-1}). These spectra were subtracted against background air spectrum. After every scan, a new reference air background spectrum was taken. The ATR plate was carefully cleaned in situ by wiping it with hexane twice followed by acetone, and dried with soft tissue before filling in with the next sample.

The cleanliness of ATR crystal was confirmed by collecting a background spectrum and comparing it to the previous one. These spectra were recorded as absorbance qualities at each data point in triplicate.

2.3 Statistical and Chemometric Analysis

PLS chemometric analysis was done using the Unscrambler software version10.3. The "leave-one-out" cross validation procedure was used to verify the calibration model. The PLS performance was assessed using the values of root mean standard error of calibration (RMSEC) and coefficient of determination (R^2). In addition, R^2 and root mean standard error of prediction (RMSEP) were used for the evaluation of validation capability of PLS.

3. RESULTS AND DISCUSSION

3.1 FTIR Spectral Analysis

FTIR spectroscopy is one of the advanced techniques that could be used for analysis of milk adultration. This technique has been widely applied, because once the instrument has been calibrated; it can be used for routine analyses. The importance of IR spectroscopy for the qualitative analysis originates from its properties especially as fingerprint technique. From the Fig.2, there was a different between the spectra of pure milk (blue) and adulterated milk with Glucose (green). The MIR spectra of control and milk spiked with Formaldehyde, Hydrogen Peroxide shown in Fig.3 and Urea are shown in Fig.4. The 3100–910 cm^{-1} region was considered to be the fingerprint region. The results were verified with statistical parameters and quantified through highly developed PLS calibration model to attain the best regression equation.
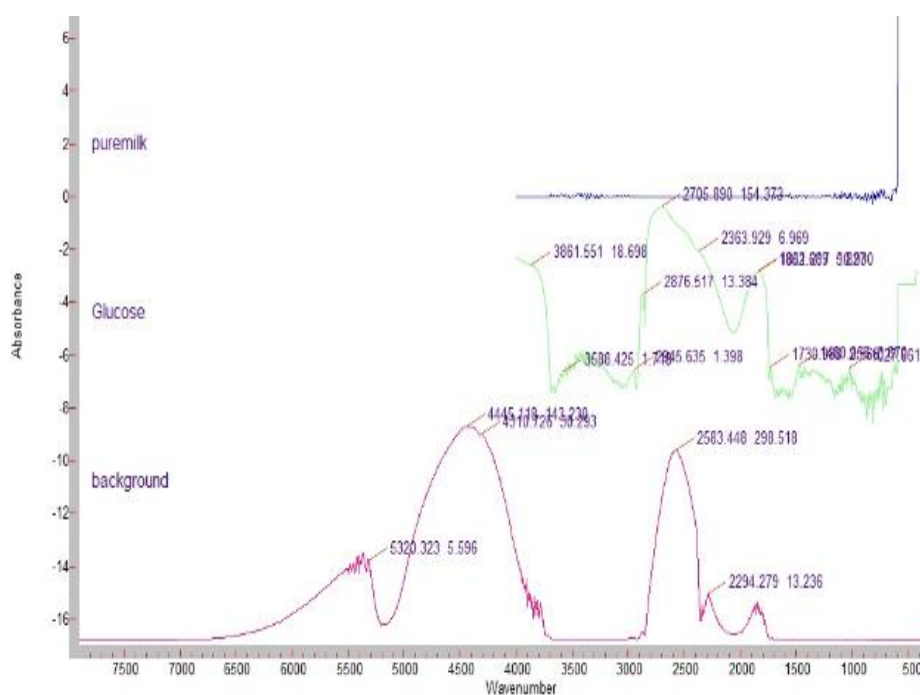

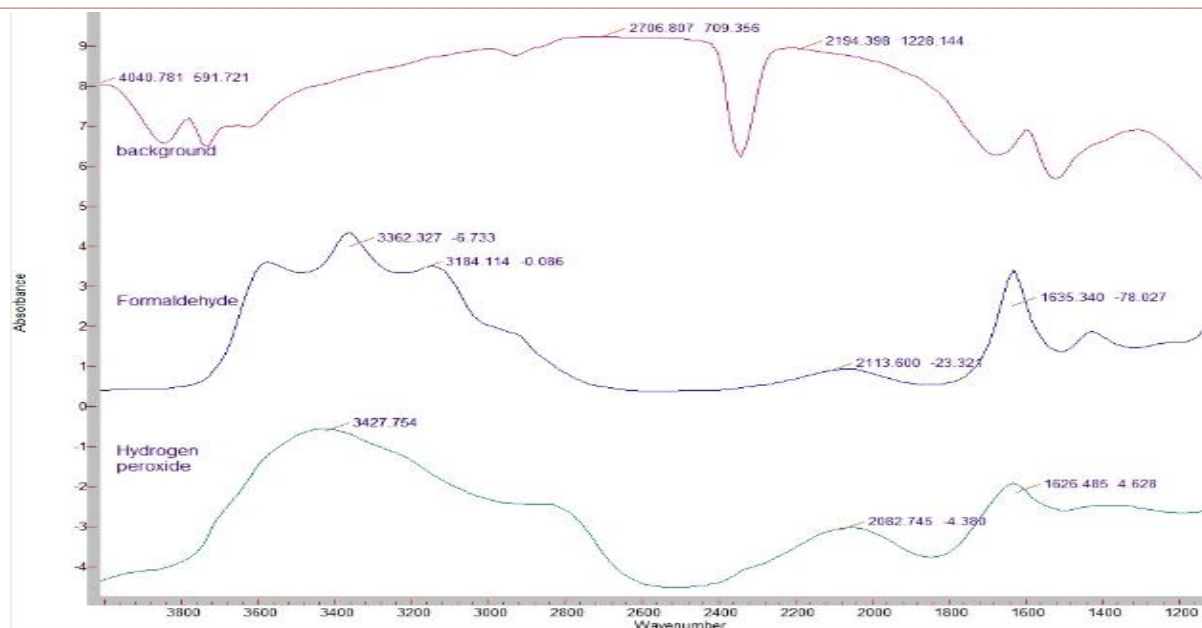**Fig. 2 FTIR Spectra of Pure milk and Glucose**

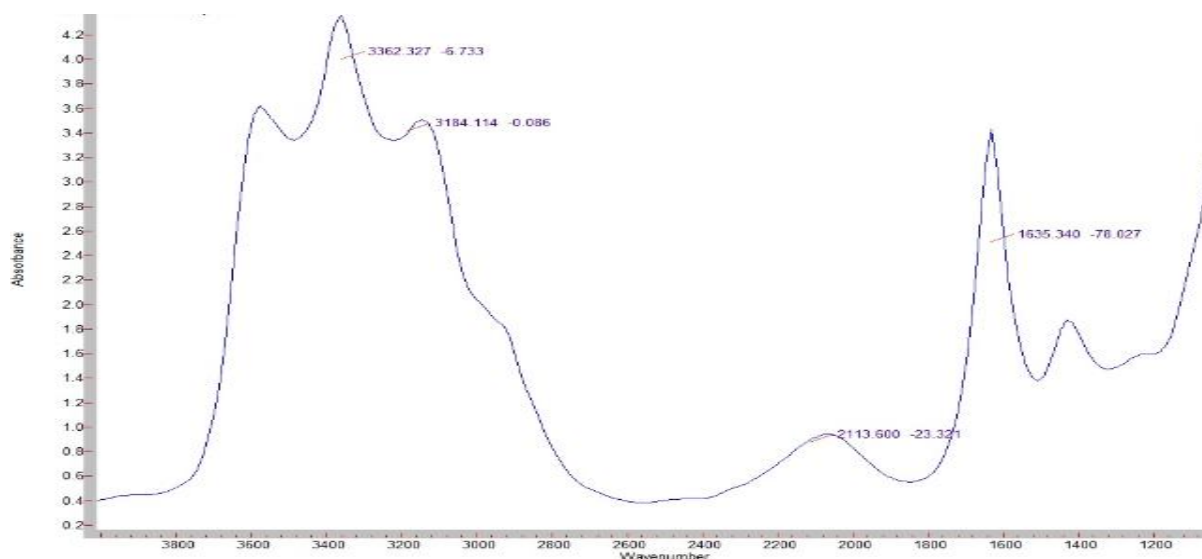**Fig. 3 FTIR Spectra of Formaldehyde and Hydrogen peroxide**

Fig. 4 FTIR Spectra of Urea

3.2 PLS prediction of added concentrations Glucose in milk. Model calibration and validation

FT-MIR spectral characteristics of different ratios of Glucose at various concentrations were investigated initially the region (3100–920 cm^{-1}) and then the regions of 1800-920 cm^{-1} from full spectrum were used to construct PLS calibration. The greatest number of components utilized as a part of PLS models were consequently chosen. The component choice depended on accomplishing least RMSEP anticipated leftover blunder of entirety of squares qualities. The RMSEP worth shows how well a model fits alignment information. PLSR scramble plot demonstrated a solid match between the reference levels and MIR anticipated qualities for milk corruption (Fig. 5). Fig. 5 plots the best performing PLS models and displays measured versus forecast of included Glucose fixation values. Alignment tests are appeared as blue and cross acceptance tests as red. It merits underscoring that both adjustment plots are fundamentally the same, with all specimens (for alignment and for cross acceptance) along the adjustment line.
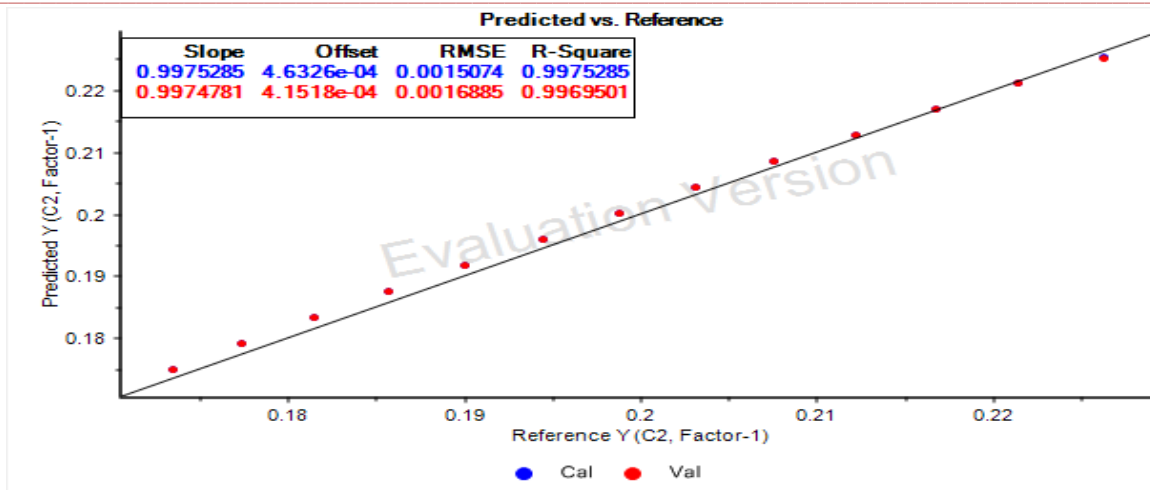
Fig. 5 PLS Calibration and Validation Model for Pure milk & Glucose

3.3 PLS prediction of added concentrations Formaldehyde and Hydrogen Peroxide in milk. Model calibration and validation

FT-MIR spectral characteristics of different ratios of Formaldehyde and Hydrogen Peroxide at various concentrations were investigated initially the region (3200–1020 cm^{-1}) and then the regions of 2100-1020 cm^{-1} from full spectrum were used to construct PLS calibration. The greatest number of components utilized as a part of PLS models were consequently chosen. The component choice depended on accomplishing least RMSEP anticipated leftover blunder of entirety of squares qualities. The RMSEP worth shows how well a model fits alignment information. PLSR scramble plot demonstrated a solid match between the reference levels and MIR anticipated qualities for milk corruption (Fig. 6). Fig. 6 plots the best performing PLS models and displays measured versus forecast of included Glucose fixation values. Alignment tests are appeared as blue and cross acceptance tests as red. It merits underscoring that both adjustment plots are fundamentally the same, with all specimens (for alignment and for cross acceptance) along the adjustment line.
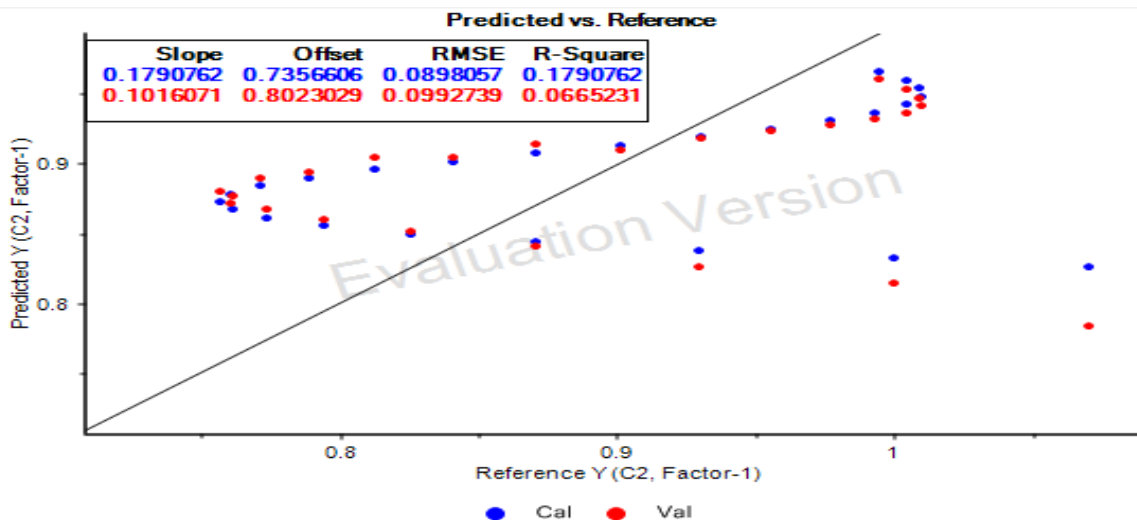

Fig. 6 PLS Calibration and Validation Model for Formaldehyde & Hydrogen Peroxide

3.4 PLS prediction of added concentrations Urea in milk. Model calibration and validation

FT-MIR spectral characteristics of different ratios of Urea at various concentrations were investigated initially the region (3800–1620 cm^{-1}) and then the regions of 2400-1620 cm^{-1} from full spectrum were used to construct PLS calibration. The greatest number of components utilized as a part of PLS models were consequently chosen. The component choice depended on accomplishing least RMSEP anticipated leftover blunder of entirety of squares qualities. The RMSEP worth shows how well a model fits alignment information. PLSR scramble plot demonstrated a solid match between the reference levels and MIR anticipated qualities for milk corruption (Fig. 7). Fig. 7 plots the best performing PLS models and displays measured versus forecast of included Glucose fixation values. Alignment tests are appeared as blue and cross acceptance tests as red. It merits underscoring that both adjustment plots are fundamentally the same, with all

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specimens (for alignment and for cross acceptance) along the   adjustment line.
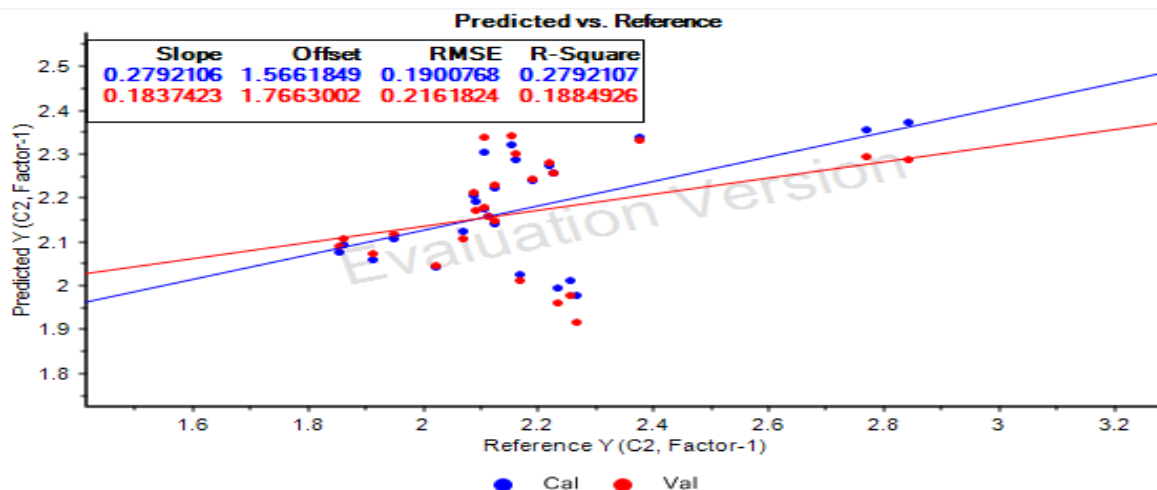

Fig. 7 PLS Calibration and Validation Model for Urea

4. CONCLUSION

Chemo metrics combined with FT-MIR spectroscopy proved to be an excellent method for identifying substances by providing unique fingerprint spectra. we have established a model FTIR-PLS able to detect adulteration of raw milk with addition of Glucose, Formaldehyde, Hydrogen Peroxide and Urea. The effect was related to the specific absorbance of Glucose, Formaldehyde, Hydrogen Peroxide and Urea in MIR spectrum. Achieved prediction results using PLS regression technique gives good estimations of the added Glucose, Formaldehyde, Hydrogen Peroxide and Urea to milk contents, with relative errors of 0.0014, 0.03162, 0.03557 and 0.3924 throughout the spectral range between 4000 and 400cm^{-1}. This study shows that the proposed procedure was an adequate method to detect the adulteration of milk from added Glucose, Formaldehyde, Hydrogen Peroxide and Urea without any sample pre-treatment and destructive manipulation. The method was calibrated and externally validated.

This method is rapid, non-destructive and easy-to-use. Great precision of method is a requirement of quality control in dairy product industry. Thus, a combination between FTIR and chemo metrics could be a powerful alternative authentication of dairy product. The propose scheme has been successfully tested in NITTTR lab.

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