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Development of new NIR-spectroscopy method combined with multivariate analysis for detection of adulteration in camel milk with goat milk

Fazal Mabood*[a], Farah Jabeen*[b], Manzor Ahmed[b], Javid Hussain[a], Saaida A. A. Al Mashaykhi[a], Zainb M. A. Al Rubaiey [a], Saim Farooq [a], Ricard Boqué[c], Liaqat Ali [e], Zahid Hussain[d], Ahmed Al-Harrasi[e], Abdul Latif khan[e], Zakira Naureen*[a], Mohammed Idrees[f], Suryyia Manzoor[g]

a) *Department of Biological Sciences & Chemistry, College of Arts and Sciences, University of Nizwa, Sultanate of Oman, (mehboob@unizwa.edu.om, fjabeen2009@yahoo.com)*

b) *Department of Chemistry, University of Malakand, KPK, Pakistan.*

c) *Department of Analytical Chemistry and Organic Chemistry, Universitat Rovira i Virgili, Tarragona, Spain*

d) *Department of Chemistry, Abdul Wali Khan University, KPK, Pakistan*

e) *UoN Chair of Oman Medicinal Plants and Marine Products, University of Nizwa, Sultanate of Oman.*

f) *Department of Chemistry, Bachakhan University, KPK, Pakistan*

g) *Institute of Chemical Sciences, Bahauddin Zakariya University, Multan*

Abstract

New NIR spectroscopy combined with multivariate analysis for detection and quantification of camel milk adulteration with goat milk was investigated. Camel milk samples were collected from Aldhahira and Sharqia regions of Sultanate of Oman and were measured using NIR spectroscopy in absorption mode in the wavelength range from 700-2500 nm, at 2 cm^{-1} resolution and using a 0.2 mm path length CaF_2 sealed cell. The multivariate methods like PCA, PLS-DA and PLS regression were used for interpretation of NIR spectral data. PLS-DA was used to detect the discrimination between the pure and adulterated milk samples. For PLSDA model the R-square value obtained was 0.974 with 0.08 RMSE. Furthermore, PLS regression

model was used to quantify the levels of adulteration from, 0%, 2%, 5%, 10%, 15% and 20%. The PLS model showed the RMSEC = 1.10% with R^2 = 94%. This method is simple, reproducible, having excellent sensitivity. The limit of detection was found 0.5 %, while the limit of quantification was 2 %.

Keywords:

NIR- spectroscopy; Camel milk adulteration; PCA, PLS-DA, PLS regression

Introduction:

In many arid and semi-arid areas of the world camel is of considerable socio-economic value and its milk has a number of nutrients beneficial to human body (Shamsia, 2009). For centuries, people have been using camel for transportation and camel milk as a source of food and also as medicines for several diseases (Gizachew, Teha, & Birhanu, 2014). In addition, it has been consumed for centuries due to its nutritional values and medicinal properties (Dowelmadina, Zubeir, Arabi, & Abaker, 2015). In the last twenty years, there have been many studies on the use of camel milk in the treatment of human diseases (Kaskous, 2015).

For both water-soluble and fat-soluble vitamins, milk is a valuable source. Because of its high concentration of vitamin C, camel milk is a kind of exception. Camel milk has 30 times more Vitamin C than cow milks, and contains 6 times more than human milk. In the desert areas this is highly important, where vegetables and fruits are scarce. Therefore, in the diet of inhabitants of these regions camel milk is often the only source of vitamin C (Gizachew, Teha, & Birhanu, 2014). The composition of camel milk is different from other ruminants' milk. It has low sugar, low cholesterol, high minerals (potassium, sodium, copper, iron, magnesium and zinc), low protein, high vitamins, and high concentrations of insulin (Yadav, Kumar, Priyadarshini, & Singh, 2015).

Being a rich source of the nutritional constituents and variety of biological activities that effect development and growth of specific body organs, metabolic responses towards nutrients absorption, digestion and fight against diseases. By the digestive action on milk biologically active peptides are produced in the gastrointestinal tract.

The positive health effects of milk proteins can be presented as anti-microbial, antioxidative, anti-thrombotic, and antihypertensive or immunomodulatory (Antanasova & Ivanova, 2010).

Camel's milk is considered as abundant source of protein for people living in arid lands of the world. This protein is rich in protective components including lysozyme, lactoferrin, Lactoperoxidase (LPS), and peptidoglycan recognition protein (PGRP) which are found only in camel's milk (Singh, Ghorui, & Sahani, 2006), IGA and IGg immunoglobulins that are suitable with human ones and provide effective defense against several viral and bacterial pathogens. The fact that camel's milk is low in different β–caseins (Beg, von Bahr-Lindstrom, Zaidi, & Jornvall, 1986) and without β-lactoglobulin (Merin et al., 2001) makes it more attractive for those suffering from milk allergies (Makinen-Kiljunen & Palosuo, 1992; Shabo, Brazel, Margoulis, & Yagil, 2005).

Milk is an important source of mineral substances, especially sodium, potassium, calcium, magnesium, phosphorus, chloride, iodine, and small amounts of iron. The key mineral compounds of milk are phosphorus and calcium, which are significant for the proper development of new borns and bone growth. The high bioavailability of these minerals effects the distinctive nutritional value of milk. Camel milk is the richest in these minerals. The mean values in mineral contents of dromedary camel milk (100g-1) for zinc (0.53 mg), manganese (0.05 mg), magnesium (10.5 mg), iron (0.29 mg), sodium (59 mg), potassium (156 mg) and calcium (114 mg) (Elamin, & Wilcox, 1992; Sawaya, Khalil, Al-Shalhat, & Al-Mohammad, 1984).

According to the previous studies, camel milk possesses nutritional and medicinal values (Gizachew, Teha, & Birhanu, 2014). It is rarely available in the market and sold with much higher prices, and thus called as desert gold because of its higher prices. Its adulteration with goat, cow, buffalo and other inexpensive commercial milks has already been started. In the present study, the focus is to develop a new NIR spectroscopy method combined with chemo metrics to authenticate as well as to check the level of adulteration in camel milk with goat milk as an adulterant. The novelty of the method lies in high sensitivity, better reproducibility, more economical, and less or no sample preparations for the overall experiment.

2. Materials and methods

2.1. Camel milk Samples preparation

Three different camel milk as well as goat milk samples were collected from Aldhahira as well as Sharqia regions of Sultanate of Oman and analysed. Those camel milk samples were then adulterated with goat milk at five different percentage levels: 2, 5, 10, 15 and 20 %. Number of total samples used was 54: 9 pure (3x3=9), 45 adulterated with goat milk. The samples were prepared in triplicate. All the samples were joined together and split into two sets (70 % of the samples) training set and (30 % of the samples) a test set for validation for PLS regression,.

2.2. Apparatus

Perkin Elmer Frontier NIR spectrophotometer was used to measure all the samples in absorption mode in the wavelength range from (700-2500 nm), at 2 cm^{-1} resolution and using a 0.2 mm path length CaF_2 sealed cell. Prominent absorption peaks were appeared in the region from 4000 to 7500 cm-1 wavenumber.

2.3. Statistical analysis

For statistical analysis the Unscrambler version 9.0 by Camo and Microsoft Excel 2010 were used The PLS-DA, PCA, and PLS regression models were built for both pure and adulterated camel milk samples. Spectral pre-treatments, such as 1st derivative with Savitzky-Golay with 5 smoothing point 2^{nd} order polynomial and baseline correction were conceded. To validate the PLS-DA models Leave-one-out cross validation was used. For PLS regression all the samples were joined together and split into two sets, (70% of the samples) a training set and (30% of the samples) a test set for validation. To validate the PLS regression models built with the training set,external cross validation was used. The RMSECV (Root Mean Square Error of Cross Validation) was used as an internal indicator of the predictive ability of the models. Using Eq. 1: RMSECV is calculated as:

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}{n}} \quad \text{----------(1)}$$

Where $\hat{y}_i$ is the % of adulteration predicted by the model, y_i is the actual % of adulteration (measured value) and n is the number of segments left-out in the cross-validation method, which is equivalent to the number of samples of the training set. RMSECV smaller values are the indicative of a improved prediction ability of the model.

The statistical measure RMSEP is used to tell how well the model predicts new samples (not used when building the model) which is calculated using Eq. 2:

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n_t}(y_{t,i} - \hat{y}_{t,i})^2}{n_t}} \quad \text{----------(2)}$$

where $\hat{y}_{t,i}$ is the % of adulteration predicted by the model, $y_{t,i}$ is the measured value (actual % of adulteration), and n_t is the number of samples in the test set. Average error expresses by RMSEP to be expected in future predictions when to the unknown samples the calibration model is applied.

3. Results and discussion:

3.1. Near Infrared spectra

Figure 1 shows the spectra of NIR for all samples ranging from (10000-4000 cm^{-1}) in term of wavenumbers while in term of wavelength ranging from (700-2500 nm) using a 0.2mm path length CaF_2 sealed cell.

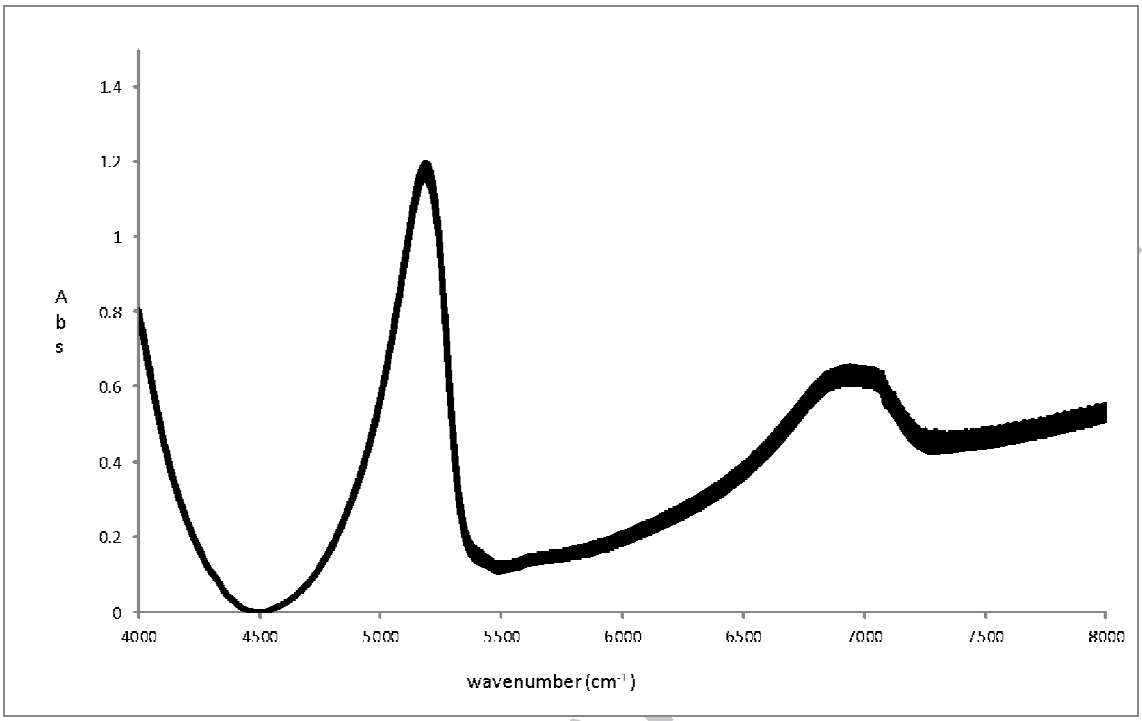


Figure 1. NIR spectra for milk samples, (point spectra) pure camel milk and (solid line spectra) adulterated with goat milk.

Figure 1 shows the NIR spectra for both pure camel milk (point spectra) and adulterated with goat milk (solid line spectra). It can be seen from the that there is a prominent absorption peaks at wavenumber 5198 cm^{-1}.

Although the spectra appear to be very similar. In order to see the difference among the unadulterated camel, cow and goat milk an alternative approach of principal componentes analysis (PCA), was applied in which a PCA model was built as shown in Figure 2. It can be seen from the PCA plot that there is complete differentiation and separation among the camel, cow and goat milk. They are spaced and grouped in the specific different regions of the PCA plot.

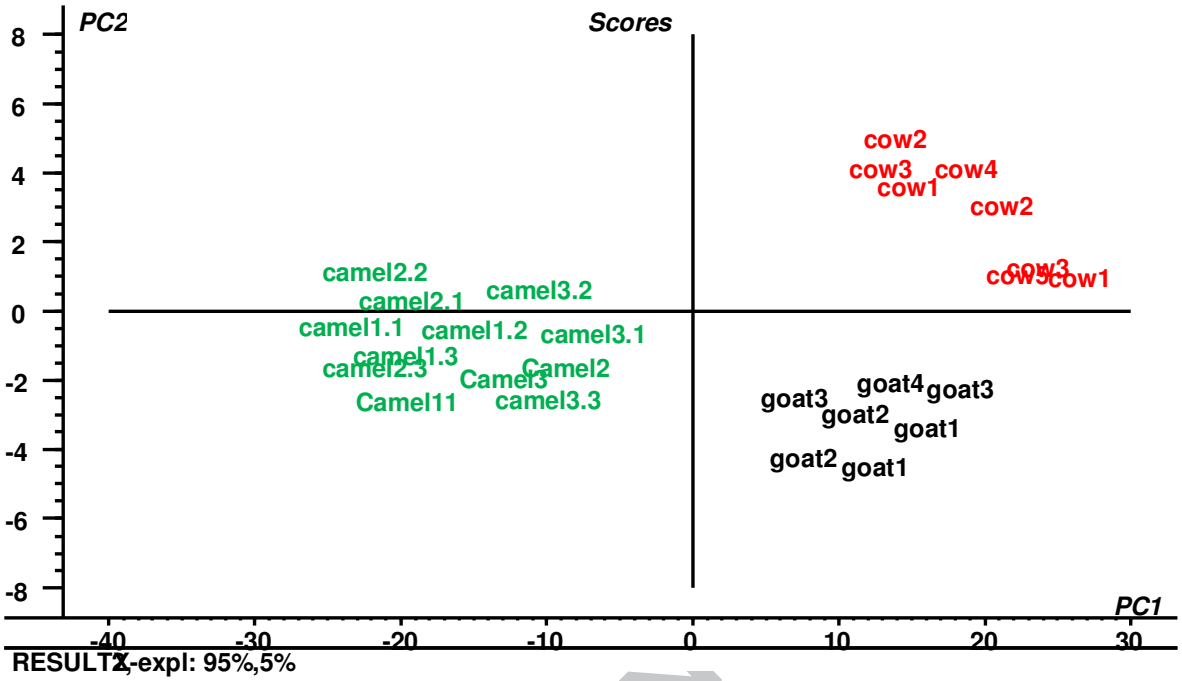
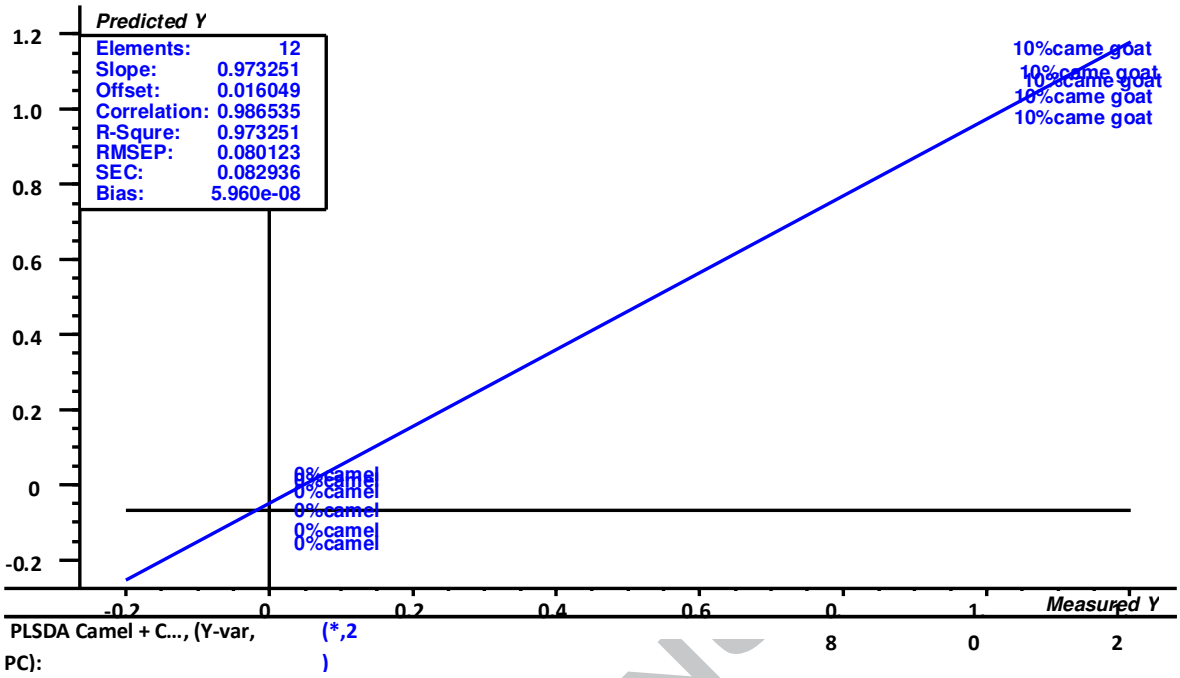


Figure 2. PCA plot for the unadulterated camel, cow and goat milk

Similarly in order to detect the discrimination between the pure and adulterated camel milk samples, PLS-DA (Partial least-squares discriminant analysis) method was applied. It was built for the spectral data between pure and with 10 % goat milk adulteration level as shown in Figure 3. Spectral pre-treatments, such as baseline correction, and Savitzky-Golay smoothing were used for building this model.



172

173 Figure 3. PLS-DA model for pure camel milk and with 5% goat milk adulteration

174

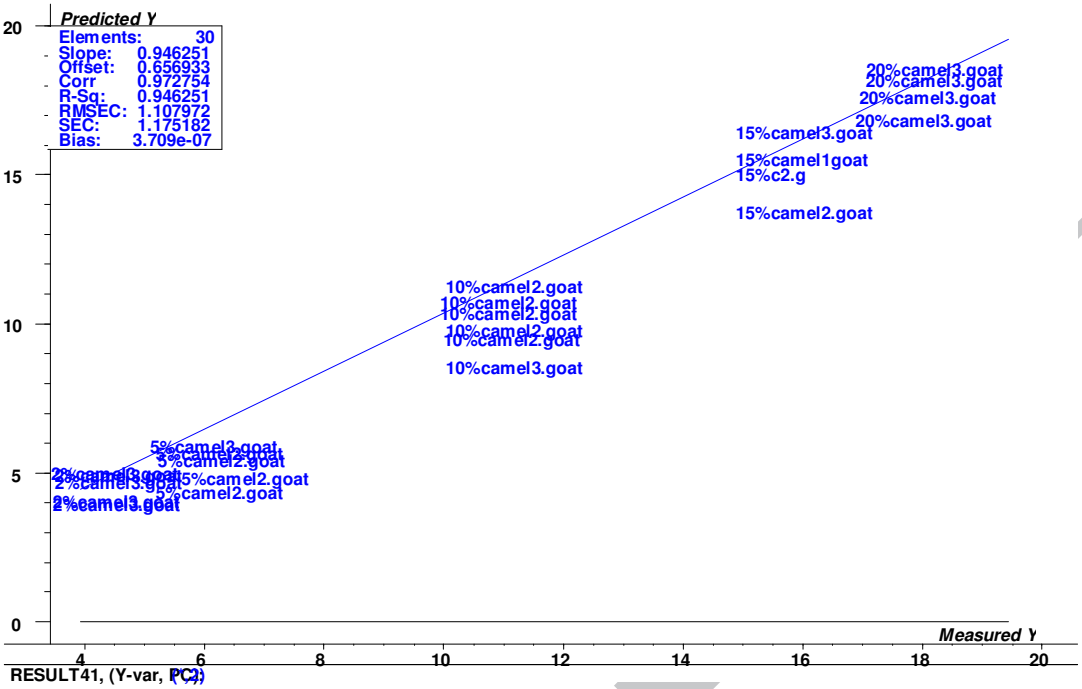
175 As Figure 3 shows a clear discrimination between the pure camel milk and with 10 %
176 goat milk adulteration. It can be used as an identification tool to check the
177 adulteration of camel milk with goat milk. If there is any amount of goat milk in
178 camel milk they will occupy the space in between the pure and adulterated samples
179 of the above PLS-DA plot. The RMSEP value for this model is 0.080 with with 0.97 R
180 square value.

181

182 **3.3 Results of PLS regression:**

183 To quantify the amount of adulteration PLS regression model was also built by using
184 70 % of the samples as a training set. It comprising both pure and adulterated camel
185 milk samples 2, 5, 10, 15 and 20 % at five different percentage levels:

186



187

188 Figure 4. PLS regression plot for pure and adulterated camel milk samples

189 It can be seen from Figure 4 that RMSEC = 1.10% has small value for 2 factors with
190 R2 = 94% and of 0.97 correlation ship.

191 To predict an independent 30% test samples set (described in the experimental
192 section PLS calibration model was then used.

193

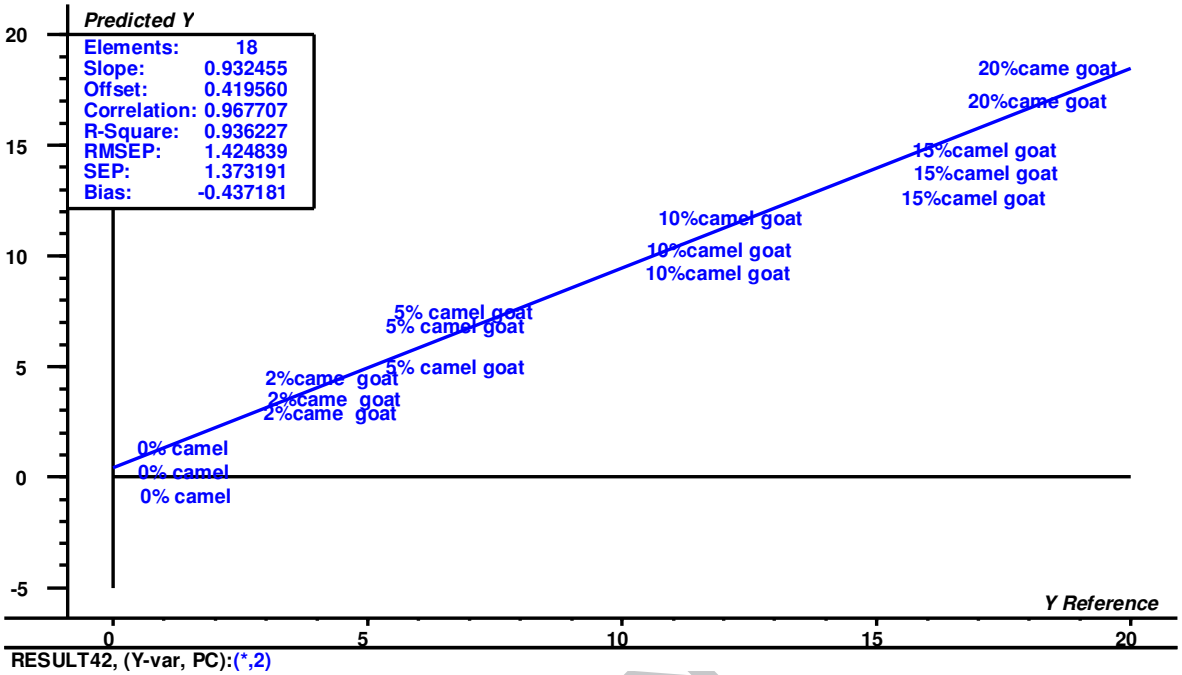


Figure 5 :Prediction plot for the Test set spectral data for both pure and adulterated camel milk samples.

It can be seen from Figure 5 that the PLS calibration model is having a very good prediction ability because it was applied to 30% test samples those were not used in building the PLS calibration model. Again it is having minimum amount of prediction error i.e. RMSEP value = 1.42% with good prediction.

4. Conclusion:

Newly developed NIR spectroscopic method combined with multivariate analysis concludes that this is a suitable method for checking the detection and quantification of camel milk adulteration with goat milk. It was further investigated that PLS-DA model can be used as an identification tool while PLS calibration model as a quantification. This method is simple, no need of much sample preparation and, having excellent sensitivity and reproducibility.

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Highlights

- Development of new NIR spectroscopic method combined with multivariate methods to detect & quantify adulteration in camel milk with goat milk
- To build PLS regression models to quantify the amount of goat milk.
- To build PCA model to explore the classification among various varieties of camel, cow and goat milks