

Rapid detection of pork gelatin in ice cream samples by using non-destructive FT-NIR spectroscopy and Partial least squares-discriminant analysis



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ABSTRACT

This study aimed at developing a fast and low-cost detection method to discriminate between ice cream samples containing pork or non-pork gelatin by using Fourier Transform Near Infrared (FT-NIR) spectroscopy and Partial Least Squares Discriminant Analysis (PLS-DA). Forty two samples of ice cream were used, among which twenty three samples were adulterated with different levels i.e. 1%, 5%, 10%, and 20 % of pork gelatin (Non-Halal). Whereas, the remaining nineteen samples containing only cow gelatin (Halal) were used as a control. All the ice cream samples were measured with the FT-NIR spectrophotometer in the reflection mode. Spectra were collected in the wavenumber range from 10000 to 4000 cm^{-1} (1000 to 2500 nm). The results show that the PLS-DA model with Unit Vector Normalization (UVN) spectral transformations for 1% pork gelatin adulteration is the optimal one which was based on a compromise between the lowest value of root mean square error of cross validation (RMSECV) for the calibration set. The lowest value of root mean square error of prediction (RMSEP) for the test set, the least number of factors and the percentage of correctly classified samples, the Halal and Non-Halal, for both calibration and test sets. This newly developed method is fast, involves simple sample preparation and is low cost.

1. Introduction

Mammalian Gelatin accounts for a vast majority of widely used Gelatin in food, pharmaceuticals and cosmetics (Djagny, Wang, & Xu, 2001; Hussain-Gambles, 2020; Sin & Sin, 2019). However, due to socio-cultural and health related concerns it has been subjected to constrain and skepticism (Eriksson, Burcharth, & Rosenberg, 2013). Gelatin, derived from porcine sources, is termed as non-halal or non-Kosher and is considered prohibited by Muslims and Jews. In Europe, about 80% of edible gelatin is produced from pigskin, but vegetarian Halal and Kosher gelatin, prepared from seaweed, fish bones or non-porcine sources, is also available (Gokhan Boran & Regenstein, 2010). Although, gelatin

must be labeled appropriately, once it has been manufactured, purified, traded and put it in the market, it is difficult to ensure its provenance or whether it has been inadvertently cross-contaminated at any point in the food chain (Soon, Chandia, & Mac Regenstein, 2017). It is therefore important to have methods available whereby Halal gelatin can be checked to ensure its authenticity and that it is free from cross-contamination with porcine gelatin. The ability to test processed food products for the presence of porcine gelatin is an essential requirement for food control in Muslim or Jewish communities (Riaz & Chaudry, 2003).

Gelatin is a highly processed protein, which is widely used as a gelling and thickening agent (E441). Gelatin is obtained by hydrolysis of collagen, which is extracted from materials such as bones, hides and

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skins from animal slaughterhouses (Karim & Bhat, 2008). Gelatin production involves controlled acidic or basic hydrolysis of connective tissue raw material, high temperature extraction with water, sterilization, and drying. These processes are not standardized and have effects on the properties of the final gelatin product which makes it difficult to differentiate. Several methods have been used to differentiate between bovine and porcine gelatins (Hidaka & Liu, 2003; Nemati, Oveisi, Abdollahi, & Sabzevari, 2004; Sin et al., 2019; Venien & Levieux, 2005). For instance recently microwave dielectric sensing system have been applied to distinguish between bovine and porcine gelatins. It is shown by Hidaka and Liu in 2002 (Hidaka et al., 2003) that bovine and porcine gelatin may be distinguished using a pH drop method after calcium phosphate precipitation. Amino acid analysis and enzyme linked immunosorbent assay (ELISA) (Nemati et al., 2004; Venien et al., 2005) can also differentiate between bovine and porcine gelatins, but both methods need repeated results and experience since the sample preparation is very sensitive and rigid. Most published methods have focused on meat species identification rather than identification of gelatin. However, extraction of good quality DNA is an important pre-requisite for PCR-based analyses and this can be a potential problem if there has been extensive heat processing which leads to degraded proteins and nucleic acids (Boran & Regenstein, 2010).

Infrared spectroscopy can offer a solution since spectra provide information on different functional groups, and so on the chemical composition of specific substances (Kumosinski & Farrell, 1993). IR spectroscopy measures the amount of light absorbed or reflected due to molecule vibrations over a range of frequencies of the incident light and thus it can be used to segregate different gelatin samples based on their absorption or reflectance pattern. For instance, Fourier transform infrared (FTIR) spectroscopy has been shown to be a very useful technique for determining a range of adulteration problems in food products, such as lard content in cakes and chocolates (Che Man, Syahariza, Mirghani, Jinap, & Bakar, 2005), lard in mixtures of animal fats (Syahariza, Man, Selamat, & Bakar, 2005), adulteration in jam (Defermez & Wilson, 1995), or extra virgin olive oil (Lai, Kemsley, & Wilson, 1995) and coffee (Paradkar & Irudayaraj, 2002). FTIR spectroscopy, together with attenuated total reflectance (ATR) or transmission accessories has been used to determine chemical, physicochemical and morphological properties, gelation and intermolecular cross-linking of collagen and proteins (Cao & Xu, 2008; Friess & Lee, 1996; Muyonga, Cole, & Duodu, 2004; Petibois & Déleris, 2006; Prystupa & Donald, 1996). In this study, reflectance FT-NIR spectroscopy, coupled with Partial Least Squares Discriminant Analysis (PLS-DA) has been applied as a fast, low-cost and non-destructive detection method for discrimination of ice cream samples containing Non-Halal gelatin, i.e. pork gelatin.

2. Materials and methods

Forty ice cream samples from commercial establishments were collected in Nizwa, Sultanate of Oman from July 2018 to January 2019. The samples were available as packet, in disposable plastic cups/container that were provided by the establishment. All samples were then immediately, transported to the laboratory in isothermal packaging and transferred to sterile flasks and processed within 24 h. The samples were used for test as external test to examine the presence of pork gelatin in all of them.

2.1. Ice cream sample preparation

The ice cream samples containing gelatin were prepared by using the method described by Muharramah, Permata, Juwono, Sugiarto, and Kurniawan (2020), Ferris (1922). In this study, forty two ice cream samples were prepared. Among them, twenty three samples were adulterated with different levels i.e. 1%, 5%, 10%, and 20 % of pork gelatin (Non-Halal), while the remaining nineteen samples, containing only cow

gelatin (Halal), were used as a control. This group of samples was used for building the models and checking their prediction ability.

2.2. FT-NIR spectroscopy

Each of the ice cream sample was scanned by using a Frontier NIR spectrophotometer (BSEN60825-1:2007 by Perkin Elmer) in reflection mode. Spectra were collected in the wavenumber range from 10000 to 4000 cm^{-1} (1000 to 2500 nm), at a resolution of 2 cm^{-1} and a total path length of 0.5 mm.

2.3. Partial least squares-discriminant analysis (PLS-DA)

PLS-DA was used for the discrimination of the two groups of ice cream samples, defined as Halal (not-containing pork gelatin) and Non-Halal (containing pork gelatin). Briefly, PLS-DA is a supervised classification technique that models the differences among several classes using a PLS regression model. The PLS-DA model relates the NIR spectra (X matrix) to the assigned dummy variables (Y matrix) by maximizing the covariance between both matrices. When there are only two classes to discriminate, as in this study, PLS-DA uses only one response variable (Y), coded as -1 for one class and $+1$ for the other class. When an unknown sample is predicted with the model, it takes a predicted value around -1 or $+1$, and it is assigned to a given class using a predefined and optimized threshold. A sample with a predicted value close to -1 will belong to one class and if the predicted value is close to $+1$ the sample is assigned to the other class. Values close to zero indicate that it is not easy to allocate it to either class, especially when the estimated deviation (uncertainty) around the predicted value includes zero. The Unscrambler software X10.3 (CAMO Software, Oslo, Norway) and Microsoft Excel 2016 software were used for building the PLS-DA models with the NIR spectral data.

2.4. Pre-processing of NIR spectral data

Before PLS-DA modeling, different spectral pre-processing techniques, such as unit vector normalization, baseline correction, multiplicative scatter correction (MSC), and standard normal variate (SNV) were used to correct both multiplicative and additive effects of the spectra and to reduce instrumental noise or background information. The accuracy of the PLS-DA models developed was evaluated based on a compromise between the lowest values of root mean square error of cross validation (RMSECV), root mean square error of prediction (RMSEP) and number of factors, and the highest values for the coefficients of determination (R^2), both for cross-validation and prediction, and the highest percentage of correctly classified samples for the external validation set.

2.5. Calibration & validation set of NIR spectral data

In this study the NIR spectra were randomly split into two sets: a training test containing 70% of the data to build the PLS-DA calibration model and a test set containing 30% of the data to externally validate the PLS-DA calibration model. The PLS-DA calibration model was also internally validated, using a full cross validation procedure and investigating a maximum of 7 latent variables (LVs).

3. Results and discussion

3.1. Spectra interpretation

Fig. 1 shows the NIR spectra of the two groups of ice cream samples defined as Halal (not-containing pork gelatin), and Non-Halal ice cream (containing pork gelatin) without and with pre-processing spectral transformation in the ranges of 10000–4000 cm^{-1} . Near-infrared

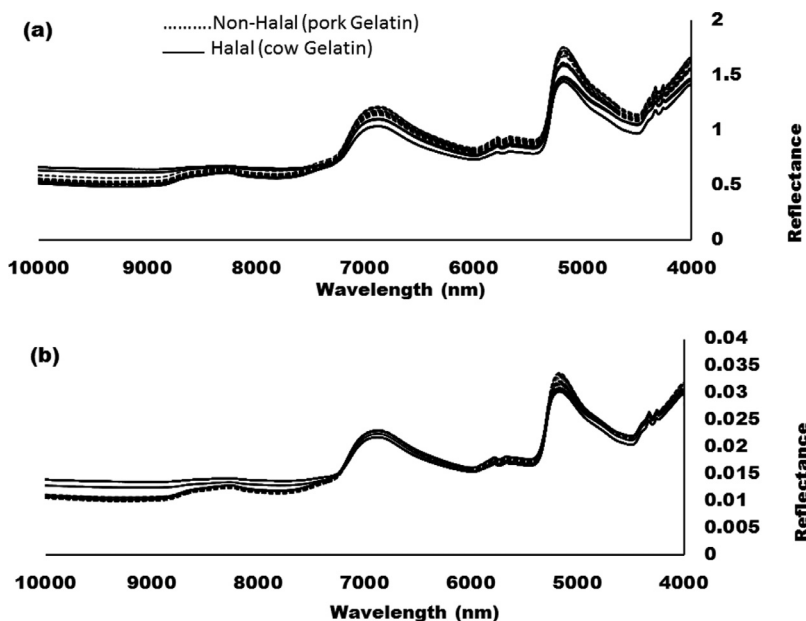


Fig. 1. NIR spectra of the two groups of ice cream samples: Halal (solid line) and Non-Halal (dashed line). (a) Raw spectra (b) Spectra pre-processed with unit vector normalization.

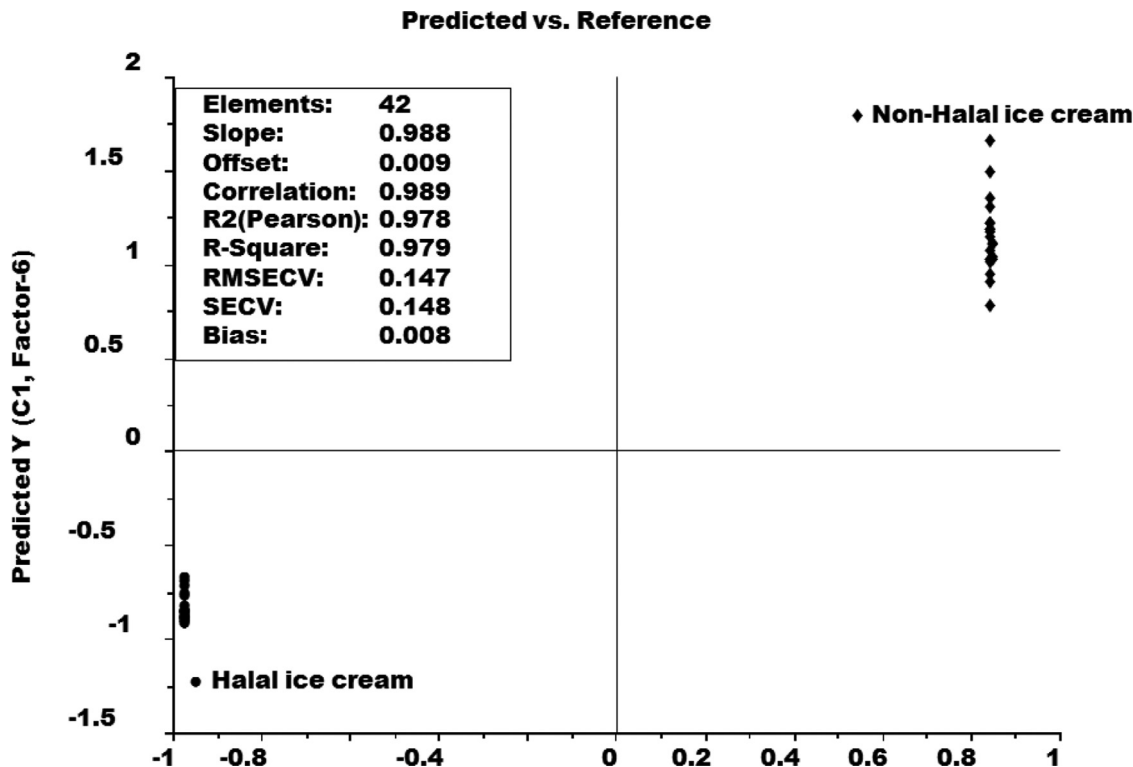


Fig. 2. Predicted versus reference values plot for PLS-DA calibration model based at optimum pre-process conditions i.e. unit vector normalization (Where C1 stands for the calibration line 1).

spectroscopy was used to measure the reflection of electromagnetic radiation. The broad band in the Near-infrared spectroscopy arises through the attenuated reflection in the overlapping wavelengths. The bands mostly correspond to overtones and combinations of vibrational modes involving C-H, O-H, and N-H chemical bonds. Recording the electromagnetic radiation absorbed from those molecular bonds in the NIR wavelengths produces spectra which are unique to a sample acting as a “fingerprint”. The absorption peaks in the spectra of Fig. 1 are almost similar for both Halal and Non-Halal samples. In order to unveil the differences in both groups of samples, PLS-DA was applied on the spectral data.

3.2. Partial least squares-discriminant analysis (PLS-DA)

PLS-DA was performed to see the differences in the spectra and to discriminate the two groups of ice cream samples, based on Halal and Non-Halal gelatin composition. During model development and optimization, the accuracy of the PLS-DA models was evaluated based on having the lowest values of root mean square error of cross validation (RMSECV), root mean square error of prediction (RMSEP), and coefficient of determination (R^2) number of factors and the highest percentage of correctly classified samples for both calibration and test sets. The re-

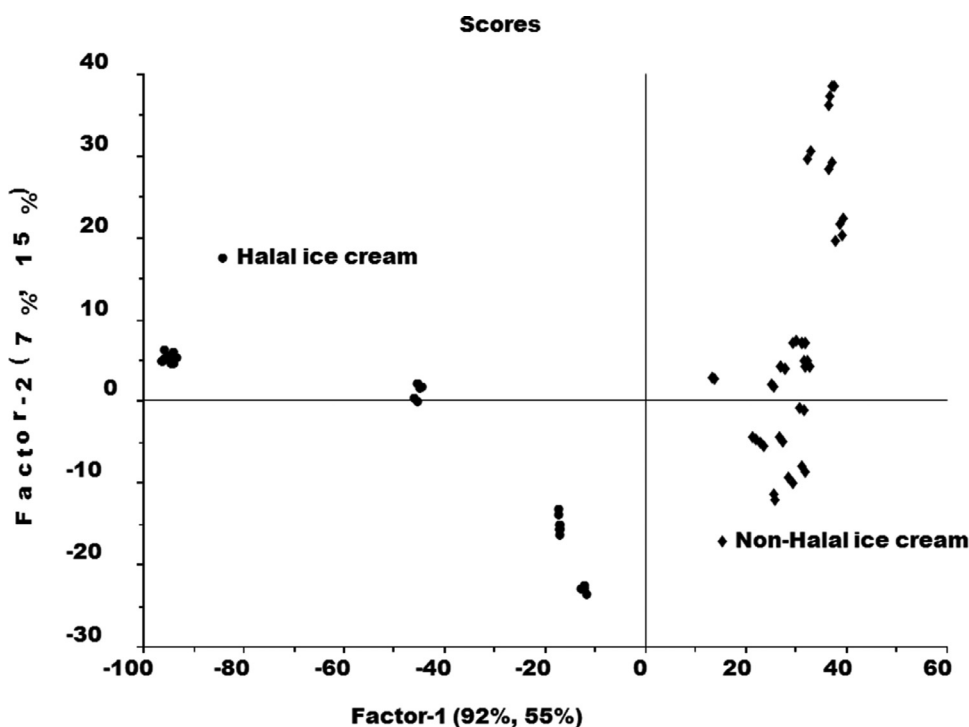


Fig. 3. Score plot of PLS-DA calibration model in between the groups of Halal & Non-Halal gelatin ice cream samples.

sults of the PLS-DA models for various types of spectral transformations are given in Table 1.

The results shown in Table 1 indicate that the PLS-DA models with unit vector normalization spectral transformations for 1 % pork gelatin adulteration is the optimum one, based on the compromise of having lowest value of errors i.e. RMSECV=0.147 for calibration set, the least number of factors i.e. 6 and 100% classification among the samples of Halal and Non-Halal ice cream samples for both the calibration and prediction sets. This calibration set, was contained 19 ice cream samples of Halal gelatin, while 23 ice cream samples of Non-Halal and all the samples were 100% correctly classified in their respective groups.

The total percentage of correctly classified samples for the prediction set using the developed calibration model was also found 100%. In prediction set, there were 11 samples of ice cream were having Halal gelatin and 7 samples were not containing Halal gelatin. Similarly, all the samples of both the types of ice cream containing Halal and Non-Halal gelatins were correctly classified 100% having the least prediction error i.e. RMSEP=0.098.

The predicted versus reference values for this calibration model are presented in Fig. 2. This plot shows how close to the ideal values of -1 and +1 (Halal & Non-Halal ice cream samples, respectively) to the predicted values.

As we can see from Fig. 2 the key indicators of PLS-DA model optimization i.e. RMSECV= 0.147, R square= 0.979 and number of factors= 6 were found for unit vector transformation as compared to other spectral transformation as shown in Table 1. These values showed the best agreement with the criteria of optimization such as that the best PLS-DA model will be the one having least error (RMSECV), highest value of R Square, less number of factor, and having the maximum classification in between the two groups of ice cream samples. This plot in Fig. 2 also shows how close to the ideal values of -1 and +1 of both the Halal gelatin ice cream samples and Non-Halal one.

The score plot (factor 1 vs factor 2) of the PLS-DA calibration model is also shown in Fig. 3.

The score plot of PLS-DA model as illustrated in Fig. 3 shows that factor-1 contributes more as compare to factor-2. It also shows a complete mapping of separation among the Halal gelatin ice cream samples

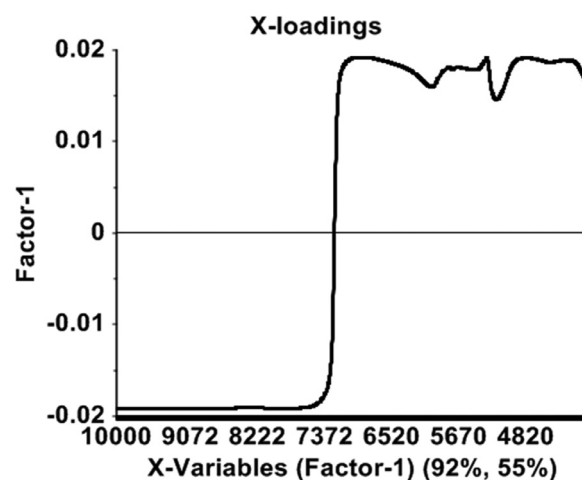


Fig. 4. X-Loading plots for factor-1 of PLS-DA calibration model.

with Non-Halal one. Factor-1 is mainly responsible for differentiation and classification among the samples of ice cream of both groups gelatin. It also shows that 92 % of the spectral variation and 55 % of the Y dummy variability were used during PLS-DA model. It is a good amount of both X and Y dummy variables and that plays a very important role in discrimination. In order to see that what part of the spectra contributed to the PLS-DA calibration model, the loading plots for factor-1 is shown in Fig. 4.

It is also evident from the X-loading plots of factor-1 of PLS-DA model as shown in Fig. 4 factor-1 explains 92% of the total spectral variability. The key bands important for the separation are in the NIR region from 4820 to 7372 cm^{-2} of factor-1. To investigate how the discriminant model will behave on the unknown samples and predict their class, and prediction error the external validation was performed on the samples using the prediction set not included during calibration model development (18 samples) as shown in Fig. 5.

Table 1
Results of the PLS-DA models in the full NIR spectral range (10000 to 4000 cm^{-1}).

PLS-DA results for ice cream samples adulterated with 1% adulterant gelatin									
Pre-processing	Calibration set Correctly classified (%)					Prediction set Correctly classified (%)			
	LVs	Total	Halal	Non Halal	RMSECV	Total	Halal	Non Halal	RMSEP
Unit Vector Normalization	6	42 19+23	19-0=19	23-0=23	0.147	18	11-0=11	7-0=7	0.098
MSC+ Unit Vector Normalization	5	42 19+23	19-0=19	23-0=23	0.144	18	11-0=11	7-0=7	0.138
SNV+ Unit Vector Normalization	5	42 19+23	19-0=19	23-0=23	0.168	18	11-0=11	7-0=7	0.133
MSC	5	42 19+23	19-0=19	23-0=23	0.178	18	11-0=11	7-0=7	0.138
SNV	6	42 19+23	19-2=17	23-3=20	0.353	18	11-1=10	7-1=6	0.460
Baseline	6	42 19+23	19-2=17	23-1=22	0.353	18	11-1=10	7-1=6	0.460
MSC+Baseline	5	42 19+23	19-1=18	23-1=22	0.290	18	11-2=9	7-0=7	0.269
SNV+Baseline	5	42 19+23	19-0=19	23-0=23	0.168	18	11-0=11	7-0=7	0.133

PLS-DA results for ice cream samples adulterated with 5% adulterant gelatin									
Pre-processing	Calibration set Correctly classified (%)					Prediction set Correctly classified (%)			
	LVs	Total	Halal	Non Halal	RMSECV	Total	Halal	Non Halal	RMSEP
Unit Vector Normalization	6	42 20+10	20-0=20	10-2=8	0.546	18	10-0=10	8-1=7	0.396
MSC+ Unit Vector Normalization	4	42 20+10	20-2=18	10-5=5	0.717	18	10-1=9	8-2=6	0.587
SNV+ Unit Vector Normalization	4	42 20+10	20-1=19	10-5=5	0.713	18	10-1=9	8-5=3	0.602
MSC	4	42 20+10	20-1=19	10-4=6	0.711	18	10-1=9	8-2=6	0.587
SNV	4	42 20+10	20-1=19	10-5=5	0.713	18	10-1=9	8-2=6	0.602
Baseline	4	42 20+10	20-2=18	10-2=8	0.585	18	10-0=10	8-2=6	0.647
MSC+Baseline	6	42 20+10	20-0=20	10-2=8	0.400	18	10-0=10	8-2=6	0.513
SNV+Baseline	4	42 20+10	20-1=19	10-2=8	0.713	18	10-1=9	8-2=6	0.602

PLS-DA results for ice cream samples adulterated with 10% adulterant gelatin									
Pre-processing	Calibration set Correctly classified (%)					Prediction set Correctly classified (%)			
	LVs	Total	Halal	Non Halal	RMSECV	Total	Halal	Non Halal	RMSEP
Unit Vector Normalization	7	42 19+23	19-0=19	23-2=21	0.315	18	11-0=11	7-0=7	0.138
MSC+ Unit Vector Normalization	3	42 19+23	19-2=17	23-2=21	0.386	18	11-3=5	7-0=7	0.491
SNV+ Unit Vector Normalization	3	42 19+23	19-2=17	23-2=21	0.387	18	11-2=9	7-0=7	0.477
MSC	3	42 19+23	19-2=17	23-2=21	0.386	18	11-3=8	7-0=7	0.490
SNV	3	42 19+23	19-2=17	23-2=21	0.387	18	11-3=8	7-0=7	0.477
Baseline	4	42 19+23	19-3=16	23-3=20	0.357	18	11-5=6	7-0=7	0.436
MSC+Baseline	3	42 19+23	19-2=17	23-2=21	0.386	28	11-3=8	7-0=7	0.490
SNV+Baseline	5	42 19+23	19-1=18	23-2=21	0.343	28	11-1=10	7-0=7	0.372

(continued on next page)

Table 1
(continued)

PLS-DA results for ice cream samples adulterated with 20% adulterant gelatin									
Pre-processing	Calibration set Correctly classified (%)					Prediction set Correctly classified (%)			
	LVs	Total	Halal	Non Halal	RMSECV	Total	Halal	Non Halal	RMSEP
Unit Vector Normalization	6	42 20+10	20-4=16	10-3=7	0.481	18	10-2=8	8-1=7	0.444
MSC+ Unit Vector Normalization	5	42 20+10	20-3=17	10-2=8	0.460	18	10-2=8	8-1=7	0.444
SNV+ Unit Vector Normalization	5	42 20+10	20-2=18	10-2=8	0.466	18	10-2=8	8-2=6	0.452
MSC	5	42 20+10	20-2=18	10-2=8	0.460	18	10-2=8	8-2=6	0.444
SNV	5	42 20+10	20-2=18	10-2=8	0.466	18	10-2=8	8-2=6	0.452
Baseline	6	42 20+10	20-2=18	10-2=8	0.489	18	10-2=8	8-1=7	0.466
MSC+Baseline	5	42 20+10	20-2=18	10-2=8	0.460	18	10-2=8	8-2=6	0.455
SNV+Baseline	5	42 20+10	20-2=18	10-2=8	0.466	18	10-2=8	8-2=6	0.452

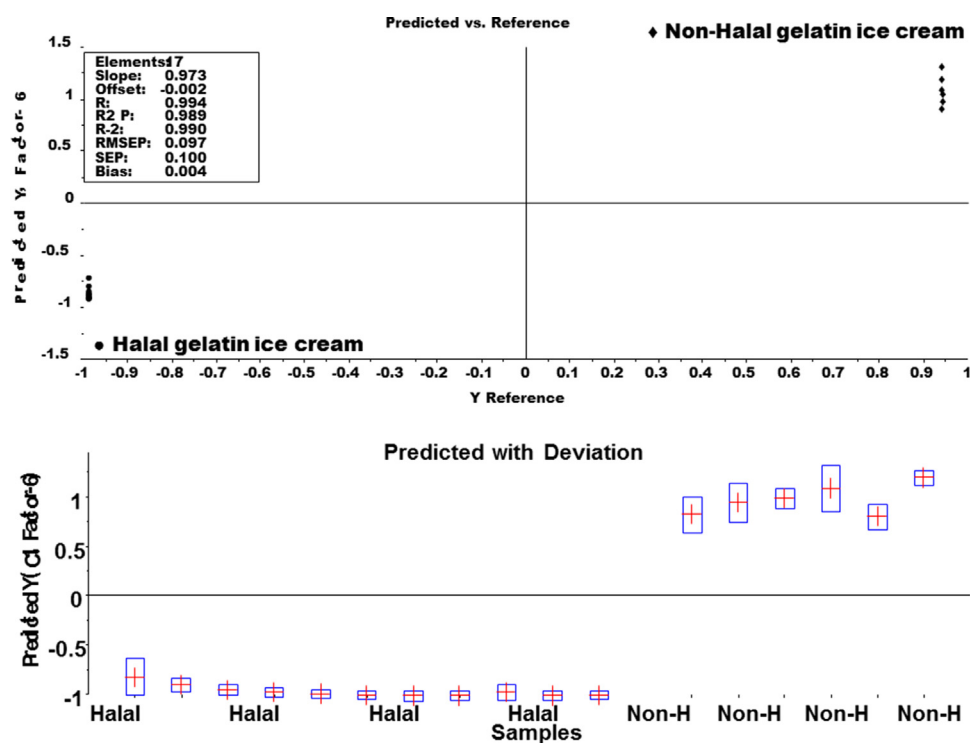


Fig. 5. Prediction plots of PLS-DA model on the test set samples of ice cream of both containing Halal & Non-Halal gelatins (Halal means not containing pork gelatin) and Non-Halal (containing pork gelatin).

Fig. 5 shows the prediction results of the PLS-DA calibration model on test set (19) samples of ice cream those were not used during building the PLS-DA calibration model. As it can be seen, all ice cream samples containing pork gelatin having predicted values close to -1 which assigns them to class 'pork gelatin ice cream'. For group of ice cream samples containing without pork gelatins having the predicted values close to +1, which was assign to them to class 'without pork gelatins ice cream samples'. The prediction error was found RMSEP=0.098, for 6 factors with 100% classification among the samples of both ice cream samples containing Halal and Non-Halal gelatins respectively.

4. Conclusions

NIR spectroscopy in reflectance mode coupled with PLS-DA could be deployed for fast, low-cost and non-destructive detection method for discrimination the ice cream samples containing Non-Halal gelatin i.e.

pork gelatin. The results indicated that the PLS-DA models with unit vector normalization is the optimum one, based on a compromise the lowest value of RMSECV=0.147 for calibration set, the lowest value of RMSEP=0.098, for 6 factors with 100% classification among the samples of both ice cream samples containing Halal and Non-Halal gelatins for both calibration and prediction set of samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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