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Determining quality of caviar from Caspian Sea based on Raman spectroscopy and using artificial neural networks

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ABSTRACT

The purpose of this study was to evaluate the feasibility of Raman spectroscopy for predicting purity of caviars. The 93 wild caviar samples of three different types, namely; Beluga, Asetra and Sevruga were analysed by Raman spectroscopy in the range 1995 cm^{-1} to 545 cm^{-1}. Also, 60 samples from combinations of every two types were examined. The chemical origin of the samples was identified by reference measurements on pure samples. Linear chemometric methods like Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) were used for data visualisation and classification which permitted clear distinction between different caviars. Non-linear methods like Artificial Neural Networks (ANN) were used to classify caviar samples. Two different networks were tested in the classification: Probabilistic Neural Network with Radial-Basis Function (PNN) and Multilayer Feed Forward Networks with Back Propagation (BP-NN). In both cases, scores of principal components (PCs) were chosen as input nodes for the input layer in PC-ANN models in order to reduce the redundancy of data and time of training. Leave One Out (LOO) cross validation was applied in order to check the performance of the networks. Results of PCA indicated that, features like type and purity can be used to discriminate different caviar samples. These findings were also supported by LDA with efficiency between 83.77% and 100%. These results were confirmed with the results obtained by developed PC-ANN models, able to classify pure caviar samples with 93.55% and 71.00% accuracy in BP network and PNN, respectively. In comparison, LDA, PNN and BP-NN models for predicting caviar types have 90.3%, 73.1% and 91.4% accuracy. Partial least squares regression (PLSR) models were built under cross validation and tested with different independent data sets, yielding determination coefficients (R^2) of 0.86, 0.83, 0.92 and 0.91 with root mean square error (RMSE) of validation of 0.32, 0.11, 0.03 and 0.09 for fatty acids of 16.0, 20.5, 22.6 and fat, respectively.

1. Introduction

The Caspian Sea is the world's biggest land-locked body of water, and it is bordered by five countries; Iran, Russia, Turkmenistan, Azerbaijan and Kazakhstan. There are several important types' fisheries in the Caspian Sea, but the greatest emphasis has always been placed on sturgeons. The most valuable caviars are derived from wild harvested sturgeons in area of the Caspian Sea; namely Beluga (*Huso huso*), Asetra (*Acipenser persicus*) and Sevruga (*Acipenser stellatus*) caviars [1]. Some research has been performed on caviars, especially from farmed sturgeons. The changes of biochemical composition associated with follicular in white sturgeon female plasma, have been determined by FT-IR [2]. Growth of organochlorine compounds in the muscle of sturgeons and their caviar was reported by Hosseini et al. [3]. On the other hand the impact of altering fatty acids composition in diet of cultured sturgeons has been studied in order to match caviar composition in wild caught sturgeons [4].

One of the most important frauds in caviar commerce involves mixing different types of caviar with the expensive one and selling it at a higher price. Due to high demand of this product, production of caviars from wild sturgeon has been decreased and some frauds happen. Sometimes, caviars from farmed sturgeon are mixed with wild roes and are presented as wild caviar. If the mixing value is not too high or both items are from the same species, such as wild or farmed Beluga, distinguishing the different kinds of origin will be impossible based on the appearance (e.g. egg size and colour). Thus, there is an increasing need for an accurate method to characterise adulteration of caviar. Several

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studies have been carried out using Raman spectroscopy as a rapid and reliable method for quality control of food. Raman is a relatively specific spectroscopic technique that measures rocking, wagging, scissoring, and stretching vibrations of functional groups. Quantitative information about food components like fat, collagen and pigment can be extracted from Raman spectra, as has been done in fish muscles [5]. High quality Raman spectra of intact salmon, ground salmon and salmon oil were obtained by Afseth [6]. Fourier Transform Raman spectroscopy has been chosen to clearly separate different classes of animal fats (poultry, pig, bovine, lamb and fish oil). PCA and Partial Least Squares Discriminant Analysis (PLS-DA) models were also chosen for discrimination between different fats [7]. A portable Raman instrument was applied for discrimination of olives according to ground and sound origin. PCA, Soft Independent Modelling Class Analogy (SIMCA), PLS-DA and K- nearest neighbours (KNN) were applied as chemometric methods for clustering the olive types [8]. Raman spectral analysis of oil extracted from freeze dried Atlantic and stored mackerel and horse mackerel also revealed significant reductions in the intensity of bands associated with CH_2 stretches [9]. Ellis studied the possibility of Raman spectroscopy to discriminate between important poultry species (turkey and chicken) and to distinguish between muscle groups with the aim of studying authenticity in muscle food products [10]. With respect to data processing, different techniques like PCA, PLS-DA, SIMCA and KNN have been used as pattern recognition methods for spectroscopic data. Nowadays, artificial neural networks (ANNs) have shown to be successful in modelling complex nonlinear systems which can perform mapping, regression, modelling, clustering, classification and multivariate data analysis [11]. Interest in using ANNs in food science has been increased in the last few years, due to the lack of restrictions on the type of relationship between the growth parameters and the desired output [12,13,14]. ANNs directly explore the knowledge contained in the input–output patterns by adjusting the highly nonlinear topology [15]. The networks most commonly used in food classification are of multilayer perceptron (MLP) trained by means of the back-propagation (BP) algorithm [16]. For instance, various beverages were recognised by sensor arrays, PCA combined with BP and neural network. The correct classification percentages obtained, were 86.3%, 86.7% and 96.7% for beer, juice and milk, respectively [17]. In another study, several wine samples were classified and different quality parameters were predicted based on gas sensor measurements [18] and ANN [19,20]. Five different tea samples have also been classified using back propagation neural network (BP-NN) with accuracy rates of 88% [21]. A MLP network was employed to correlate FT-IR spectral data with beef spoilage during aerobic storage at chill and abuse temperatures. This network was able to classify 91.7%, 81.2% and 94.1% of fresh, semi-spoiled (semi-fresh) and spoiled samples, respectively [22]. Soluble solid content (SSC) of 38 novel orange fruits has been predicted by PCA and BPNN [14]. Radial basis function neural network (RBF-NN) and BP-NN models were also developed and evaluated for discrimination of a spirituous beverage based on concentration of selected elements like Cu, Fe, Zn. RBF model showed high overall predicting performances ($>97\%$) higher than BP-NN used for the same database [23].

The objective of this article is to demonstrate that Raman spectra contain useful quantitative information about constituents related to the purity of caviars, and that Raman spectroscopy thus is a potential method for rapid and non-destructive analysis of the adulteration of caviar. In our study, Raman spectra of three caviar types were treated by Principal Component Analysis (PCA) in order to visualise the natural grouping of pure and mixed samples. Linear discriminant analysis (LDA) algorithm and non-linear methods like Artificial Neural Networks (ANNs) were applied to assess discrimination of the caviar's purity.

2. Materials and methods

2.1. Caviar samples

High quality caviar was made from fresh sturgeon roes. These sturgeons were caught using Gill nets with the standardized mesh and dimensions set by the Iran fisheries organisation. Caviar samples were derived from them among the daily catch during spring 2011. The roes were withdrawn from the opened abdominal cavity and delicately massaged to release the eggs, which were undergone a brief washing stage followed by dry salting with 3.5–5% salt. The caviars were packed in closed commercial glass cans, and stored at 0–4 °C. A total of 93 pure samples (31 samples from each type of sturgeon; Beluga, Asetra and Sevruga) and 60 mixed samples were packed in glasses of 16 g each and stored in the refrigerated storage room (±2 °C) prior to analysis. Two groups of samples were considered for this study. The first one was composed of 31 samples of three pure caviars in order to identify different types. The second was formed by six groups of mixed caviars (Table 1). Each group is identified by two letters, the first letter (capitalised) denotes the main constituent and the second letter (lower case) is related to impurity of the caviar. The main type was always present at 11 g, whereas the impurity was present at 5 g. For example, Bs (Beluga-Sevruga) means that Beluga is the main caviar with 11 g weight with 5 g impurity of Sevruga.

2.2. Raman spectroscopy

A Raman spectrum was measured directly from intact caviar, but the spectra were noisy with high background fluorescence. In order to overcome this problem and improve the spectra, one layer of sample was used. Raman spectra of each sample were obtained, on a Kasier Optical Systems Hololab Series 5000 instrument consisting of a Holoprobe transmission spectrograph. Measurement was carried out in the wavenumber range 1955–550 cm^{-1} with two replicates for each sample. The Raman system was equipped with a 785 nm stabilised external cavity diode laser operating at the average power of 90 mW at the sample. All spectra were collected using 1000 μm slit width and a detector temperature of −40 °C. Each sample spectrum was an average of two accumulations in 5 s acquisition time at the same point.

2.3. Reference method

Water content was determined by freeze-drying; until a constant weight was reached. Protein content was estimated according to the Kjeldahl method [24]. For the estimation of crude fat, the Bligh and Dyer extraction was used [25]. The fatty acids were determined by gas–liquid chromatography [26]. The samples were homogenised in trichloromethane by means of Ultra-Turrax and the total lipid was extracted as described by Folch [27]. The lipid sample was dissolved in 10% methanolic hydrogen chloride (2 mL). A 0.1 ml solution of tricosanoic acid (910 mg mL^{-1}) was added as internal standard.

Table 1
Experimental samples.

Group1	Pure Asetra	31 samples
	Pure Beluga	31 samples
	Pure Sevruga	31 samples
Group2	Asetra + Sevruga (As)	10 samples
	Asetra + Beluga (Ab)	10 samples
	Beluga + Asetra (Ba)	10 samples
	Beluga + Sevruga (Bs)	10 samples
	Sevruga + Beluga (Sb)	10 samples
	Sevruga + Asetra (Sa)	10 samples

Table 2
Mean and standard deviations for fatty acids and other parameters (%) of pure caviars.

	16:0	18:1(*n*−7)	20:1(*n*−9)	20:5(*n*−3)	22:6(*n*−3)	Total fat	Protein	moisture
Beluga	24.27±0.8	7.68±2.3	0.41±0.16	5.21±1.6	3.46±1.0	16.4±1	21.6±0.3	50.9±3.8
Asetra	25.49±1.2	4.24±3	0.68±0.18	5.86±0.7	2.63±0.9	15.2±1.7	24.9±0.1	52.5±4
Sevruga	29.11±0.4	4.39±2.1	2.28±0.47	10.68±1.0	4.14±1.4	18.5±2	23.5±0.3	44.4±2.7

The samples were sealed and heated at 50 °C, and 2 mL of a 1 M potassium carbonate solution was added to each sample. The Fatty Acid Methyl Esters (FAMEs) were extracted with 2 mL × 2 mL of hexane and 1 μL was injected to the gas-chromatogragh, in split mode. Fatty acids analysis was carried out on an Agilent gas-chromatogragh (Model 6890 Series GC) fitted to an automatic sampler (Model 7683 Series Injector) and FID detector. The relative proportion of each fatty acid in the fatty acids patterns was expressed as percentage of the sum of fatty acids resolved. Two replicate analyses per sample were performed. All analyses were carried out on pure caviars (Table 2).

2.4. Data analysis

Raman spectra were exported to the Unscrambler software (X10, CAMO, AS, Norway). A data pre-treatment routine was applied to remove the fluorescent background intensities from Raman spectra. This method was described by Brennan et al. [28]. A forth order polynomial was fitted to the raw spectra. The fitted polynomial was then subtracted from the original spectrum. Thereafter the replicates of every sample of every data set were averaged. The polynomial fitting routine was written by Matlab code (ver. 7, USA). To optimise the accuracy of calibration, several scattering corrections and mathematical pre-processing methods were tested. Subsequently, Extended Multiplicative Scatter Correction (EMSC) was used for normalisation of the spectra. PCA was applied to the spectral data in order to obtain the best possible view of the spectral structure [29]. It allows converting original and correlated variables into uncorrelated variables called principal components (PCs) which contained the main information and reduces data redundancy. Loading plots were used to interpret the spectral information contained in each PC. Supervised pattern recognition techniques, LDA, are used in a wide range of applications where classification of certain criteria is needed. Some examples are detection of eggshell crack [30], food of animal origin [31] and geographical and floral origins of honeys based on volatile compounds [32]. LDA is simple, mathematically robust and often produces models whose accuracy is as good as more complex methods according to previous researches. LDA performs classification based on finding optimum linear boundaries among different classes by maximising the ratio between class variance and minimising the ratio within class variance. This is the well-known Fisher criterion [33]. This method establishes PCA for each class and the corresponding models are developed individually [34]. LDA Projections exhibit a high degree of class separation, whereas PCA preserves the original structure of the data. For the PC-ANN models, PCA was performed first to extract information from the whole spectral regions, and a few PCs were used to be the neurons of network input layer. Two types of neural networks were tested for classification: Multilayer Perceptron with Back propagation training algorithm (BP) and Probabilistic Neural Networks with Radial Basic Function (PNN). The BP network architecture is formed by three layers: the input layer has seven neurons corresponding to the seven first principal components, a variable number of neurons with *tansig* function for the hidden layer (the optimum number was 16), and 3 neurons with *hardlim* function for the output layer, the same number of existing classes. It has a learning rate of 0.01 and training times less than 3 min. On the other hand, PNN is a kind of feed forward neural network. The original PNN structure is a direct neural network implementation of Parzen nonparametric Probability Density Function (PDF) estimation and Bayes classification rule [35,36]. The standard training procedure of PNN requires a single pass over all the patterns of the training set [34]. It is composed of three layers, with *radial basis* transfer functions in the hidden layer and a competitive one in the output [37,38]. Also, Leave One Out (LOO) cross validation was applied in both cases in order to check the performance of the networks [39]. LOO consists of training *N* distinct nets (where *N* is the number of measurements) by using *N*-1 training vectors, while the validation of the trained network is carried out by using the remaining vector, excluded from the training set. This procedure is repeated *N* times until all vectors are validated [40]. Matlab software (R2009) was used for developing the PC-ANN models.

3. Results and discussion

Fig. 1 shows the raw and preprocessed Raman spectra. The major Raman bands of caviars are found at 932 cm^{-1} (C–C stretch), 974 cm^{-1} (=C–H out of plan deformation), 1266 cm^{-1} (symmetric =C–H rock), 1302 cm^{-1} (CH_2 twist), 1442 cm^{-1} (CH_2 scissoring) and 1662 cm^{-1} (cis C=C stretch). The PCA score plot (two first PCs) of different types of the pure caviar samples is shown in Fig. 2a. The first PC represented 43%, while the second PC explains 22% of the total variance. Sevruga is well distinguished by PC2 and has the highest positive score values. Some physical differences between species were also observed, Sevruga's eggs are the smallest and have the thinnest membrane, and thus most of the membranes were broken down even before starting the measurement. Fatty acids also showed differences in palmitic and EPA compositions for Sevruga samples, as shown in Table 2. Beluga samples are placed near Asetra (Fig. 2a). This fact may be explained by the great similarity in the fatty acids (16:0, 20:5). From the loading plot corresponding to the second PC (Fig. 2b), it is possible to determine the Raman peaks responsible for the distinction along PC2. The major bands are associated with the unsaturated structures, which is in a good agreement with caviar composition as known from the literature [1,4] and as seen in Table 2. The positive part of the loading plot is mostly presented by Sevruga, whereas the negative part is characterised by Asetra. the band at 1662 cm^{-1} associated with stretching of the C=C group, whereas the band at 1262 cm^{-1} can be associated with the symmetric rocking of the =C–H group, thus samples along PC2 are ordered according to increasing levels of fatty acid unsaturation, as confirmed by Table 2.

Table 2. Mean and standard deviations for fatty acids and other parameters (%) of pure caviars

The collected data of pure and mixed caviar samples were analysed by the application of PCA in order to visualise variation occurring on samples of pure caviar and mixed one, respectively. The score plots of PC1 versus PC2 for Beluga, Asetra and Sevruga samples from Raman spectra are represented in Fig. 3. Some

samples grouped together creating clusters that correspond to the samples of pure and impure caviars, whereas it is, as expected, more difficult to distinguish between the impure groups. The pure samples of Beluga (Fig. 3a) and Asetra (Fig. 3b) grouped in the negative part of the PC1 axis. The Ba and Bs samples were placed in the positive part of PC1 (Fig. 3a). Loading plots of the PCA analysis of Beluga and Asetra have peaks in same regions as shown in Fig. 3d. For Sevruga, the score plot pattern is opposite (Fig. 3c) compared to Beluga and Asetra. A region between 1470 and 1430 cm^{-1}, representing the scissoring vibration of CH_2, and 1670–1640 cm^{-1} associated with stretching C═C, were important bands along PC1.

LDA was used to discriminate different types of Caspian caviars and distinguish the purity of them. Table 3 shows the classification results of group one (Table 1) in the form of a confusion matrix of a 3PCs-LDA. The number of correctly classified (CC%) for three kinds of caviars are also shown. The LDA models of Asetra and Beluga have an efficiency of 93.5% which is related to two and one incorrectly classified samples as Sevruga. Five samples were also misclassified for Sevruga

LDA was also used to classify caviars of the second group according to Table 1. Six different classes of mixed samples (Ab, As, Ba, Bs, Sb and Sa) were created in order to distinguish impurity of the samples. Table 4 shows the results of the LDA analysis. Correct classification as a function of the number of PCs used in LDA models, was between 100% and 70%. Two samples of the Ab class were identified as members of As class and one of them was classified as pure Asetra. No misclassification was seen between group one and two (pure and impure caviars), except for Asetra, which was also shown in Fig. 3b.

These results were also confirmed by ANN analysis. The first seven PCs, which accounted for 99.2% of the original variation, were used as input to the neural networks models. These two networks were used to discriminate among different types of pure caviars (group 1 according to Table 1). The results show that the BP network classified different types of caviar more accurately than PNN (Table 5), while PNN has more accurate performance for discrimination of a spirituous beverage [23] and detection of wine ageing [41]. Two misclassifications for Asetra and three misclassifications for both Beluga and Sevruga were represented in BP. On the other hand, distinguishing pure sample from the impure one in each type (Asetra, Beluga and Sevruga) was performed with PC-ANN. The success rate of classification for different types of caviars in pure and impure situations is depicted in Table 6. The worst classification of impure samples was related to Asetra's group (success rate = 66.66%) as shown in LDA results for Ab (Table 4). According to the research of Cajka, the prediction ability for classification of Trappist and non Trappist bier was 93.9% (PLS-

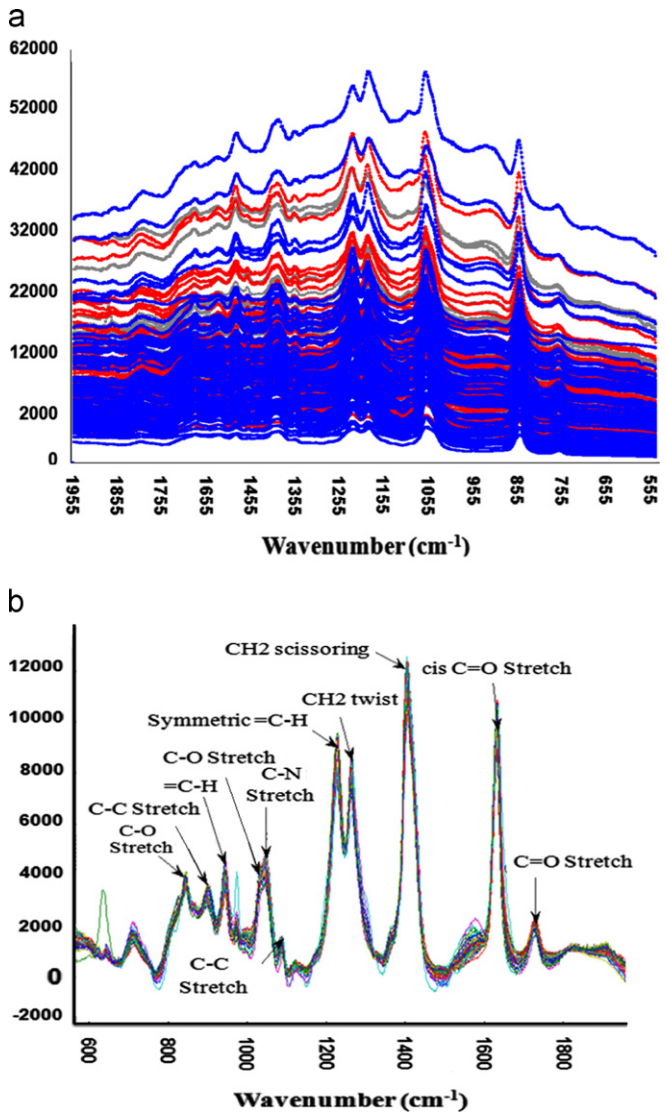

Fig. 1. (a) Raw and preprocessed (b) Spectra of caviars.

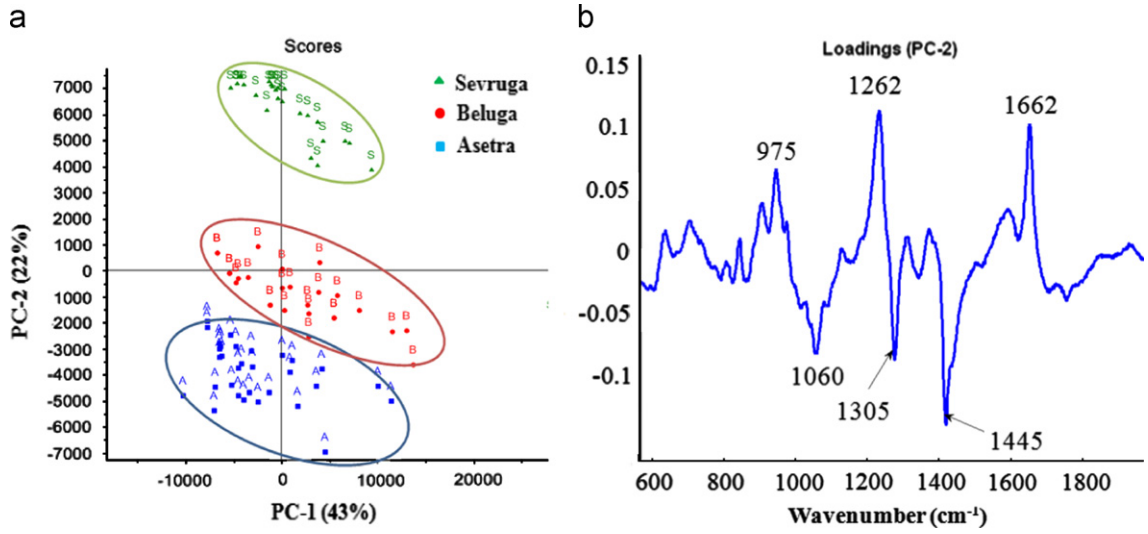

Fig. 2. Discrimination between pure Beluga, Asetra and Sevruga in PCA score plot (a) and the corresponding loading plot of PC2 (b).

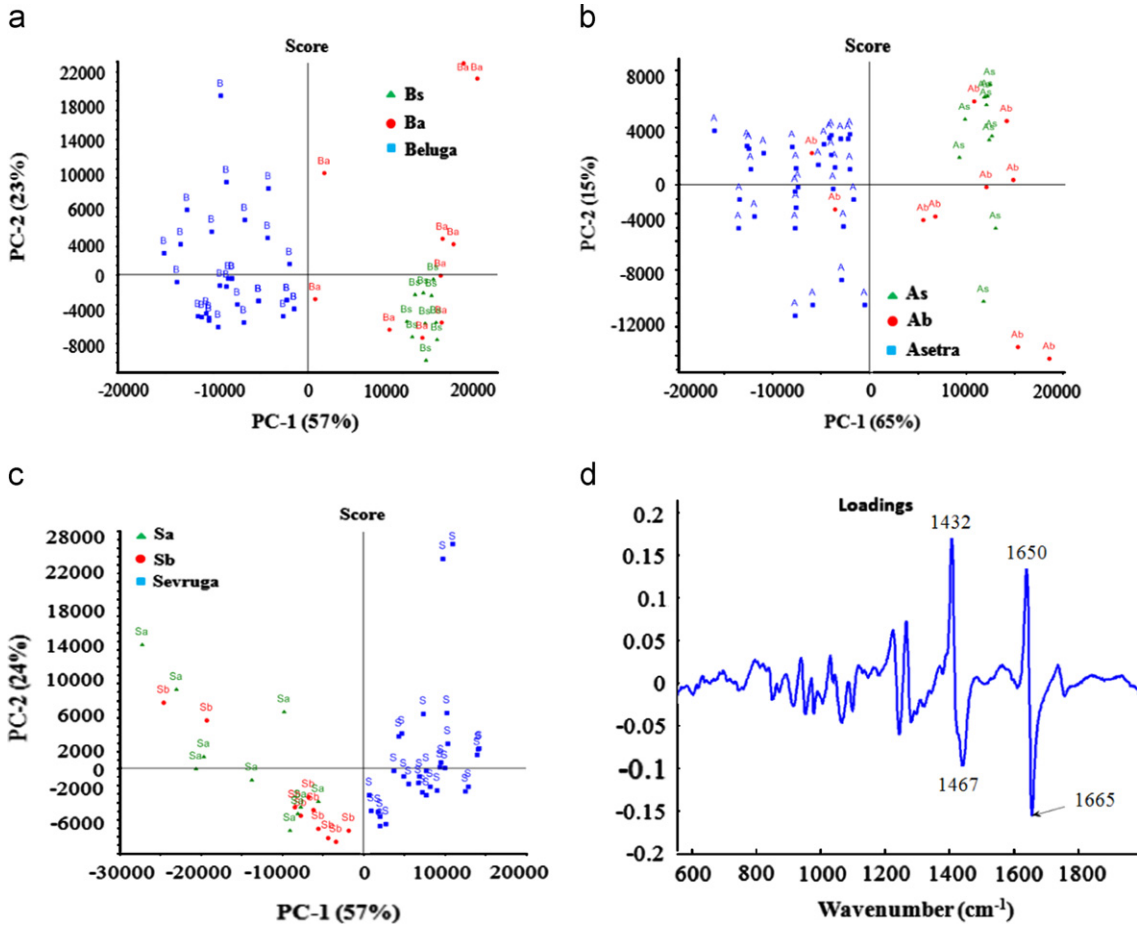

Fig. 3. PCA plots shows impurity in Asetra (3a), Beluga (3b), and Sevruga (3c). The PC2 loading plot of Asetra and Beluga is also shown (3d).

Table 3
LDA classification of caviar types based on Raman spectra.

	Asetra	Beluga	Sevruga	Correct classification (CC%)
Asetra	29	0	5	93.5
Beluga	0	29	0	93.5
Sevruga	2	2	26	83.8

Table 4
LDA analysis for distinguishing pureness in different types of Caspian caviar.

	A	B	S	Ab	As	Ba	Bs	Sb	Sa	CC%
A	30	–	5	1	–	–	–	–	–	96.77
B	–	31	–	–	–	–	–	–	–	100
S	–	–	26	–	–	–	–	–	–	83.77
Ab	1	–	–	7	–	–	–	–	–	70
As	–	–	–	2	10	–	–	–	–	100
Ba	–	–	–	–	–	10	–	–	–	100
Bs	–	–	–	–	–	–	10	–	–	100
Sb	–	–	–	–	–	–	–	10	–	100
Sa	–	–	–	–	–	–	–	–	10	100

Table 5
PC-ANN classification of caviar types.

Caviar types	Probabilistic neural network			Back propagation		
	Asetra	Beluga	Sevruga	Asetra	Beluga	Sevruga
Asetra	23	6	5	29	1	1
Beluga	3	23	2	2	28	1
Sevruga	3	6	22	2	1	28
CC%	74.19	74.19	71.00	93.55	90.32	90.32

Table 6
Performance of the PNN and BP network for prediction of the optimal model in caviar samples analysed by Raman spectroscopy.

Caviar samples	Total number of samples	Success rate (%)	
		BP	PNN
Group1 (pure)	93	80.39	66.66
Asetra+Ab+As	51	82.35	68.63
Beluga+Ba+Bs	51	90.20	78.43
Sevruga+Sa+Sb	51	91.40	73.12

DA), 91.9% (LDA) and 97.0% (ANN-MLP) [42]. The LDA, PNN and BP-NN models for predicting caviar types have 90.3%, 73.1% and 91.4% accuracy, respectively (comparing data in Table 3 and 5). Therefore the BP model, obtained more accurate results.

For several of the analytical parameters of the samples, regression models were developed based on Partial Least Squares Regression (PLSR) and the preprocessed Raman specta. Generally PLSR is used to transfer a large set of highly correlated and often collinear spectral variables into independent latent variables. When applied to spectra of the present study, the aim of the PLSR analysis was to find a mathematical relationship between the Raman scattering at different Raman shifts and the contents of fats and fatty acids of caviars. The optimal calibration models were constructed based on the lowest RMSE and the highest correlation

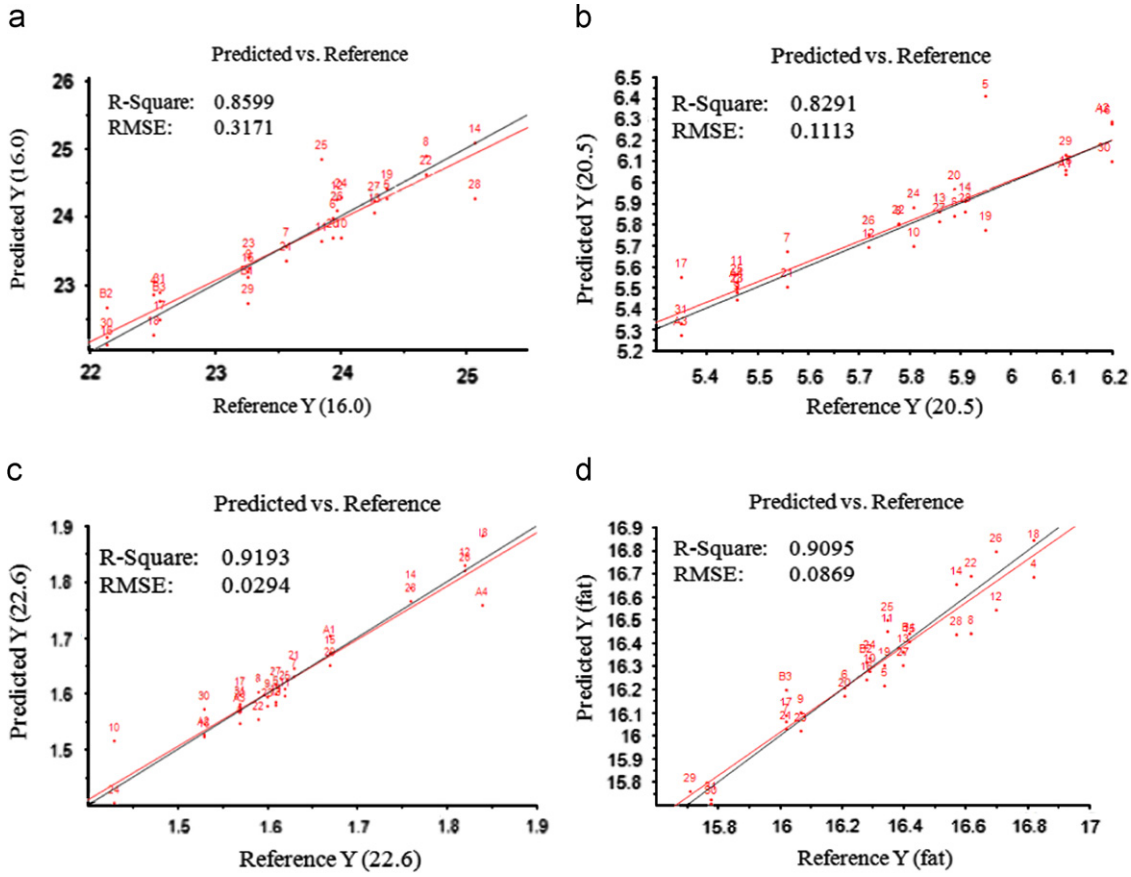

Fig. 4. Regression plot from the PLS models for C16.0, C20.5, C22.6 and fat of Beluga caviar.

coefficient based on cross validation (R^2_{cv}). Fig. 4 shows some plots of predicted vs. the reference values of selected analytical parameters of Beluga caviars based on PLSR calibration models. The determination coefficients (R^2) between the actual and the predicted values are marked on the plots. It is obvious to notice that the developed PLSR models had good performance in predictiong both fatty acid features and fat contents. It should be noted, however, that these regression models are based on a limited number of samples with a constricted range of variation, thus an independent set of samples is needed to check the validity of the present calibration models. But still the results indicate good quantitative relations between the Raman spectra and the selected analytical parameters as measured chemically. These results also justify the validity of such techniques for the proper quantification of caviar in a non-destructive manner.

4. Conclusion

This study has shown that, Raman spectroscopy is a promising technique for rapid and non-destructive classification of Caspian caviars. Raman spectra in the wavenumber range of 1995–545 cm^{-1} can be used for measuring fatty acids of pure sample, and PCA results indicated that the Raman spectra provide a clear discrimination of different types of caviars. The PCA findings were further elaborated using linear (LDA) and nonlinear (ANN) classification models. The LDA and artificial neural network of both types (PNN and BP) distinguished three classes of caviar types and also discriminated impurity present in each type. More accurate predictions were achieved by using the BP network. It is also shown that Raman spectroscopy combined with PLS regression analysis can be used as an accurate tool for quantifying the chemical parameters like fatty acids and fat contents in caviars.

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