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Raman spectroscopy and discriminant analysis applied to the detection of frauds in bovine meat by the addition of salts and carrageenan☆

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ABSTRACT

In the last years, there has been an important and growing concern about food authentication due to the increasing number of occurrences of new types of food frauds. Recently, some frauds have been reported describing the injection of non-meat ingredients, such as salts and polysaccharide compounds, into bovine meat *in natura*, aiming at increasing its water holding capacity (WHC) and obtaining economic fraudulent gains. Thus, this paper developed a simple and rapid analytical method based on a multivariate supervised classification model (partial least squares discriminant analysis, PLS-DA) and Raman spectroscopy for tackling this problem. Sixteen vacuum-packed pieces of the same cut, eye of the round (*semitendinosus*), of approximately 4 kg were obtained from different origins. According to an experimental design, each piece was divided into 11 parts, providing control and adulterated samples. Single, binary and ternary mixtures of adulterated samples were prepared by injecting NaCl, sodium tripolyphosphate and carrageenan in the meat pieces. A total of 165 samples were produced (54 controls and 111 adulterated) and their purges, the exudated liquid extracted from the meat after thawing, were obtained. Raman spectra of these purges were recorded between 1800 and 700 cm^{-1}. The whole data set was split into 112 samples for the training set and 53 for the test set. The best PLS-DA model was built with 4 latent variables and successfully discriminated adulterated samples at relatively small rates of false negative and false positive results, which varied from 8.0 to 11.7%. As an additional validation step, confidence intervals were calculated by bootstrap algorithm.

1. Introduction

In recent years, the concerns about food authenticity have been growing. The determination whether a product is, in fact, what it is declared to be is important to ensure consumer confidence and to detect possible changes in original food properties. Food fraud can be characterized by the use of a mechanism to mask or omit inappropriate sanitary conditions of products, giving them attributes that aim to increase the profits of their commercialization [1]. The U.S. Food and Drug Administration (FDA) defines economically-motivated adulteration (EMA) as the fraudulent action of intentional addition or substitution of a substance in a product in order to obtain economic gain due to the reduction of production costs or to the increase of the apparent value of the product. In this definition, EMA includes the addition or substitution of substances in order to mask product undesirable properties, the substitution of a substance for a cheaper one or the omission of any component added to the product without declaration on its label [2].

Specifically for meat fraud, four major types can be categorized: meat origin, meat substitution, meat processing treatment and non-meat ingredient addition [3]. Other authors have categorized food frauds in general in only three types, replacement, addition and removal [4]. The most common type of food fraud reported in the literature, described in 95% of the publications, is the substitution of tissues, breeds or species by other tissues, breeds or species. Only less than 5% of the reported publications have referred to the addition of

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ingredients and only less than 1% to the removal (data from 1980 to 2010) [5]. One of the most famous incidents of meat fraud, a case of meat substitution, was the horsemeat scandal occurred in Europe in 2013 [6,7]. Methods based on DNA analysis, such as polymerase chain reaction (PCR) [8], are among the most important ones for detecting meat substitution frauds. However, these methods are very laborious, demanding time and qualification of involved professionals. Thus, several simpler alternative analytical methods have been developed for this aim based on molecular spectroscopy, in the majority of cases associated with chemometric tools. Frauds involving a variety of meat species have been detected using mass spectrometry [9–11], Raman [12,13], nuclear magnetic resonance (NMR) [14], near infrared (NIR) [15,16], visible [17], and mid infrared (MIR) [18,19] spectroscopies; NIR hyperspectral imaging [20], multispectral imaging [21] and data fusion of UV–visible, NIR and MIR spectra [22].

On the other hand, the least common type of meat fraud reported in the literature has been the addition of non-meat ingredients [3]. The addition of vegetable proteins is a common practice in fraud of meat products. Articles describing the detection of soybean frauds in hamburgers using multiplex-PCR [23], the development of a screening method for the simultaneous detection of soy, pea and lupine in meat products applying high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) [24], and the use of vibrational spectroscopy combined with chemometrics to detect and quantify the adulteration of minced meat with textured soy protein [19] have been reported in the literature.

Another type of meat fraud consists of adding water aiming the economic growth of the sellers. The quality control regulations describe the allowed limit of addition of exogenous water to meat. However, the addition of water is prohibited for meats *in natura*. Exogenous water in meat can be determined by a standard method based on the water/protein ratio [25]. With the addition of water, this ratio becomes too high and can evidence the meat fraud. However, the addition of other sources of protein and salts increases water binding, and consequently, the water holding capacity (WHC) [26]. This practice leaves the water/protein ratio close to the natural ratio and hides the fraud. Thus, in order to provide a proof of fraudulent practices, it is necessary to detect the presence of external proteins or salts in the meat [3].

The increase of WHC can naturally occur by the addition of exogenous substances in different ways (heating, injection, milling), leading to the increase in the pressure at the meat surface. When its WHC increases, meat becomes tenderer due to the relaxation of muscle fibers. The effect of the addition of salts, such as NaCl, KCl and $MgSO_4$, in the WHC of bovine meat is dependent on their concentrations and has been studied by NIR/MIR spectroscopies combined with principal component analysis (PCA) [27,28]. These articles have used vibrational spectroscopy to elucidate proteins conformational changes due to the addition of salts. Other additives can be used to increase meat WHC, such as a xanthan gum, a high molecular weight polysaccharide gum, and carrageenan, a polysaccharide extracted from edible seaweeds [29].

In Brazil, official methods [30] have employed classical techniques for meat quality control, determining physico-chemical and microbiological parameters based on the so called target analysis. However, individual parameters are inappropriate to characterize frauds due to the wide variation in chemical composition of bovine meat, as a function of sex, cut, breed, feed intake, slaughter age, among others [3]. On the other hand, the utilization of the non-target analysis of food (food fingerprinting) presents the advantage of evaluating complex food matrices in terms of multiple characteristics (geographical origin, species variety, possible adulterations, etc.) using the same analytical method [31]. This strategy invariably combines spectroscopic techniques with chemometrics.

Despite the large number of recent publications focusing on developing analytical methods for detecting food frauds, few of them have been devoted to real food fraud incidents [4]. In a previous paper, we have analyzed 55 samples of bovine meat *in natura* (43 adulterated and 12 controls) originated from criminal networks dismantled by the Brazilian Police [32]. Seized adulterated samples had been injected with aqueous solutions of salts, such as sodium chloride, phosphate, tripolyphosphate (STPP) and acid pyrophosphate, carrageenan, maltodextrin and collagen. Meat samples were directly analyzed and a partial least squares discriminant analysis (PLS-DA) model was built with data fusion of five physico-chemical parameters (protein, sodium, chloride, phosphate and ash) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra. Low level data fusion PLS-DA model was able to discriminate samples with efficiency rates between 85% and 93%. Nevertheless, a discriminant model built with only FTIR spectra failed to provide good predictions, with efficiency rates around 50%.

Considering the limitations observed in our previous paper for developing FTIR models to detect adulterations directly in the pieces of meat [32], the present work choose to analyze purges, the exudated liquid obtained from the meat after thawing. Instead of FTIR, other vibrational technique, Raman spectroscopy, was used for this work due to its advantage of not suffering interference of water. Raman scattering spectroscopy has been very used for determining structural information in different types of samples, presenting great potential for investigations of forensic cases. This technique has also been considered a rapid tool to study food composition, particularly to detect species in meat products [33].

Thus, the aim of this article was to develop a rapid and reliable screening method using Raman spectroscopy and PLS-DA in order to detect frauds by the addition of NaCl, STPP and carrageenan in bovine meats, through the analysis of their purges. The importance of this paper as compared to the previous ones is related to the detection of meat frauds by the addition of salts and carrageenan, which has been a problem very rarely mentioned in the scientific literature. In relation to the only paper that have previous addressed this problem [32], the present work employed Raman spectroscopy, which provides analytical signals free of water interference, and originally analyzed purges, since the direct determination in the meat pieces by FTIR has been demonstrated to be unfeasible. For an in-depth study, samples of eye of the round were adultered under very controlled conditions of injected volume, weight gain and adulterant concentrations (5% and 10%, including binary and ternary mixtures). The developed model was validated with an independent test set and also the estimation of appropriate figures of merit (FOM). Finally, confidence intervals were determinated for each sample prediction from the PLS-DA model using a bootstrap resampling methodology [34].

2. Materials and methods

2.1. Reagents and samples

All reagents used were of analytical grade. Water was purified by deionization using a Milli-Q system with a resistivity of 18.5 MΩ cm (Millipore, Bedford, MA, USA). NaCl was purchased from Química Moderna (Barueri, SP, Brazil), sodium tripolyphosphate from Synth (Diadema, SP, Brazil) and kappa carrageenan from GastronomyLab (Brasília, DF, Brazil). Adulterants were injected with disposable luer lock tip syringes of 60 mL (SR Prodcutos para la Salud, Paraguay) using fuchsia needles of 40 mm × 1.20 mm (BD PrecisionGlide, Curitiba, PR, Brazil).

Sixteen vacuum-packed pieces of eye of the round (*semitendinosus*) of approximately 4.0 kg were purchased in the local commerce, from suppliers we can trust. Each piece was divided into eleven fractions of about 2.5 cm wide. These pieces of meat were subdivided into two groups, G1 and G2, with eight pieces/samples each. Since the anatomy of the bovine eye of the round presents greater concentrations of connective tissues at the extremities as compared to the central region of the cut, four unadulterated fractions were selected as control samples: two fractions at the extremities and two intermediate fractions. The other seven fractions were adulterated by injections of 5% w/v aqueous

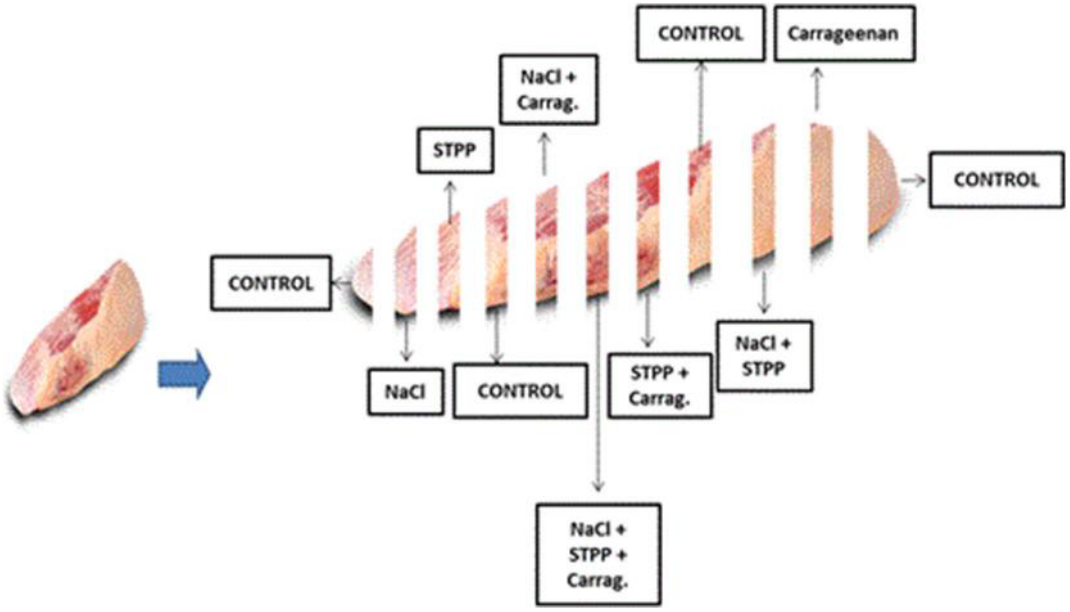

Fig. 1. Scheme of the experimental design used for obtaining control and adulterated samples from the partition of each piece of meat.

solutions of the following adulterants: sodium chloride (NaCl), sodium tripolyphosphate (STPP), carrageenan (Carrag), NaCl:STPP (1:1), NaCl:Carrag (1:1), STPP:Carrag (1:1), NaCl:STPP:Carrag (1:1:1). The eye of the round pieces of groups G1 and G2 were adulterated until gaining 10% and 5% of mass, respectively. After injecting the adulterant solutions, samples were centrifuged at 10,000 rpm for 10 min and stored under refrigeration in a vertical freezer (Consul, Brazil) at −12 °C for approximately 7 days. For obtaining the purges, samples were subjected to controlled thawing in refrigerator at 4–10 °C for 48 h. Purges were then collected and stored in Eppendorf tubes for further analysis. Fig. 1 displays a scheme showing the experimental design used for obtaining samples from the partition of each piece of meat. A total of 165 meat samples were obtained, 54 controls and 111 adulterated.

2.2. Raman spectra acquisition and data processing

Raman spectra were recorded using an FT-Raman Vertex 70 spectrometer (Bruker, Massachusetts, USA), equipped with a Raman module – RAM II (Bruker). Purges were centrifuged in an Eppendorf centrifuge for 10 min at 10,000 rpm. Centrifuged purge fractions were stored in Duran tubes duly identified and sealed with Parafilm plastic. Duran tubes were placed in the spectrometer sample holder containing a back mirror in order to increase the amount of scattered radiation. Raman spectra were obtained from 3600 to 710 cm^{-1} with 4 cm^{-1} of resolution, 512 scans accumulation and 1 W laser power (1064 nm). Mean spectra (triplicates) of each sample were used for building the models. Chemometric models were built using MATLAB software, version 8.4 (The MathWorks, Natick, USA), and PLS Toolbox, version 7.0 (Eigenvector Technologies, Manson, USA).

2.3. Chemometric analysis

PLS-DA is the most popular discriminant classification method in the chemometric literature [35]. It is derived from PLS regression, correlating independent spectral variables, contained in the X matrix, with a dependent dummy variable vector, in the y vector. In this work, dummy reference variables in the y vector were arbitrarily defined as 1 for adulterated samples and 0 for authentic meat samples. Since PLS-DA provides predicted y values that are not exactly 1.0 or 0.0, a Bayesian threshold was estimated [36,37].

In the development of a robust supervised classification model, a systematic criterion needs to be applied aiming at selecting representative samples for the training set and to ensure their homogeneous distribution in the multivariate space. The whole data set was split into training and test sets, corresponding to two thirds and one third of the samples, respectively. The Kennard and Stone (KS) algorithm [38] was applied for the selection of training samples separately in each class, control/authentic and adulterated. In this way, the original spectral data matrix was split in 112 (75 adulterated and 37 control) samples for the training set and 53 (35 adulterated and 18 control) samples for the test set. In the sequence, data were preprocessed. Unit vector normalization was applied for the correction of baseline deviations in the Raman spectra, followed by mean centering. The number of latent variables (LV) was selected by cross-validation using venetian blinds with 10 splits, based on the smallest cross validation classification error (CVCE).

The analytical validation of the constructed PLS-DA model was performed by estimating appropriate FOM, such as sensitivity (SEN), specificity (or selectivity) (SPE), false positive rate (FPR), false negative rate (FNR) and reliability or efficiency rate (EFR) [36,37]. SEN indicates the ability of the model to detect true positive (TP) samples as positive, while SPE demonstrates its ability to detect true negative (TN) samples as negative. EFR is calculated as the difference between the total of results (100%) and the sum of FPR and FNR. Equations defining all of these FOM used to assess the quality of supervised classification methods are shown in Table 1.

Other important aspect related to the validation of the PLS-DA model is the uncertainty estimation of the predicted values. This estimation can be performed by resampling, employing the residual bootstrap method [35]. In spite of the importance of this aspect for the validation of qualitative models, the number of papers that have applied it for PLS-DA results is small [39–42]. Confidence intervals for y predicted variables are calculated resampling new data sets obtained from the original data by random perturbations. Unknown distributions of the parameters are obtained by mimicking the random resampling mechanism. The concept of pseudo-degrees of freedom [43], which considers the difference between mean square errors of calibration and cross validation, was employed in the calculations. The confidence interval estimates for each sample were obtained with 1000 replications.

Table 1
Qualitative figures of merit for PLS-DA model.

FOM	Equation	Training set	Test set
FPR	$\frac{FP}{TN+FP}$	8.1%	11.7%
FNR	$\frac{FN}{TP+FN}$	8.0%	8.5%
SEN	$\frac{TP}{TP+FN}$	92.0%	91.5%
SPE	$\frac{TN}{TN+FP}$	91.9%	88.3%
EFR	$\frac{TN+TP}{TN+FP+TP+FN}$	83.9%	79.8%

FN = number of false negatives; FN = number of false positives; TN = number of true negatives; TP = number of true positives.

3. Results and discussion

3.1. Raman spectra and the effect of meat adulterants

Raman spectroscopy allows to study modifications in the secondary protein structures, such as α-helix and β-sheets, as well as to provide information about the amino acid residues. The Raman spectrum of a sample of purge of bovine meat *in natura* (Fig. 2a) presents bands at various wavenumber regions related to different functional groups of amino acids, lipids and proteins. Some spectral bands can be assigned to the CONH group, such as the two NH stretching peaks in the region between 3500 and 2900 cm^{-1}, which were associated with amide A and amide B vibrations, respectively. The region between 1800 and 700 cm^{-1} provides information about the structure of proteins, such as α-helix and β-sheets structures. The main modes are amide I (1640–1690 cm^{-1}) and amide III (1230–1300 cm^{-1}). Amide *I* shows stretching vibrations of C═O, while amide III is characterized by coupled C–N stretching and N–H bending vibrations of peptide groups. Other important Raman signals can be observed in this region, particularly assigned to the aromatic moieties of amino acid residues, such as tryptophan at 880, 1345 and 1557 cm^{-1}, phenylalanine at 1003 and 1045 cm^{-1}, and tyrosine around 850 cm^{-1} [44,45].

The addition of salts to meat, such as NaCl and STPP, or polysaccharide gums, such as carrageenan, changes the conformation of proteins causing specific α-helix, β-sheet and β-turn vibrations that can be detected by Raman spectroscopy [45,46]. The effect of the addition of NaCl in the chicken batters has been studied by some authors [47]. The most important spectral bands related to changes in the secondary structure of proteins have been described as an intense band characteristic of the amide I vibration mode (centered near 1660 cm^{-1}) and other signals in the amide III region (1225–1350 cm^{-1}). The amide I band involves C═O stretching, C–N stretching, Cα-C-N bending and N–H in-plane bending from peptide groups. In the amide III region, the main bands involve C–N stretching and N–H in-plane bending vibrations of the peptide bonds. The characterization of changes in the protein tertiary structures has been related to the stretching vibrations of tryptophan residues ring (760 cm^{-1}) and double Raman bands assigned to the para-substituted benzene ring of tyrosine residues, which are centered at 830 and 850 cm^{-1} [45,47]. The addition of STPP to meat produces the same significant bands as sodium chloride, including additionally the characteristic band of P═O stretching at 1102 cm^{-1} [48].

Another meat adulterant evaluated in this research was carrageenan, a linear sulfated polysaccharide extracted from red seaweeds. The identification of carrageenan structures has been performed by FTIR and FT-Raman spectroscopies [49]. The most important Raman bands characterizing commercial kappa-carrageenan (used in our work) has been reported at 1075–1085 cm^{-1}, assigned to C–O bond of 3,6-anydrogalactose, at 1240–1260 cm^{-1}, related to S═O bond of sulfate

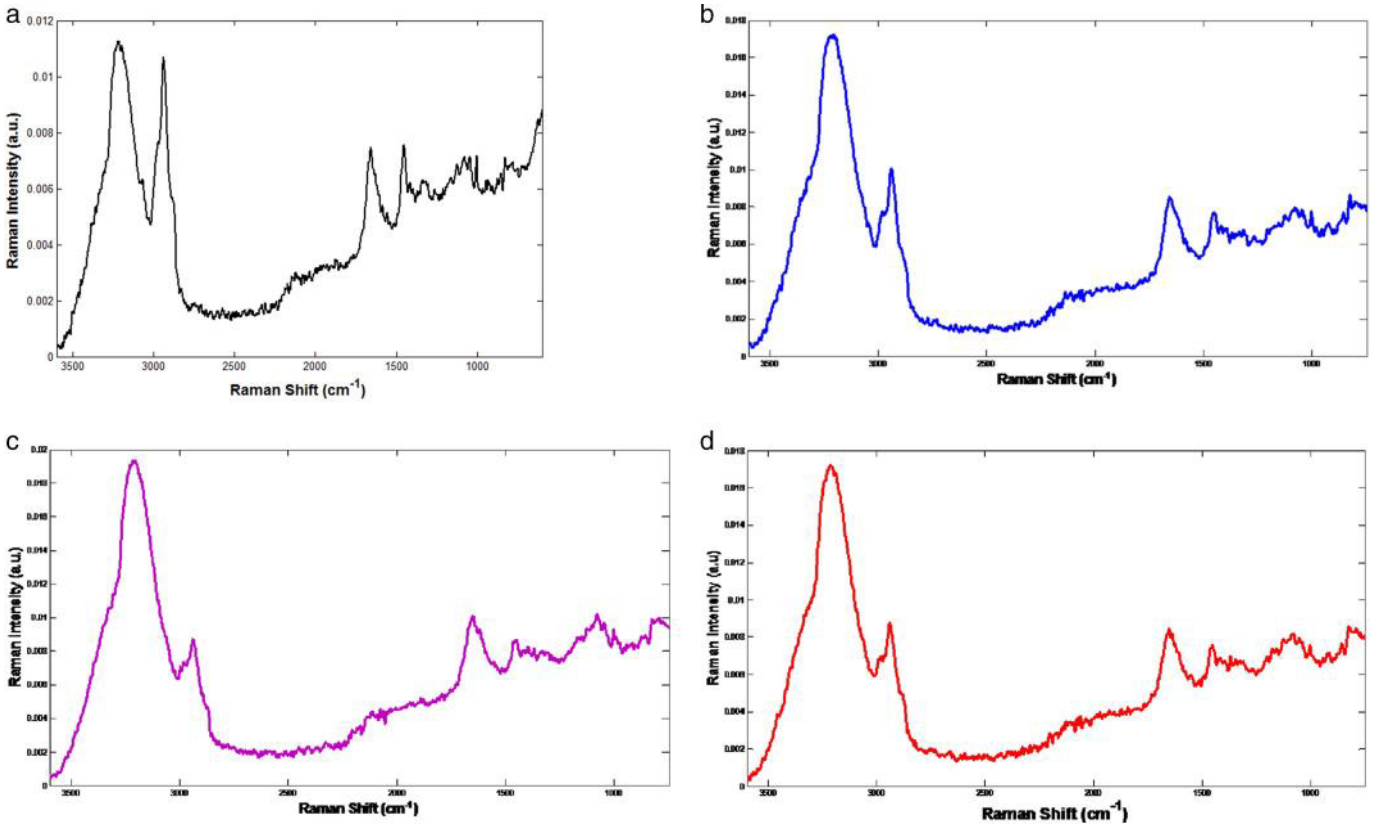

Fig. 2. Raman spectra of purges of (a) a sample of unadulterated bovine meat *in natura*, (b) a sample adulterated with NaCl 10%, (c) a sample adulterated with STPP 10%, and (d) a sample adulterated with carrageenan 10%.

esters, and at about 845 cm^{-1}, which can be assigned to D-galactose-4-sulfate. The combination of carrageenan and soy protein isolate has increased the gel strength and water retention of salt-soluble meat proteins. In this study, Raman spectroscopy was used for detecting the β-sheet structure as the base for protein aggregation and gelling [50]. Fig. 2b-d show Raman spectra of purges of samples adulterated at the level of 10% with NaCl, STPP and carrageenan, respectively. Visually, it is very difficult to note spectral differences between the adulterated and non-adulterated samples, with the exception of the band centered around 3200 cm^{-1}, which is more intense for the adulterated samples.

Since the addition of salts causes changes in the structures of meat proteins, Raman spectroscopy shows strong potential for providing noninvasive information for the detection of frauds in bovine meat *in natura* by non-meat ingredient additions (NaCl, phosphates, and carrageenan). Preliminary studies with ATR-FTIR [32] and Raman (not shown) spectra have indicated the unfeasibility of obtaining accurate discriminant models by analyzing directly the meat pieces with vibrational techniques, especially when the model included different meat cuts causing a matrix effect. Therefore, the alternative of building a chemometric model with the spectra of the purges was adopted, since purge analysis is commonly performed for meat quality control.

Raman spectra from all of the 165 samples used in this study are shown in Fig. 3. Spectral regions corresponding to the stretching of the CH bonds (3589–2736 cm^{-1}), and the bending vibrations of the CH_2 bonds and amides I and III (1868 to 712 cm^{-1}), have been identified as the most important ones for quality assessment of meats [45]. By observing Fig. 3, these same spectral regions can also be considered of higher potential for the construction of the classification model.

3.2. PLS-DA model

As it has been already mentioned (Section 2.3), the whole data set was divided into 112 samples for the training set and 53 samples for the test set. Previous outlier detection was carried out. Samples presenting spectral Q residuals or leverage values above the thresholds estimated at 95% confidence levels were detected as outliers and removed from the model. A total of eight samples, seven in the training set (3 control and 4 adulterated samples) and one in the test set (control) were removed from the model, corresponding to 4.8% of the original samples. Thus, the best PLS-DA model was built with 157 samples and using 4 LV, which accounted for 95.21% of the variance in the X-block and 60.50% in the Y-block. For this model, Y predicted values are shown in Fig. 4. The Bayesian threshold was estimated at 0.5923.

As can be seen in Fig. 4, three control samples were predicted as adulterated (false positives) and six adulterated samples were predicted as control (false negatives) in the training set. These results correspond to FPR of 8.1% and FNR of 8.0%. In the test set, two false positives (11.7%) and three false negatives (8.5%) were observed. As a consequence, SEN, the rate of true positives, was equal to 92.0% and 91.5% for training and test sets, respectively. Reciprocally, SPE, the rate of true negatives, was equal to 91.9% and 83.9% for training and test sets, respectively. Finally, EFR, a global FOM that encompasses both the FN and FP rates, was estimated as 86.6% and 79.8% for training and test sets, respectively. Since EFR includes the effects of both types of error, it is a FOM more appropriate for comparison with other classification methods published in the literature. All of these FOM are shown in Table 1, jointly with the respective equations used to estimate them.

Classification models built in this paper aimed at preliminary forensic discrimination. Thus, the developed screening method should primarily minimize false negatives, because false positive results can be circumvented by complementary analysis for confirmation with conventional reference methods, before a judicial decision be finally made [51]. Taking this into account, a FNR below 10% for the test set (8.5%) was considered acceptable for our goals.

For the spectral interpretation of the developed PLS-DA model, it is interesting to observe the VIP scores, which are contained in an important informative vector that is shown in Fig. 5. Variables with VIP scores higher than a threshold of 1.0 are considered to contribute significantly to the model. The three Raman peaks that most contributed for discrimination are marked in Fig. 5(a-c). The highest VIP scores are associated with a spectral band centered at 3200 cm^{-1}, assigned to the NH stretching of amides (Fig. 5 - a) [13], which could be related to changes in the conformation of proteins as a function of the addition of salts to meat. The second most intense peak of VIP scores, at 845 cm^{-1} (Fig. 5 - c), can be associated with the effect of carrageenan and the presence of D-galactose-4-sulfate residues [49,50], as previously mentioned (Section 3.1). The third most intense peak of VIP scores, centered around 2930 cm^{-1} (Fig. 5 - b), is assigned to a CH stretching and could be related to the meat quality [45]. By observing VIP scores, it can be realized that many variables/peaks presented values higher than 1.0, thus contributing significantly for detecting meat adulteration.

3.3. Attempts to build one-class models

Some authors have criticized the predominance of discriminant models, such as PLS-DA, applied to food authentication problems, advocating the preferential use of one-class modeling [52]. These authors have argued that in the detection of food adulteration, the acquisition of a sample set representative of all of the possible types of frauds is unfeasible, making the modeled adulterated class non-representative. Nevertheless, every multivariate model is a local model, and other authors defended the alternative of combining PLS-DA with outlier

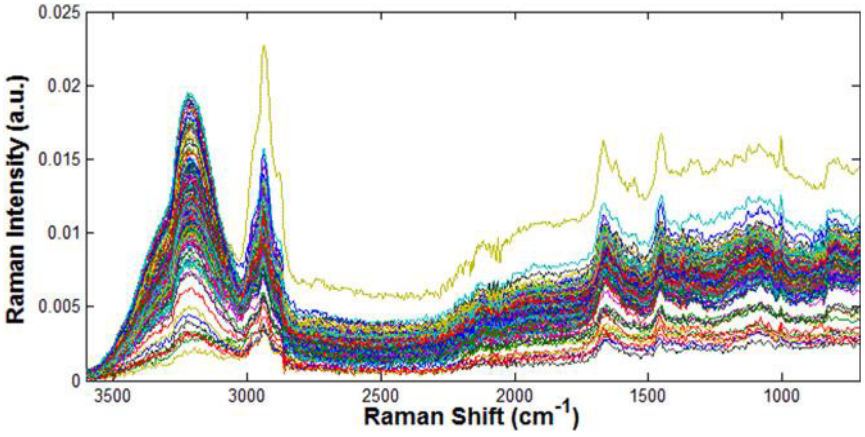

Fig. 3. Raman spectra of the 165 analyzed samples.

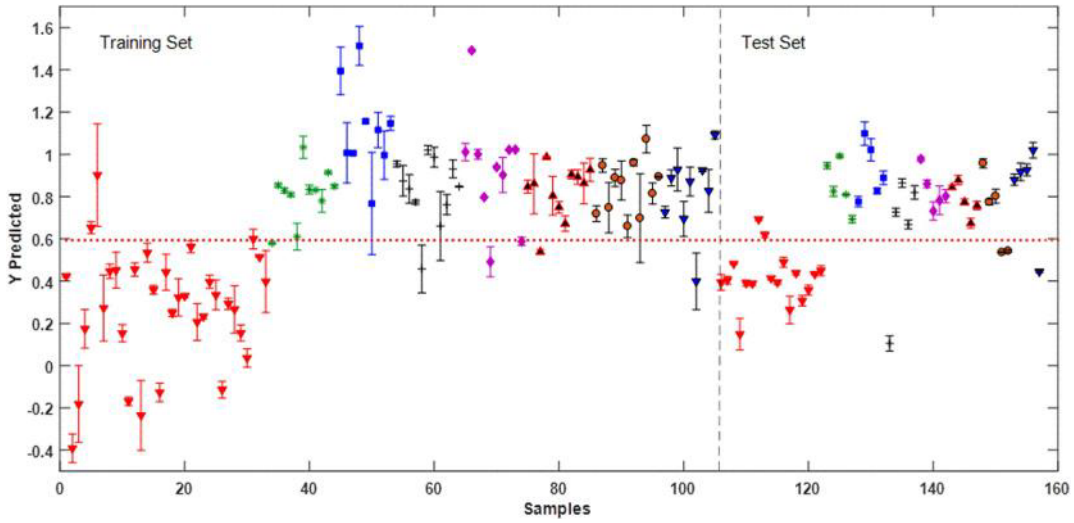

Fig. 4. Predicted Y values for the PLS-DA model. Horizontal dotted line indicates the classification threshold. Vertical dashed line divides training and test samples. Class symbols: control samples (red down triangles), samples adulterated with NaCl (green asterisks), with STPP (blue squares), with carrageenan (black crosses), with NaCl plus STPP (purple diamonds), with NaCl plus carrageenan (up triangles), with STPP plus carrageenan (circles) and with ternary mixtures of the three adulterants (blue down triangles).

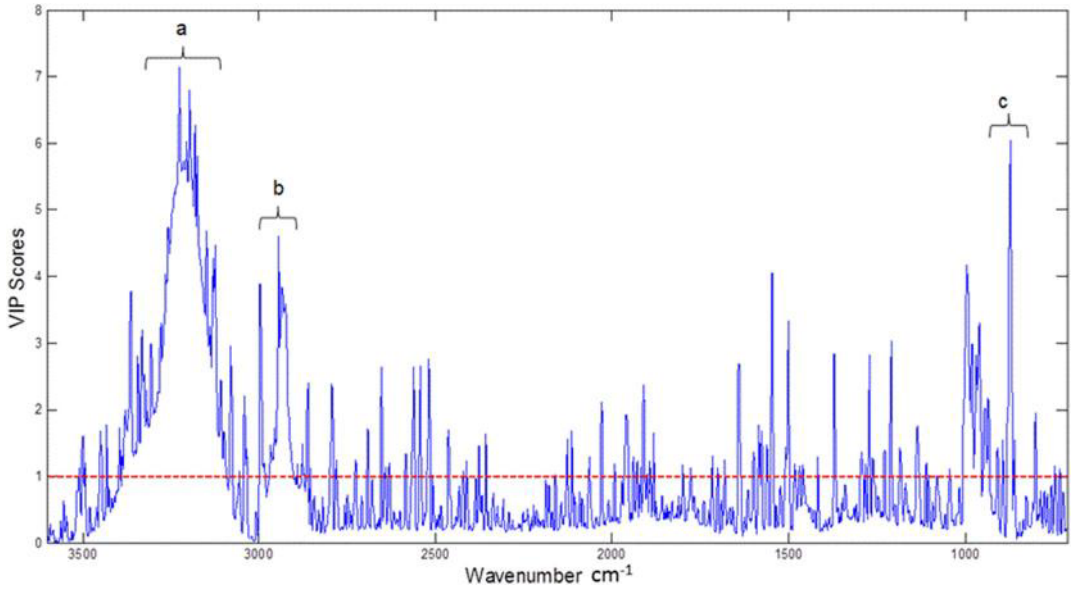

Fig. 5. VIP scores for the PLS-DA model. Horizontal dashed line indicates the threshold of 1.0. Arrows indicate the three most important Raman peaks (a, b and c) for the model.

detection [53]. In this way, future samples very different from the original ones (new types of frauds) will be detected as outliers and not assigned to any of the original classes. In this sense, class modeling problems can be defined in terms which closely resemble outlier detection: for each class as a function of method-specific criteria, it is verified whether a sample is fitted well by the respective class model (accepted) or not (reject) [54].

Data driven soft independent modeling of class analogy (DD-SIMCA) [55] and one-class PLS (OCPLS) [56] models were built for our data. The best models were obtained with 5 principal components for DD-SIMCA and 7 LV for OCPLS. These models presented similar results, with high SEN (around 94%), but poor SPE (below 40%). Therefore, they were considered unsatisfactory. Similar poor results were obtained by other authors in building one-class models to detect infested rice grains, what was justified due to the high heterogeneity of the samples [54]. In our case, original meat samples were obtained from different origins without control of the ante-mortem factors that can influence meat composition, such as breed, sex, age, feed intake and handling. Thus, it was not possible with these data to obtain one-class models with the same good performance as was provided by PLS-DA.

3.4. Bootstrap uncertainty estimates

In order to complete the analytical validation of the method, uncertainties were estimated for specific sample predictions by employing bootstrap resampling with 1000 replications. Error bars for each predicted value are shown in Fig. 4. Six samples in the training set presented confidence limits exceeding the threshold. Two of these samples had been wrongly predicted by the model (one false positive and one false negative), whereas the other four samples were true positives. For the test set, no sample presented a confidence interval that exceeded the threshold. Thus, inconclusive result rates could be estimated for the model as 3.8% for the training set and 0% for the test set.

4. Conclusions

This paper was based on a police report that happened a few years ago in Brazil, when some slaughterhouses were fined and processed for frauds in bovine meat *in natura* by the addition of non-meat ingredients, such as NaCl, sodium tripolyphosphate and carrageenan. The goal of this fraud was to increase the meat water holding capacity for obtaining economic fraudulent gains. Preliminary studies have indicated limitations for detecting this type of fraud directly in the solid meat by using vibrational techniques alone. A good discriminant model was only obtained with data fusion models merging vibrational spectra and physico-chemical variables [32]. Thus, the alternative of analyzing the purges of meat was adopted. A rapid, simple and low cost method was developed and validated based on a multivariate discriminant PLS-DA model built from Raman spectra of purge samples. A robust model was constructed incorporating variability from meat pieces of the same cut (eye of the round) obtained from different origins and adulterated by the injection of the investigated adulterants according to an experimental design. The developed method detected samples injected with single, binary and ternary mixtures of adulterants, providing false negative and false positive rates varying from 8 to 12%. As an additional validation step, uncertainty confidence intervals were determinated for each sample prediction provided by PLS-DA using bootstrap resampling. This type of screening method has shown a great potential to be applied in real situations to detect meat frauds and adulterations. Regulatory agencies and criminal/forensic investigators can use this methodology for obtaining fast results and improving their analytical capacity.

Conflict of interest

All of the authors declare no conflicts of interest.

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