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Surface-enhanced Raman spectroscopy for classification of testosterone propionate and nandrolone residues in chicken

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ABSTRACT

There is a critical need for a rapid and simple screening method of androgens in chicken. In this study, we evaluated surface-enhanced Raman spectroscopy (SERS) coupled with multivariate techniques for the classification of two androgens (i.e., testosterone propionate and nandrolone) in chicken from 294 samples. Raw Raman spectra were pretreated by using the methods of baseline correction, normalization and second derivative. Support vector machines (SVM) model using the score values of the first four principal components as the inputs was developed to classify all the chicken samples into the four groups (i.e., control, nandrolone, testosterone propionate, and testosterone propionate combined with nandrolone groups) with accuracy of 96.9%. Furthermore, the particle swarm optimization (PSO) was adopted to automatically optimize the penalty parameter C and the kernel parameter g of SVM model for improving the classification accuracy. The experimental results demonstrated that SERS, in combination with multivariate methods, could be utilized as a rapid and simple classification assay of androgens in chicken and exhibited great potential in practical applications as a screening tool to better serve customers.

1. Introduction

Androgens, such as testosterone propionate and nandrolone, are extensively used as growth promoting agents in food production [1,2]. These androgens can exist in different tissues of animals including poultry and pork, and then enter human body through the food chain. The possibility of adverse effects on human consumers of androgen residues has aroused the people's attentions. Many countries and regions, such as the European Union and China, have prohibited or restricted the use of androgens for growth promotion in food-producing animals [2]. Liquid chromatography-tandem mass spectrometry (LC–MS) [3–5] and gas chromatography-mass spectrometry (GC–MS) [2,6–8] are highly useful for the detection of androgen in animal tissues, but these assays are too laborious and time-consuming to be used as rapid and on-site screening approaches. Therefore, a rapid and efficient detection approach is required to determine whether there are androgens in animal tissues or not.

Both testosterone propionate and nandrolone belong to androgens. The presence of these two androgens in chicken, which is the largest poultry consumer goods in China, has potential negative consequences for consumers, since it has been shown that the residues of these two androgens in human body can be found following consumption of chicken.

Surface-enhanced Raman spectroscopy (SERS) is an extension of standard Raman spectroscopy with an extremely weak optical effect that can amplify Raman intensities by a factor of 10^4-10^6 to enhance the throughput and sensitivity of the signal [9]. In the past decades, SERS technology has been attracted much attentions and intensively applied in chemistry and food science etc. owing to its sensitivity and specificity attributes [10,11]. Until today, there have been only a few reports of the use of UV resonance Raman spectroscopy for analysis of nandrolone [12,13]. So, it is an extremely meaningful work to investigate the SERS screening approaches of testosterone propionate and nandrolone residues in chicken.

Herein, we demonstrated the potential of using SERS to rapidly classify testosterone propionate and nandrolone in chicken. Support vector machines (SVM) is a new generation of classification method based on statistical learning theory and has a good discrimination power owing to its nonlinear characteristics [14,15]. To achieve this objective, we developed SVM model to classify all the chicken samples

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into the four groups. Additionally, principal component analysis (PCA) was used to extract key features as the inputs of SVM model.

2. Experimental

2.1. Materials and reagents

Chicken was purchased from the vegetable market of Jiangxi Agricultural University. Testosterone propionate (99.0%), trisodium citrate, magnesium sulfate, acetonitrile, methanol and hexane were obtained from Nanchang Precision Scientific Instruments Co., Ltd., China. Nandrolone (98.1%) was bought from Standard Substances Network of China. Tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$) with gold content of not less than 49% was purchased from Sigma-Aldrich.

2.2. Synthesis of gold nanoparticle colloids

The gold nanoparticle colloids were synthesized by a typical trisodium citrate reduction method with slight modification [16,17]. In brief, 0.9 mL of 1% trisodium citrate was added into 100 mL of boiling 0.01% chloroauric acid aqueous solution. The whole mixture was stirred during the addition of trisodium citrate. Next, the synthesized gold nanoparticle colloids were cooled to room temperature after the whole mixture was boiled for 9 min with continuously stirring. Before use, the colloids were transferred into a brown bottle with stopper and kept at room temperature.

2.3. Preparation of standard solutions

Approximately 10.0 mg of testosterone propionate was dissolved in 100 mL of acetonitrile to obtain 100 mg/L testosterone propionate standard solution. 100 mg/L nandrolone standard solution was obtained by dissolving about 10.0 mg of nandrolone in 100 mL of acetonitrile.

2.4. Sample preparation

Chicken breasts were mashed with tissue disintegrator (JJ-2B, Jintan Jinnan Instrument Factory, China) after the membranes were removed from chicken breasts. Subsequently, testosterone propionate and nandrolone were extracted from chicken breasts based upon a LC−MS/MS method with slight modification [4,5,18,19]. Briefly, about 5 *g* of chicken breasts were spiked with 2 mg of testosterone propionate or nandrolone with 20 mL of acetonitrile, and the spiked samples were vigorously whirled (VOTRER-5 whirlpool mixer, Haimen Kylin-Bell Lab Instrument Co., Ltd., China) for 1 min, oscillated ultrasonically (JK-50B ultrasonic cleaner, Hefei Jinnike Machinery Co., Ltd., China) for 10 min and centrifuged (JW-1024 low speed centrifuge, Anhui Jiawen Instrument Equipment Industry Co., Ltd., China) for 10 min (4200 r/min). Next, the residue was extracted with 20 mL of acetonitrile again, and then the above-mentioned supernatants were merged together. Subsequently, the supernatant was evaporated to about 10 mL with nitrogen at 60°C (HSC-24B nitrogen evaporation, Tianjin Heng Ao Technology Development Co., Ltd., China), and centrifuged for 10 min (4200 r/min) after it was vigorously whirled with 2 *g* of magnesium sulfate for 1 min. After the obtained supernatant was filtered using 0.45 μm filter membrane, 10 mL of hexane was added in the above solution to whirl for 1 min, oscillate ultrasonically for 10 min and centrifuge for 10 min (4200 r/min). Lastly, the acetonitrile phase was evaporated to nearly dry at 30°C and fixed the volume to 20 mL with methanol. The above-mentioned solution was diluted to different levels of testosterone propionate and nandrolone by using chicken extract without testosterone propionate and nandrolone. Chicken extract without testosterone propionate and nandrolone were obtained in the similar steps of the above-mentioned method by using chicken breasts spiked without testosterone propionate and nandrolone. All the chicken extract samples were divided into four groups, i.e., chicken extract samples without testosterone propionate and nandrolone (control group), chicken extract samples containing 0.5 to 25 mg/L testosterone propionate (testosterone propionate group), chicken extract samples containing 0.1 to 25 mg/L nandrolone (nandrolone group) and chicken extract samples containing 0.5 to 25 mg/L testosterone propionate and 0.5 to 25 mg/L nandrolone (testosterone propionate combined with nandrolone group).

2.5. Spectral measurement

SERS measurements were performed on a portable Raman system (Ocean Optics Co., Inc., USA). This system consists of a QE65 Pro Raman spectrometer, a diode laser with 785 nm excitation wavelength, a Raman coupled fiber probe for 785 nm with SMA-SMA connectors, a Raman sample holder and a computer. The laser power was fixed at 650 mW with an integration time of 10 s.

Approximately 500 μL of gold nanoparticle colloid, 10 μL of the analyzed sample solution and 100 μL of magnesium sulfate solution (0.1 mol/L) were added into 2 mL quartz sample vials. The SERS spectra were measured to establish the analysis model after mixing well. One spectrum was collected for each sample. Raman spectra of a total of 294 chicken extract samples were measured, including 102 samples of control group, 47 samples of testosterone propionate group, 43 samples of nandrolone group and 102 samples of testosterone propionate combined with nandrolone group.

The ultraviolet-visible (UV–vis) spectra of gold nanoparticle colloid and magnesium sulfate-aggregated gold nanoparticles were measured in the spectral range of 400 ~ 900 nm with a UV–vis spectrometer (T6, Beijing Purkinje General Instrument Co., Ltd., China) using quartz cuvettes of 1 cm light path.

2.6. Data analysis

Raw Raman spectra were first baseline corrected to reduce the baseline variability at the region between 400 and 1800 cm^{-1}, and normalized against the maximum Raman peak (i.e., the intensity of maximum Raman peak was set to 1) to remove laser power fluctuation and the interference of background noises [13,14]. Next, the second derivatives of the Raman peak intensities versus wave numbers were performed to remove the additive effects from Raman spectra, such as overlap of Raman peaks and baseline offsets [20]. These processes were processed by The Unscrambler 9.7 (CAMO Software AS, Norway) software. Subsequently, spectral classification was accomplished by SVM with a radial basis function (RBF) kernel in MATLAB environment (R2010b, The Mathworks Inc., Natick, MA). Two-thirds of samples (n = 196), which were chose from all the samples on a random basis, were used to establish the prediction model and the rest (n = 98) were used to test the model performance. Accuracy and sensitivity and specificity [21] were calculated to evaluate the model performance. The particle swarm optimization (PSO) was adopted to automatically optimize the penalty parameter C and the kernel parameter g of SVM model for improving the classification accuracy. Before performing the SVM-based classification, PCA was used to extract key features and reduce dimensionality [20] using The Unscrambler 9.7 software. The classification was carried out on the score values of the first four principal components (PCs).

3. Results and discussion

3.1. SERS spectra characteristics of samples

The surface plasmon resonance peaks of gold nanoparticle colloid and magnesium sulfate-aggregated gold nanoparticles were showed in Fig. 1. The absorption band of gold nanoparticle colloid corresponded to the maximum of the surface plasmon resonance which was around