

## PREDICTING AUTHENTICITY AND PHYSICOCHEMICAL CHARACTERISTICS OF MEAT THROUGH FT-IR SPECTROSCOPY COUPLED WITH MULTIVARIATE ANALYSIS

Muneeb Khan<sup>1</sup>, Muhammad Issa Khan<sup>1,\*</sup>, Amna Sahar<sup>1</sup> and Amer Jamil<sup>2</sup>

<sup>1</sup>National Institute of Food Science and Technology, Faculty of Food Nutrition and Home Sciences, University of Agriculture, Faisalabad, Pakistan; <sup>2</sup>Department of Biochemistry, University of Agriculture Faisalabad, Pakistan

\*Corresponding author's e-mail: drkhan@uaf.edu.pk

Major issues related to meat in our modern society are its authenticity and traceability. Meat consumers are facing problems related to addition of low value meat to high quality meat. The current study was designed to explore the potential of analytical method for the rapid detection and identification of meat. Meat of four species (beef, pork, chicken and turkey) were collected for the chemical (moisture content, ash content, crude fat and crude protein), quality (pH, color and drip loss) and spectral analysis through Fourier transform infrared spectroscopy (FT-IR). Pork meat showed highest crude protein (22.20%) and crude fat contents (5.85%). Highest pH (6.14) and drip loss (1.84%) was seen in turkey meat and pork meat, respectively. Spectral data collected from FT-IR spectroscopy was analyzed through principle component analysis and partial least square regression model. The FT-IR spectra obtained after absorption in infrared region explained that all the meat samples were different based on their OH, CH and CH<sub>2</sub> stretching in bonds. The near infrared region around 2300-1400 cm<sup>-1</sup> had maximum spectral information required for the discriminant investigations based on the pigments contained in different species of meat to the physicochemical characteristics (moisture, intramuscular fats and fatty acids). Coefficients of determination for calibration (R<sup>2</sup>C) and validation (R<sup>2</sup>V), root mean square errors of calibration (RMSEC) and root mean square error of prediction (RMSEP) for moisture, crude protein, crude fat, ash, pH, drip loss and color L\*, a\*, b\* of different meat samples were measured. The color value b\* developed strong prediction with R<sup>2</sup>C = 0.941 and R<sup>2</sup>V = 0.872.

**Keywords:** FT-IR spectroscopy, meat authenticity, meat species identification, meat adulteration.

### INTRODUCTION

Meat contains high biological value proteins and micronutrients (e.g. vitamins A, D, E, B<sub>6</sub>, B<sub>12</sub>, selenium, zinc, iron). Animal sourced protein are of high quality as it contains all essential amino acids required for the maintenance, growth and biological functioning in human body (Williamson and Manach, 2005). Amino acids are the basic building blocks of proteins and their composition varies widely in different sources of proteins. Therefore, eating meat gives particular benefit in the body where muscle tissues are built (Lombardi-Boccia *et al.*, 2005). Meat consumption in daily diet improves exercise performance by strengthening muscle functioning. Essential dietary amino acids (carnosine; dipeptide of  $\beta$ -alanine and histidine) are present only in meat and fish. These dipeptides are primarily responsible for the improved immune responses and help in reducing muscle fatigue (Sale *et al.*, 2010; Derave *et al.*, 2019). Conjugated linoleic acid (CLA), which is mainly found in lamb and beef has health benefits in weight loss but overdose of CLA leads to injurious metabolic disorders (Benjamin and Spener, 2009). The demand for meat and its products is increasing at rapid rate due to increased population (Delgado, 2003). It is one of the most desirable foods sold at higher rates specifically in developed countries.

The quality, safety and price along with nutritional properties of fresh and processed meat products must be assured before it is introduced in the market (Taljaard *et al.*, 2006).

The issues related to meat authenticity and its adulteration in meat processing industries is getting more attention. The problem in authentication concerned with substitution of high value raw material with low quality cheaper materials (Nakyinsige *et al.*, 2012). The consumption of pork and non-Halal animal meat is prohibited in Islam for religious reasons. Therefore, analytical and molecular methods are focused on the identification of meat species in raw, processed and cooked meat products (Ali *et al.*, 2012). In order to detect adulteration in various meat products, molecular (PCR) and spectroscopic techniques (FT-IR, fluorescence spectroscopy, etc.) have shown their potential for assessing authenticity. Several studies have proved that these methods as reliable, comprehensive as well as efficient when compared with traditional methods of finding the meat authenticity. FT-IR spectroscopy when used in combination with multivariate analysis is a good option in order to identify the quality of meat along with its specie authentication in a most reliable way. Moreover, Fourier transform infrared spectroscopy (FT-IR) is a well-known spectroscopic technique in terms of detection and identification of different species of meat and

its product (Karthek *et al.*, 2011). Research findings are primarily based on the chemical composition of food commodities. In the current study meat chemical composition and quality were assessed and the results of these traditional methods were cross verified with spectral data in statistical analysis (principle component analysis and partial least square regression) to assure authenticity and detect adulteration.

## MATERIALS AND METHODS

**Procurement of raw materials:** Meat samples from leg portion (40 samples, each muscle weighed about 200 g) of beef, pork, chicken and turkey were purchased from the local market in Columbus, Ohio (IRSIP period). The samples were immediately transferred to Meat Science Laboratory in packed form. Samples were aerobically enclosed into polyethylene pouches. Each pouch contains two samples, one for chemical analysis and one for spectroscopic and were stored at  $-20 \pm 2^\circ\text{C}$ . Chemicals, reagents and solutions were purchased from registered suppliers of Merck and Sigma.

**Chemical analysis:** Meat samples were analyzed for chemical compositional analysis according to the method described in AOAC (2006). The moisture content was estimated by hot air oven at  $105 \pm 2^\circ\text{C}$ . Ash content was measured by charring on flame and complete burning in furnace at  $550 \pm 5^\circ\text{C}$  to greyish end point. Percent nitrogen was determined by Kjeldhal method in three stages consisting of digestion with acid, distillation with base and boric acid and titration with acid neutralization. Crude fat was measured by extraction with n-Hexane in Soxhlet apparatus.

### Quality analysis of raw meat

**pH measurement:** The meat pH was calculated by using digital pH meter (Hanna instruments) by following the method described by Hossain *et al.* (2012). Before taking pH measurements the instrument was calibrated with buffers of pH-7 and pH-4. Samples were prepared by homogenizing 1 g of fresh meat with 9 mL of distilled water at 12000rpm. The probe of pH meter was directly immersed into the homogenized meat solution and sample readings were taken in triplicate at room temperature ( $20 \pm 2^\circ\text{C}$ ).

**Drip loss measurement:** Drip loss of raw meat samples was calculated by following the method illustrated by Honikel (1998). The meat samples ( $2.5\text{ cm} \times 2.5\text{ cm} \times 12\text{ cm}$ ) were weighed and hanged in refrigerator. After 24 h the weight loss during storage was calculated in percentages.

**Color analysis:** Meat samples were placed in refrigerator at  $4 \pm 1^\circ\text{C}$  for 30 min. in order to take color values. The oxygenation of myoglobin was facilitated on the surface of meat. The color values were measured by Hunter Lab Mini Scan XE Plus apparatus with CIELAB  $L^* a^* b^*$  scale (Commission Internationale de l'Eclairage (CIE), 1976). The instrument was standardized by reference black and white color standards.

**Spectroscopic analysis through FT-IR:** The spectroscopic data were generated by following the method described by Rahman *et al.* (2018). The prepared meat samples were analyzed for species identification by taking spectra in multiple scans through infrared spectroscopy (Bruker Tensor 27). The samples were scanned in the range between  $4000\text{--}650\text{ cm}^{-1}$  wave number with the resolution  $4\text{ cm}^{-1}$ . The instrument was equipped with dehumidifier (silica gel) to reduce interference by water vapors. The background air spectrum was removed from obtained spectrum before every scan and reference air spectrum was taken. Before filling in next sample the surface was dried with soft tissue to protect scratching of lens. Lens purity was tested every time by taking background spectrum before sample loading on ATR lens. Averaged spectra of ten scans were taken as absorbance values in triplicate in each data set.

**Spectral data processing and PLSR model:** The Unscrambler X 10.5 software was used for principle component analysis (PCA) and chemometrics analysis of partial least square regression (PLSR). Calibration + validation model was developed for the estimation of best model results and it was established by executing cross-validation as "leave one out" method (Geladi *et al.*, 1985). The values obtained for root mean square errors of calibration (RMSEC) and coefficient of determination ( $R^2$ ) were implemented as the rationality criteria for the calibration. In addition, to eliminate the undesirable X data variations (scattering effect) the dataset was subjected to pre-processing in standard normal variate (SNV) correction. Unscrambler X 10.5 was used for PCA analysis followed by PLS regression model.

## RESULTS AND DISCUSSION

**Chemical analysis:** The mean values for chemical composition of four species meat (beef, pork, chicken and turkey) are given in Table 1. It is evident from the results that crude protein and crude fat percentages had significant variations while moisture and ash content showed non-significant variation. The highest crude protein value (22.20%) was found in pork meat whereas turkey meat had lowest crude protein contents (19.12%). Similar findings for crude protein were observed by Dawood and Alkanhal (1995) who studied the effect of physicochemical characteristics of meat during processing and storage on meat of animal species. The highest value for crude fat (5.85%) was obtained in pork meat whereas lowest crude fat (2.37%) was recorded in chicken meat. It is evident from the findings that moisture makes the major component of meat, but it had non-significant variations among species meat. Turkey meat had highest moisture content (75.78%) while beef had lowest moisture (72.68%). The finding of the present study is in line with the work of Okrouhla *et al.* (2009) who documented 73.07% moisture content in pork which are quite closer to

**Table 1. Mean values for chemical analysis of species meat (beef, pork, chicken and turkey).**

| Animal Species | Moisture (%) | Crude Protein (%)         | Crude Fat (%)           | Ash (%)   |
|----------------|--------------|---------------------------|-------------------------|-----------|
| Beef           | 72.68±1.79   | 22.17 <sup>ab</sup> ±0.37 | 4.85 <sup>b</sup> ±0.73 | 1.07±0.06 |
| Pork           | 74.19±1.19   | 22.20 <sup>a</sup> ±0.60  | 5.85 <sup>a</sup> ±0.77 | 1.07±0.14 |
| Chicken        | 74.38±0.75   | 21.83 <sup>b</sup> ±0.86  | 2.37 <sup>d</sup> ±0.20 | 1.19±0.13 |
| Turkey         | 75.78±0.84   | 19.84 <sup>c</sup> ±0.52  | 4.24 <sup>c</sup> ±0.37 | 0.98±0.14 |
| SEM            | 0.7274       | 0.3963                    | 0.3584                  | 0.0664    |

**Table 2. Mean values for quality analysis of species meat (beef, pork, chicken and turkey).**

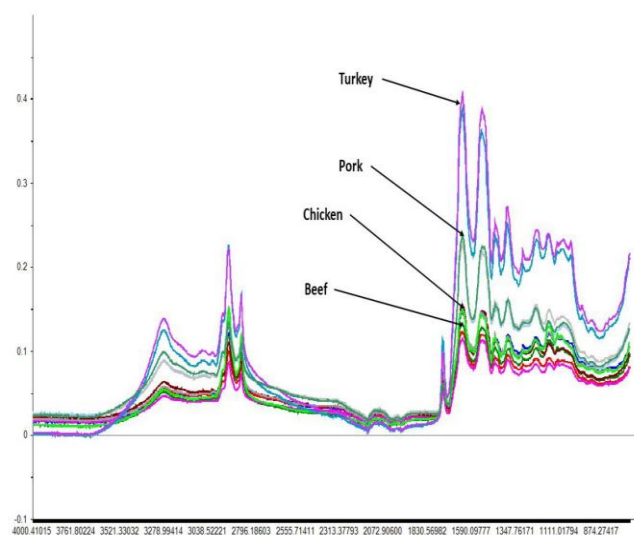
| Animal Species | pH                       | drip loss                | color L*                 | color a*                 | color b*                |
|----------------|--------------------------|--------------------------|--------------------------|--------------------------|-------------------------|
| Beef           | 5.53 <sup>c</sup> ±0.14  | 1.53 <sup>b</sup> ±0.11  | 37.56 <sup>c</sup> ±0.91 | 17.69 <sup>a</sup> ±0.46 | 6.09 <sup>c</sup> ±0.79 |
| Pork           | 5.64 <sup>bc</sup> ±0.05 | 1.84 <sup>a</sup> ±0.07  | 34.76 <sup>d</sup> ±0.46 | 12.28 <sup>c</sup> ±0.74 | 4.20 <sup>d</sup> ±0.46 |
| Chicken        | 5.87 <sup>b</sup> ±0.13  | 1.18 <sup>c</sup> ±0.22  | 39.60 <sup>b</sup> ±1.94 | 16.04 <sup>b</sup> ±0.91 | 8.12 <sup>a</sup> ±0.95 |
| Turkey         | 6.14 <sup>a</sup> ±0.19  | 1.72 <sup>ab</sup> ±0.24 | 41.48 <sup>a</sup> ±0.95 | 11.99 <sup>c</sup> ±0.45 | 7.10 <sup>b</sup> ±0.61 |
| SEM            | 0.0606                   | 0.0493                   | 0.6705                   | 0.4764                   | 0.4664                  |

current findings. A non-significant variation exists for ash content in meat of all four species.

**Quality analysis (pH, drip loss and color):** Meat pH and color has developed a strong relationship to the quality of red and white meat. The dark, firm, dry (DFD) and pale, soft, exudative (PSE) has direct link with the water holding capacity and drip loss. The meats with the lower drip losses at refrigeration temperature has higher water holding capacity and it has direct effect on the improved cooking yield (Wang *et al.*, 2019). Mean values of pH, drip loss and color for the species meat have been presented in Table 2. Quality parameters (pH, drip loss, color L\*, a\*, b\*) significantly varied among the meat samples. The pH values were ranging between 5.53-6.14 in which turkey meat had higher pH (6.14). The current findings for pH are supported by the results of Sales and Mellett (1996) and Hoffman *et al.* (2008) who reported significant differences in muscle pH of different origin meat. Pork meat exhibited the highest drip loss (1.84 %) which showed its less water holding capacity with PSE type of meat, whereas chicken meat showed lowest value (1.18 %) for drip loss among the four species meat. Drip losses of all meat samples observed in present study (Table 2) are comparable to study conducted by Hong *et al.* (2005) who found drip losses in the range between 1.28-4.28% at different thawing levels in white meats. The color values for the meat lightness, redness and yellowish (L\*, a\* and b\*) of meat samples has been presented in Table 2. It is obvious from the lightness (L\*) values that among different species meat it varied significantly. However, compared to beef, pork and chicken meat, the turkey meat exhibited higher intensity of lightness. Although the current values are in accordance to the findings of Hoffman *et al.* (2008) who studied 10 different muscles of their subspecies. The a\* values of meat samples were ranging from 17.69-11.99 showing a significant relation. The highest (17.69) and lowest (11.99) a\* values were found in beef and turkey meat, respectively. The results found in present study for the lightness are in line with the findings of

Morris *et al.* (1995) who observed significant difference in lightness values of animal muscle.

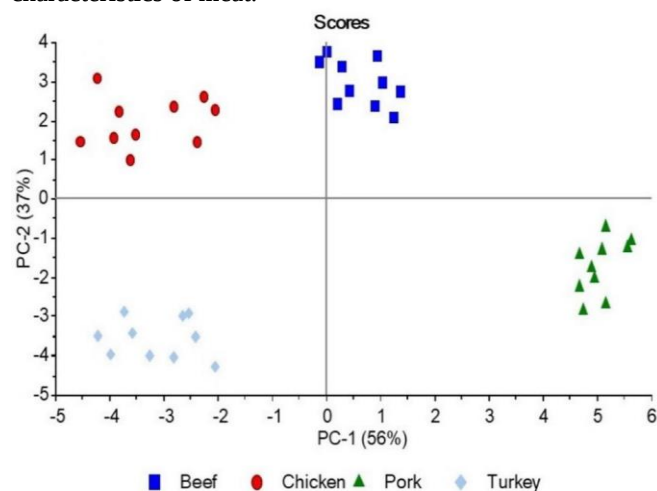
**Prediction of instrumental characteristics from Fourier transform infrared spectroscopy (FT-IR):** Animal species meat identification is important in establishing meat standards for the prevention of adulteration and authentication issues in meat industries. Spectroscopy along with chemometrics analysis has been proven an optimistic approach for the detection of undeclared meat species and fraudulent products in market. FT-IR spectra obtained from different species meat (beef, pork, chicken and turkey) is presented in Fig. 1.

**Figure 1. FT-IR spectra of beef, pork, chicken and turkey illustrating different peak heights at 4000-650 cm<sup>-1</sup>.**

It is evident from figure that meat possesses dominant aqueous contents in the range from 4000 cm<sup>-1</sup> to 650 cm<sup>-1</sup> (Ripoche and Guillard, 2001). Absorption bands in infrared region were seen at 1150 nm, 1460 nm and 2910 nm

(wavelengths) that are mainly associated to the presence of water content related to the third, second and first OH stretch implication (Sahar and Dufour, 2014). Water constitutes the major portion of fresh meat samples ranging between 70-80%. Absorption bands of CH bonding in second overtone were seen around  $1350\text{cm}^{-1}$  while, absorption of  $\text{CH}_2$  stretching was observed at  $1783\text{cm}^{-1}$  which represent the presence of fat and fatty acids. Moreover, at  $2310\text{cm}^{-1}$  saturated and unsaturated fatty acids with the CH combination were identified. It is obvious from the spectra obtained after absorption in infrared region that all the meat samples differentiated based on their OH, CH and  $\text{CH}_2$  stretching in bonds (Boubellouta and Dufour, 2012). In the near infrared region around  $2300\text{--}1400\text{cm}^{-1}$  (Fig. 1) had maximum spectral information required for the discriminant investigations based on the pigments contained in different animal and bird species to the physicochemical characteristics (moisture, intramuscular fats and fatty acids). These results are in line with the investigations of several other researchers (Downey, 1999; Alomar *et al.*, 2003).

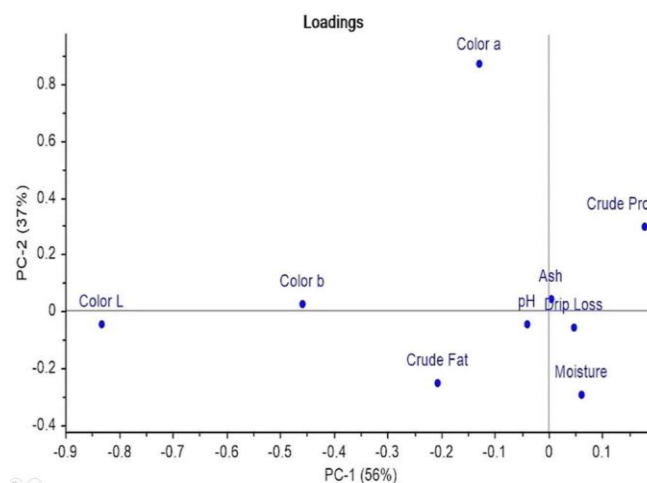
Principle component analysis was performed on spectral data obtained from beef, pork, chicken and turkey meat to be differentiated into groups. The results regarding PCA are presented in Figure 2 showing the PC1 and PC2 comparisons. All the PCs loadings presented comparable spectrum in PC1 and PC2 in which PC1 describes 56% and PC2 describes 37% of the overall variance in samples. Sample loadings were performed to filter the variations based on principle components (PC1, PC2,) in Fig. 2. PCA score plots of physicochemical characteristics on pure species meat appeared in distinct groups based on differences in muscle characteristics of meat.



**Figure 2. PC1 and PC2 score plots for beef, pork, chicken and turkey obtained after PCA applied on FT-IR spectra with SNV correction.**

It is obvious from the PCA score plots that color values developed strong relation with species meat differentiation.

These muscle characteristics based on the moisture content, meat pigments, lipids, fats, pH, drip loss and fatty acids. Among the pure meat species, turkey and pork meat exhibit major distinction from other meat based on bands in moisture level and fatty acids appearing in different groups. The score plots related to the meat products showing grouping in separate region due to vibrational bonds of their composition. The uppermost loadings on PC1 were observed at  $1980\text{cm}^{-1}$  and  $980\text{cm}^{-1}$  related to the water content and around at  $1800\text{--}1500\text{cm}^{-1}$  and  $2304\text{cm}^{-1}$  with intramuscular fats and fatty acids. Whereas, meat pigments showed the peak loadings at  $650\text{cm}^{-1}$ . All the PCs loadings showed more negative dependence but strong variance towards species identification as it can be seen in Fig. 3. These sample loadings of individual meat spectra are highly variable showing that each pure meat species spectra have distinction in moisture level, intramuscular fat, fatty acids and meat color. Several scientists have reported the absorbance bands between  $1700\text{--}1900\text{cm}^{-1}$  related to the fats type in muscular samples (Rahmania and Rohman, 2015). This spectral information arising from the absorption bands in different infrared regions were found to identify pure meat species by most authentic discriminate model using statistical software (Ding and Xu, 1999; Alexandrakakis *et al.*, 2012).



**Figure 3. PC1 and PC2 for traditional chemical and quality parameters (crude fat, crude protein, moisture content, ash content, pH, drip loss and color) for PLSR model of meat obtained from beef, pork, chicken and turkey.**

The spectral data obtained from all the meat samples of four species (beef, pork, chicken and turkey meat) were subjected under PLSR (partial least square regression) model for the species meat classification. However, these four species meats could be identified based on their structural and chemical composition of their major constituents. In the PLSR model building nine components of chemical and quality parameters were analyzed. The values for root mean

**Table 3. Performance of PLSR model validation by dividing data into calibration dataset and test samples dataset for prediction of moisture, crude fat, crude protein, ash, pH, drip loss and color L\* a\* b\* of meat obtained from beef, pork, chicken and Turkey.**

| Parameter        | Calibration |           | Prediction |           |
|------------------|-------------|-----------|------------|-----------|
|                  | $R_c^2$     | RMSEC (%) | $R_p^2$    | RMSEP (%) |
| Moisture content | 0.642       | 0.724     | 0.647      | 0.644     |
| Crude fat        | 0.749       | 0.866     | 0.721      | 0.753     |
| Crude protein    | 0.565       | 0.635     | 0.513      | 0.561     |
| Ash content      | 0.465       | 1.248     | 0.460      | 0.875     |
| pH               | 0.674       | 0.824     | 0.651      | 0.961     |
| Drip loss        | 0.729       | 1.574     | 0.762      | 1.134     |
| Color L*         | 0.811       | 2.119     | 0.919      | 2.431     |
| Color a*         | 0.745       | 1.265     | 0.977      | 1.032     |
| Color b*         | 0.941       | 2.017     | 0.872      | 1.854     |

square error of calibration (RMSEC) and root mean square error of prediction (RMSEP) were found high for color b\* (Table 3). However, PLSR model showed less prediction with the eight out of nine components (moisture, crude protein, crude fat, ash, pH, drip loss, color a\* and color L\*). This less variation may cause by less variant non-linear prediction model. Although, spectral data confined to many linear variants like color b\* that has developed high variant prediction model. However, obtained coefficient of determination in Table 3 for calibration ( $R^2C$ ) and validation ( $R^2V$ ), RMSEC and RMSEP for moisture, crude protein, crude fat, ash, pH, drip loss, color a\* and color L\* were very low as compared to color b\* ( $R^2C=0.941$ ,  $R^2V=0.872$ , RMSEC=2.017, RMSEP=1.854). Quite a lot number of pre-processing trials has presented excellent performance of the PLSR model for the given parameter of color b\* for the all species meat. Rinnan *et al.* (2009) discussed about the second derivative transformation resulting in the high differences between  $R^2C$  and  $R^2V$  values that indicates the increased noise level, hence, it should be avoided. Similarly, tenderness was predicted with linear regression model showed very poor results with simplistic components as compared to the combination of complex factors (Barlocco *et al.*, 2006; Cluff *et al.*, 2008). De Marchi *et al.* (2011) had studied the chicken attributes and used the complex component factors and it has revealed excellent PLSR model for differentiation. Hence, FT-IR data obtained from different animal and bird meats could easily be identified by using PLSR model based on various traditional chemical and quality parameters.

**Conclusions:** The major issues related to meat in our modern society are the authenticity and traceability of meat. Spectroscopy has potential to differentiate animal species based on electromagnetic radiations interaction with meat components. This study addressed the potential of FT-IR spectroscopy in identification of four species meat (beef, pork, chicken and turkey). Mathematical modelling (PLSR) was applied in combination with information acquired from

different instrumental sources. The prediction results showed this methodology gives low root mean square errors (RMSE) and high coefficients of determination ( $R^2$ ) for four chemical and three quality characteristics. PLS results showed FT-IR as rapid, non-destructive and real time monitoring tool for assessing meat authenticity for species meat identification.

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