Molecular Authentication and Spectroscopic Techniques used for species identification of Meat and meat products:A review

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Abstract

Due to its superior proteinious nature, vitamin, and mineral content, Meat is the one of the primary sources of nutrition in the human diet. As meat is the one of expensive food commodity which make it open to adulteration. Meat products are available in different varieties of food categories including different meat species. Laws, don't always permit the blending of two or more different types of meat. On the other hand, producers can reduce costs by substituting low grade meats with high quality meats. Different types of meat and meat products are consumed more frequently in different countries, but generally, economic, racial, and religious considerations are top of mind. Techniques for species identification are becoming more significant in this situation. The optimal method for identifying species is still debatable in the modern era, despite the fact that various approaches relying on DNA or spectroscopy been established for a long time. As a result, this review covered both the effective techniques now in use and the most promising new approaches.

Introduction

Meat is an excellent protein source and a fundamentally healthy food that significantly contributes in the diet of humans (Arihara, 2006; Zhang et al., 2010; Hathwar et al., 2012). West Europe consumed 6.94 million tons of beef in 2012, and it is widely identified that over the past fifty years, these numbers have rather stable (Zhao et al., 2013). Despite the fact that there are many different species of meat that may be consumed by humans, beef, chicken, and pork still make up the lion's share of all meat consumption worldwide. While some of these meats can be managed in various behaviors into a varied range of treated meat goods that account for a sizeable portion of the worldwide market. For using meat as a fresh these meat can be used for this purpose.

Because of concerns about animal welfare, health and safety issues, and reasons connected to food quality, consumers are becoming more interested in learning where their food comes from. The meat producers must adhere to several regulations imposed by the legal organisations due to some cost and religious considerations (Murugaiah et al., 2014). For these causes, the validity of meat, which focuses on identifying species or origin, has received a lot of attention as a developing field of study with relevant scientific and technical advancements (Ayhan, 2013). Since this issue

has long been a popular one in science, several methods have been created till today.(Esslinger et al., 2014).

Those methods may divided into 4 groups: mass spectrometry methods, spectroscopic methods, separation methods like HPLC and GC, and DNA technologies) (Luykx et al., 2008).

Currently, electrophoresis, liquid chromatography, randomly amplified polymorphic DNA PCR, and classes precise PCR are employed to identify various species (Aida et al., 2005; Syahariza et al., 2005; Che Man et al., 2007).

Though, given the necessity for developing quicker, less expensive, and secure ways, molecular approaches are regarded as reliable means to identify different types of meat. Due to their low cost and ease of use, spectroscopic methods are becoming more popular in addition to histology tests, immunoassays, and DNA analysis (Hashim et al., 2010; LermaGarcia et al., 2010; Zhang et al., 2012). Strength of spectroscopies is in the ability to identify or measure the characteristics of materials centered on their spectral sign. Image then converts this data into chemical maps for spatial visualisation. As a result, these techniques may be used to identify the qualities, their quantities, and their locations in trial (Kamruzzaman et al., 2013). The DNA based technology known as (PCR) has been shown to be actual for detecting tiny quantities of DNA by quickly and sensitively intensifying a board area of the pattern DNA (Kesmen et al., 2012). Numerous DNA orders from diverse animals, including game species, and several local animals, have been examined. In comparison with other approaches for specie documentation employing DNA based methodologies, (PCR-RFLP) examination has previously been effectively employed for species difference in heated and treated products of meat. (Aida et al., 2005).

According to estimates, cooked meat products are more likely to be adulterated than raw meats. It is typical practice to reduce expenses by blending Deboned mechanically chicken tissue containing pulverised or comminuted animal products. (Doosti et al., 2014). The United States has adopted a new trend that holds that all foodstuff contaminations might pose a food safety risk, on the grounds that consumers of contaminated food may be unaware of any possible risks to their health (Zhao et al., 2014).

In contrast, there are 3 key challenges in creating an validity technique. The first is the necessity for a legal need, norm, or advice in all situations. The second challenge is identifying a sign that distinguishes the relevant food. The requirement for genuine samples to be accessible for the method's development and assessment is the third (Primrose et al., 2010).

DNA Based Methods to Identify the Meat Types

Compared to other food kinds, meat and meat products are quite costly as well as highly perishable. They have thus always been concerned in their composition and quality. When seen from an industrial and commercial viewpoint, carcasses of beef and pork appear to be the main ingredients in the production of meat (Kartheek et al., 2011).

Amongest the methods for identifying kinds, PCR is a DNA type method that enables the discovery of incredibly small sums of nucleic acid inquiries and the purpose of their order through the magnification of certain DNA or RNA strains. That approach has certain benefits, including excellent compassion and quick presentation with large trial sizes. However, the target DNA must be recognized at both ends and resistant material is required for the PCR procedure (Luykx and van Ruth, 2008). Additionally, certain treating methods may degrade DNA, which lowers the magnification degree of PCR. This appears to be one of the drawbacks of DNA based techniques, although often, tainted DNA may produce beneficial outcomes, as in the work done by Che Man et al (2007). The findings of this learning demonstrated that PCR might be carried out effectively using DNA recovered from sausages, a processed pork product.

While ostrich meat's nutritional profile is comparable to that of chicken, its sensory qualities are more like those of beef. So, without labelling, ostrich meat may be substituted for beef in meat mixes. According to a research by Abdulmawjood et al. (2014), a loopmediated to identify ostrich meat in meat mixtures, an isothermal amplification (LAMP) test based on the cytochrome b gene of the mitochondrial DNA of the species Struthio camelus was created. With a sample time of 15 to 20 minutes, the LAMP approach paired with a real time fluorometer enabled for the detection of roughly 0.01% ostrich product of meat (Abdulmawjood et al., 2014).

Typical sort of meats used in food adulteration is pork. A research was led by Che Man et al. (2007) with the goal of identifying pig in specific culinary products, such as sausages. Food items' genomic DNA was isolated before being exposed to species-specific PCR that focused on certain 12S rRNA gene regions. This method was recommended as a trustworthy method for Halal certification since it produced outstanding results for identifying hog derivatives.

Direct DNA sequencing needs high-quality DNA, however denaturated DNA might produce confusing sequence findings in mixed species or spurious bands. Currently, PCR-RPLF offers a number of benefits, including being a cost-effective, accurate detection method that may be used with mixed meals comprising different species (Murugaiah et al., 2009).

Aida *et al.*, (2005) introduced a technique for identifying pig and lard samples utilising PCR of the cytochrome b gene's conserved region. Pork genetic DNA was isolated and tested using PCR. Following the use of restriction enzyme (BsaJI) to cut the PCR products, RPLF was used. Determining the species of pig using the PCRRFLP methodology produced good findings, indicating a possible application for Halal verification.

Another factor is the need of detecting fraud or adulteration in Muslim nations for religious reasons. Despite being the most popular world's supply of beef (Murphy et al., 2014), pork regarded as illegal or forbidden in Islamic nations. 224 meat items from various Iranian businesses and marketplaces, including 68 sausages, 48 frankfurters, 55 hamburgers, 33 hams, and 20 cold cut meats, were collected (Doosti et al., 2014). Following the extraction of the samples' genomic DNA, the PCR-RFLP method was used to classify the presence of beef. Six fermenting sausages,

four frankfurters, four hamburgers, two hams, and one icy cut meat were said to forbidden or illegal meat.

The PCR-RFLP approach proved successful in identifying the species of beef, hog, in a research by Murugaiah et al. (2009). After PCR products from the meat samples' cyt b gene were produced, the restriction endonucleases AluI, BsaI, RsaI, MseI, and BstUI enzymes was selected. By using this technique, it was possible to correctly prove the genetic distinctions between those six varieties of meat, and a trustworthy system for classifying the species was created. Kesmen et al. used a quick Taq Man-based duplex real-time PCR technique (2014). Pork meat in raw cooked donkey beef and pork beef mixes are identified and measured were accomplished using this technique, which was based on the concurrent intensification of mitochondrial ND2 and ND5 gene fragments. This investigation enabled the successful detection of 0.001% of the meat types in uncooked and oven cooked combinations and 0.01% of the meat types in autoclaved meat combinations.

Although DNA and ELISA based approaches are undoubtedly the greatest precise and subtle ways for detecting food mechanisms, these methods are more difficult to execute than chromatographic, electrophoretic tests (Murugaiah et al., 2009; Boyaci et al., 2014). The USDA provides a research laboratory guidance for using ELISA for animal types identification in prepared and preserved meat and hen. The USDA created an ELISA-based test that is extensively used in industry to specify the varieties of meat. Although ELISA and PCR procedures are regarded as being difficult, they can be made simpler by being packaged in a kit. This will not require a qualified personnel and is more appropriate to ELISA. Random Amplified Polymorphic DNA, or RAPD, is a relatively quick, simple, and inexpensive equipment-free approach. This technique uses PCR to examine genomic variation without having to know the DNA orders beforehand (Huang et al., 2003). When processing items with DNA that has only minimally degraded, such smoked or salted goods, RAPD PCR can be utilised to classify the meat classes and create accurate impressions.

Spectroscopic Methods to Identify Meat Types

The demand for novel and quick logical techniques in the field of food contamination, spectroscopic techniques, with (FT-IR), have been reserved into account. These techniques often rely on transmittance or reflectance data and need no sample preparation or very little (Reis et al., 2013). Due to its environmental friendliness and lack of complex sample preparation requirements, infrared (IR) spectroscopy is a widely used analytical method (Al-Jowder et al., 1997; Hashim et al., 2010; Liu et al., 2012; Liu et al., 2013).

The meat ball is a well-liked meat dish that is eaten worldwide. The difficulty of substituting pig for beef for certain financial reasons affects both individuals and organisations. In a study to quantify the quantity of pig fat (lard) in meatballs, chemometrics of PLS and principal component analysis were combined with FT-IR spectroscopy (PCA) (Kurniawati et al., 2014). In fact, using FT-IR for a quantitative examination of fats and oils is an excellent choice (Che Man

et al., 2005; Kuligowski et al., 2008; Jaiswal et al., 2015). The number of wave range of 1018–1284 cm-1 was identified as the quantitative documentation opinion of lard in meatballs in the learning conducted by Kurniawati et al. (2014). In addition, the area between 1200 and 1000 cm-1 was described for the categorization of beef and lard in meat ball broth. Kurniawati et al. (2014) employed FTIR spectroscopy and then PLS to demonstrate pork meat contamination in meatballs in a research comparable to that of Rohman et al. (2011). Spectroscopically similar to The spectral bands linked to pure PF and BF, as well as the PF, BF, and their mixtures in meatball composition, were scanned and named. The FTIR projected standards in the PLS calibration model were y= 0.999x+ 0.004 and were used for quantitative analysis on fingerprint sections of 1200-1000 cm1. For the purpose of Halal verification, FT-IR spectroscopy was therefore advised for the identification and measurement of pork in beef meat ball preparation. The prediction of independent samples using laboratory-made meatball samples, including the mixtures of BF, was afterwards performed using this model.

Many nations do not incorporate horsemeat in their food chains because they believe it is inappropriate for human consumption. Even though it is illegal to use horsemeat in meat products in Europe, it was found in many beef burgers. Additionally, the UK's Food Values Agency (FSA) discovered horsemeat in various beef products and pulled them off the shelves of supermarkets (Boyaci et al., 2014; Jakes et al., 2015).

Jakes et al. presented a dependable methodology for differentiating between beef and horsemeat varieties (2015). Based on a comparison of the meats' triglyceride fingerprints, the researchers carried out 60 MHz 1H NMR spectroscopy. Peak integrations were found to be sufficient for deciphering the different meat varieties, leading to the calculation of 62 and 76 extractions for horsemeat and fresh beef, respectively. Boyaci et al. created a novel method for identifying horsemeat in adulterated beef (2014). Data was collected using Raman spectra, and chemometric calculations were made using PCA. This method was done to evaluate pure fat samples taken from 49 samples of beef and horse. It was claimed that the categorization of the meats was accomplished in a brief examination time and deprived of the need of time consuming sample research procedures.

Since biological resources are often impervious, highly sprinkling, and contain a considerable quantity of water, they provide challenges in the mid-IR region. At present time, effective use of such materials for attenuated total reflection (ATR) sample attachments (Downey, 1998). Zhao et al. (2014) produced true and offal contaminated beef burger trials in lab medium and investigated adulteration in fresh and frozen beef burger. Frozen then thawed material both had 100% accurate classification correctness, according to mid infrared ATR spectroscopy and multipurpose data analysis.

The creation of minced meat alters the morphological features of muscles, hence this type of Meat may be cheated. Due to its lower price compared to beef, meat of turkey is one of the candidates to be chosen for contamination. chemometric approaches in conjunction with

spectroscopic methods (UV-visible, near-infrared, and mid-infrared) to identify adulterated minced beef The data was evaluated using 154 mixes of minced beef adulterated with turkey meat in quantities ranging from 5 to 50%. Analysis by Principal Components (PCA), To analyse the findings, partial least squares (PLS) regression and linear discriminant analysis (LDA) were used. According to reports, NIR and MIR spectroscopy produced the greatest findings, whereas UV-vis readings were fewer than ideal (Alamprese et al., 2013).

Lamb mince might also be used with less expensive meats. By adding minced pork to minced lamb in the range of 2-40% (w/w) in around 2% increments, Kamruzzaman et al. employed a quick analytical method founded on near infrared (NIR) hyper spectral imaging to identify contamination (2013). We chose four significant wavelengths (940, 1067, 1144, and 1217 nm) and used prejudiced reversion coefficients (Bw) and a numerous Model linear regression to assess adulteration. The created model made it possible to find contamination in samples of minced lamb. FT-IR and PLS were used to look for rat meat contamination in meatballs (Rahmania et al., 2015). Rat meat's relationship between actual and anticipated values according to FT-IR is quantitatively given by the equation $y = 0.9417x + 2.8410$, which has a coefficient of determination of 0.993 and a root mean square error of calibration of 1.79%. Moreover, the categorization of rats and beef meatballs might be accomplished using PCA.

Conslusion

Consumers are very concerned about high-quality goods with transparent origins. The legal authorities must devise effective strategies for conducting product analyses and enforcing legal regulations on producers. In order to maintain autocontrol, both the producers' and the government's control mechanisms require straightforward, affordable, and useful techniques. Due to their dependability and certainty, DNA-based technologies have been in demand up to this point. These methods require skilled workers and are too expensive. At this stage, the researchers focused on spectroscopic methods like NIR, MIR, and FT-IR, which if used in conjunction with chemometric tests might provide reliable findings for determining the origins of foods. In fact, the approach used to identify species should be chosen based on the goal. Spectroscopic approaches look to be helpful in regular analysis, DNA constructed technologies may yield more accurate findings on a lab scale.

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