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Determination of Turkey Meat Adulteration in Cooked Ground Beef Using Fluorescence Spectroscopy with Machine Learning

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Keywords

Beef, Fluorescence spectroscopy, Machine learning, Meat adulteration, Turkey **Abstract:** Beef adulteration with turkey meat is typically driven by financial motives. Since turkey meat is less expensive than beef, producers aiming to cut costs and boost profits blend turkey meat into beef products in certain ratios. This study aimed to investigate the use of fluorescence spectroscopy as a fast, non-destructive, and comprehensive method, combined with multivariate analysis, to predict meat adulteration. Raw turkey ground was combined with raw beef ground in concentrations from 0-100% (w/w) in 10% increments and then cooked. Fluorescence measurements of the cooked samples were taken (Ex 200-500 nm, Em 525 nm). The resulting spectral data were analyzed using chemometric tools, such as principal component analysis and partial least squares regression, and error metrics (Table 1 and 2) were calculated. For the training, validation, and test datasets, R² values of 0.941, 0.922, and 0.916, and RMSE values of 8.124, 10.856, and 8.456 were identified, respectively. This research demonstrated that fluorescence spectroscopy and multivariate analyses can serve as rapid, non-destructive, and effective methods for detecting a 20% turkey meat adulteration in meat products.

Makine Öğrenmesi Destekli Floresans Spektroskopisi ile Pişmiş Kıyma Sığır Etinde Hindi Eti Tağşişinin Belirlenmesi

Anahtar Kelimeler

Sığır eti, Floresans spektroskopisi, Makine öğrenmesi, Et tağşişi, Hindi eti Öz: Sığır etine hindi eti karıştırılması genellikle finansal nedenlerle gerçekleştirilmektedir. Hindi eti, sığır etine kıyasla daha ucuz olduğundan, maliyetleri düşürmek ve kâr oranlarını artırmak isteyen üreticiler, belirli oranlarda hindi etini sığır eti ürünlerine karıştırmaktadır. Bu çalışma, et hilelerinin tespiti için hızlı, tahribatsız ve kapsamlı bir yöntem olarak floresans spektroskopisinin çok değişkenli analizlerle birlikte kullanımını araştırmayı amaçlamıştır. Çiğ hindi kıyması, %0–100 (a/a) aralığında ve %10'luk artışlarla çiğ sığır kıyması ile karıştırılmış, ardından pişirme işlemi uygulanmıştır. Pişmiş örneklerin floresans ölçümleri (Ex: 200–500 nm, Em: 525 nm) alınmıştır. Elde edilen spektral veriler, temel bileşen analizi (PCA) ve kısmi en küçük kareler regresyonu (PLSR) gibi kemometrik araçlar kullanılarak analiz edilmiş, hata metrikleri (Tablo 1 ve 2) hesaplanmıştır. Eğitim, doğrulama ve test veri kümeleri için sırasıyla 0.941, 0.922 ve 0.916 R² değerleri ve 8.124, 10.856 ve 8.456 RMSE değerleri elde edilmiştir. Bu araştırma, floresans spektroskopisi ve çok değişkenli analizlerin, et ürünlerinde %20 oranındaki hindi eti taklitçiliğini tespit etmek için hızlı, tahribatsız ve etkili yöntemler olarak kullanılabileceğini ortaya koymuştur.

1. INTRODUCTION

The adulteration of meat products is not only an economic problem but also a serious concern in terms of public health, religious sensitivities, and consumer rights. The addition of low-cost meat types to beef, which has high economic value, is a common example of food adulteration [1].

Grounded and cooked meat products are adulterated more easily than fresh meat. This is because size reduction, cooking, and use of spices may reduce the effects of 67

adulteration. Detecting adulteration in minced beef usually becomes more difficult after cooking because thermal process causes denaturation of the protein and loss of the chemical bonds of the meat [2], which makes the detection method more difficult. Therefore, prevention of meat adulteration, proper labelling of food, and prevention of unfair competition in the meat sector have become important issues for the competent authorities of the meat sector [3]. The detection of adulteration in meat products is of great importance for ensuring consumer safety and determining compliance with legal regulations [4].

Different analytical techniques have been used to determine adulteration. Polymerase chain reaction (PCR) is a well-known molecular method involving the chemical amplification of specific DNA sequences [5]. This technique is useful for the identification of different species in a mixed meat sample or for the determination of meat origin based on specific DNA sequences that are unique to different meat species [6]. Chromatographic, immunological, and electrophoresis methods are commonly used for detecting foreign compounds [7,8]. Hoffmann et al. [9] investigated the ability of HPLC-MS/MS scanning to simultaneously detect plant proteins (soya, pea, and lutein) in meat products.

Although the methods described above provide high accuracy and demonstrate robustness, they still have numerous shortcomings; for example, they are more expensive, labourious, and time-consuming, experts are required to perform the experiment, and a considerable amount of time is required to prepare the sample and perform the analysis. Therefore, there is a need to develop fast and accurate techniques suitable for online authentication that are easy to implement by government agencies and quality assurance engineers to monitor counterfeiting in processed meat products. Increasing efforts have been made to introduce fast, innovative, and reliable detection techniques to ensure the authenticity, quality, and safety of meat and meat products [10,11].

Fluorescence spectroscopy has attracted attention as a noninvasive, rapid, and economical method for food analysis. The characteristic fluorescence properties of biomolecules found in meat products are considered potential tools for distinguishing different species [12]. However, the high-dimensional and complex nature of fluorescence data makes it difficult to effectively analyse this information. Machine learning algorithms are powerful tools for pattern extraction, classification, and prediction processes from large datasets [13,14]. This study aimed to develop a new, fast, practical, and low-cost analytical method as an alternative to traditional analysis methods for the determination of adulteration of turkey meat mixed with beef at different ratios.

2. MATERIAL AND METHOD

2.1. Material

Postmortem beef (Longissimus thoracis et lumborum) and turkey breast meat (Musculus superficiolis) were obtained

from a local meat supplier, transferred to the laboratory under aceptic conditions, and stored in a freezer (-20 °C) until use.

2.2. Sample Preparation

Water 10% (w/w) and NaCl 2% (w/w) were added to the beef/turkey minced meat mixture, in 50 mL centrifuge tubes (Isolab, Turkey). The tubes were placed in a water bath set at 60°C and the temperature of the water bath was increased to 85°C. The centre temperatures of the experimental groups were monitored using a thermometer (ISOLAB, Turkey). The cooking process was terminated when the centre temperature of the samples reached 74°C (Fig. 1). The cooked samples were kept at room temperature for 30 min after the cooking liquid was removed [15].



Figure 1. A representative sample preparation study (The photograph was taken by A. Soyuçok for this study).

2.3. Fluorescence Spectral Analysis

A fluorescence spectrophotometer (Varioscan Lux, Thermo Fisher. Inc, USA) was used for spectral measurement. To determine the wavelength at which the highest emission occurred, the fluorescence emission spectra of the meat samples were recorded at a constant excitation wavelength (460 nm) and emission values in the range of 480-840 nm and the highest emission value was determined. All spectra were recorded at room temperature and each treatment was performed in triplicate. The excitation spectra were acquired at a fixed emission wavelength of 525 nm over an excitation range of 200-500 nm.

2.4. Chemometric Analysis

The Unscrambler X 10.5 software was employed to preprocess the fluorescence spectra, thereby correcting the initial values. Initially, the raw spectral data were smoothed using a Savitzky-Golay derivative filter (second-order polynomial, 11-point window) to mitigate the signal noise and eliminate variations attributable to light scattering. Subsequently, the data were normalised using a Standard Normal Variate (SNV) transformation. The data were then structured in a mean-centred format by averaging, rendering them suitable for Partial Least

Squares (PLS) modelling. The Partial Least Squares Regression (PLSR) method was utilized in the modeling process, with a kernel-based approach selected as the algorithm. Model accuracy was assessed through the cross-validation method, and the performance evaluation criteria included R², RMSE, slope, and offset values.

3. RESULTS AND DISCUSSION

The present study leveraged fluorescence spectroscopy as a rapid, non-destructive means to detect turkey adulteration in cooked ground beef. The highest emission of cooked beef mince containing turkey meat adulteration was observed at 525 nm (Fig. 2A). The spectrum was scanned at 525 nm excitations (Fig. 2B). As the percentage of adulteration of turkey meat varied, the changes in the spectrum increased, indicating that the wavelengths were suitable for the determination of adulteration (Fig. 3) and were also due to the tryptophan content in the myofibril protein and the free amino acids in the meat [16]. The wavelengths at which this change occurs serve as fingerprints for the identification of turkey meat [17].

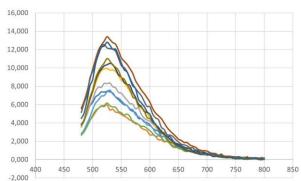


Figure 2A. Emission spectrum (Ex. 460nm).

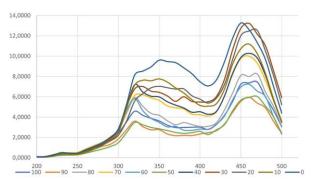


Figure 2B. Fluorescence spectrum (Ex 200-500 nm, Em 525 nm).

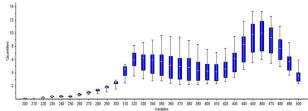


Figure 3. Effect of turkey adulteration at different wavelengths.

Various analytical methods exist for meat fraud detection, each with trade-offs. DNA-based methods (PCR) are gold-standard for species identification and can detect adulterants down to very low levels (often <1%) [18].

Real-time PCR or multiplex PCR reliably confirm species in raw or lightly processed meats. However, PCR requires DNA extraction and thermal cycling instrumentation, and the target DNA can be degraded by intensive processing. For example, Aprilia et al. [19] found that conventional PCR failed to detect pork in heavily heated beef-floss products, whereas an ELISA immunoassay succeeded. Thus, PCR may miss adulterants in highly processed foods, necessitating complementary methods.

Near-Infrared (NIR) spectroscopy is a rapid alternative. NIR (and Vis-NIR) spectroscopy measures overtone vibrations of O-H, C-H, and N-H bonds; it is nondestructive and can be implemented in portable probes. In adulteration studies, NIR (often with PLSR) technique has quantified turkey or other meats in beef with good success. For example, Alamprese et al. [20] used FT-NIR (1000-2500 nm) and PLSR to predict turkey in minced beef, achieving R2>0.884 and RMSE <10.8%. Similarly, Weng et al. [21] applied Vis/NIR (300-2500 nm) plus chemometrics and reported ~99% accuracy in classifying pork or organ adulteration in ground beef. NIR spectroscopy's advantages include fast scanning and minimal sample prep, but its broad overlapping bands can limit specificity. Preprocessing (e.g. SNV, derivatives) is crucial in NIR spectroscopy just as in fluorescence. Notably, studies show NIR spectroscopy often outperforms UV-Vis spectroscopy for meat fraud: Alamprese et al. [20] reported that NIR/MIR spectra gave better turkey detection than UV-Vis spectra.

Liquid Chromatography–Mass Spectrometry (LC-MS) techniques (including proteomics and metabolomics) are highly sensitive and specific. LC-MS can identify speciesspecific peptide or lipid markers even in processed meat. State-of-the-art targeted proteomics methods achieve detection limits on the order of 0.2-1% in cooked products [18]. For example, Prandi et al. [22] developed an LC-MS/MS assay with LOD ≈0.2–0.8% for eight species (including turkey and chicken) in complex meat matrices. High-resolution LC-MS has also mapped adulteration in multi-component meat meals. These approaches yield definitive results and can handle very low adulteration levels, but they are costly, require skilled operation and heavy sample preparation, and are not suited for on-site or rapid screening. LC-MS is best viewed as a confirmatory or research tool rather than a routine field test.

Immunoassays (ELISA, lateral-flow "dipstick" tests) target specific proteins and can offer rapid, user-friendly detection of particular species (e.g. antibodies against pork myoglobin). ELISAs for meat species are available and can detect ~1% adulteration under ideal conditions [19]. They require no complex optics or chemometrics. However, immunoassays depend on intact antigen epitopes, which may be denatured by cooking. As observed by Aprilia et al. [19], an ELISA detected pork in heat-processed beef where PCR failed, implying ELISA can sometimes outperform DNA methods on cooked products. On the other hand, if proteins are heavily modified, **ELISA** give may false negatives. Immunoassays are also limited to pre-chosen targets; each assay detects one species (or one allergen), so screening for multiple adulterants requires multiple tests.

Fluorescence-PLSR occupies a middle ground between these techniques. It is quicker and cheaper than LC-MS and PCR, yet more general (and often more quantitative) than single-target ELISAs. Our approach achieved comparable accuracy to published NIR models [20,23], confirming its viability. However, unlike targeted assays, it does not identify species by unique markers; it instead uses statistical patterns. This means it works well for known adulterations (beef vs turkey), but its generalizability to unknown adulterants must be validated separately. Complementary use (e.g. following up a positive fluorescence screen with PCR or MS for confirmation) would combine the strengths of these methods.

Despite the encouraging performance, several limitations must be acknowledged. First, the detection limit of $\sim 20\%$ turkey content means that very low-level adulteration (below 10-20%) was not reliably quantified. This is evident from the test-set bias (Table 2): the regression slope was well below unity (0.789) and the offset high (≈16%), indicating substantial underestimation at low adulteration levels. One cause is spectral masking: at small percentages, turkey's fluorescent signature is weak relative to the dominant beef background, making it hard to distinguish. The fixed-emission setup (Em = 525 nm) captures only one slice of the fluorescence landscape, possibly missing other differentiating peaks. It's known that overlapping fluorophores (e.g. tryptophan, NADH, collagen crosslinks) can yield broad, featureless spectra in mixed meats [14]. Our preprocessing (SG smoothing, SNV) improves precision but cannot recover information lost in broad overlap.

Table 1. Error metrics for calibration and validation data set.

	Slope	Offset	RMSE	\mathbb{R}^2
Calibration	0,941	2,633	8,124	0,941
Validation	0,901	4,573	10,856	0,922

Table 2. Error metrics for test data set.

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	SEP	Bias	Slope	Offset	RMSE	R ²			
Test	8.96	5.98	0.789	16,544	8,456	0,916			

Processing effects also complicate the task. All samples were cooked, which generates Maillard products and denatures proteins. These chemical changes may reduce species-specific contrast. For example, Aprilia et al. [19] noted that intensive heat can degrade DNA, affecting PCR, but also it could alter protein fluorescence. By removing cooking juices, we reduced variability due to moisture and soluble compounds, but this also eliminated any adulterant signals in the liquid fraction. In some cases, juices might carry pigment or vitamin differences (though that fraction was deliberately excluded here to mimic a typical solid-recipe analysis).

Another limitation stems from the PLSR model itself. Partial Least Squares assumes a linear relationship (albeit in latent-space) between spectra and concentration. We used a kernel-PLSR to allow nonlinearity, but extreme

still concentrations exhibited bias. The high calibration/validation R2 and low RMSE suggest the model fits the training data well, but some overfitting or calibration transfer issues are possible. In particular, the steep drop in test slope hints at calibration-prediction differences, perhaps due to sample-to-sample variation not captured in the calibration set (Table 1). For example, heterogeneity in fat content or the microdistribution of turkey meat could lead to spectral inconsistencies. Ensuring sample homogeneity and averaging multiple scans per sample can help mitigate this issue.

Fluorescence intensity can be affected by instrument factors (lamp stability, detector sensitivity) and by matrix scattering. We attempted to minimize these by using front-face optics and SNV normalization [24], in addition, our study focused only on turkey in beef; detecting other adulterants (e.g. pork, chicken, lamb) would require retraining the model with corresponding mixtures and possibly different spectral features. While our method shows strong accuracy for moderate to high adulteration levels, its sensitivity limit (~20%) and potential biases mean it is not a definitive single solution. Instead, it is best viewed as a rapid screening tool: samples flagged by fluorescence should be further analyzed by confirmatory methods (PCR, LC-MS). Understanding these limitations guides realistic interpretation of the results and directs improvements.

Food adulteration in Türkiye has been a significant public health and regulatory concern. Turkish law (the Turkish Food Codex) explicitly prohibits mislabeling and mixing of meats. For example, Regulation Notification 2012/74 forbids producing "prepared meat mixtures" by mixing beef with poultry or pork without declaration [25]. Violating this rule not only deceives consumers (especially on religious grounds) but also can endanger health if allergens or unsanitary meats are involved. documented non-compliance: Studies nationwide survey of processed beef products found that 34% of samples were adulterated, predominantly with cheaper poultry (32.9%) and occasionally horsemeat (1.3%) [25]. No pork was found in that study, reflecting consumer sensitivities to pork; indeed, while pork is legal, it must be explicitly labeled (due to the majority-Muslim population). Earlier work also found adulteration rates on the order of 15-50% in various regions of Türkiye, underscoring its pervasiveness.

In the past year Turkish authorities have intensified enforcement. In late 2024, Türkiye Ministry of Agriculture and Forestry, publicly released lists of hundreds of food products failing standards. These included cases of beef products containing undeclared pork, equine meat sold as beef, and processed foods with unauthorized additives. For instance, a popular pizzeria was found to serve "beef" pizza laced with pork, causing a social outcry. In one high-profile campaign, over 500 companies and nearly 1,000 product batches were flagged for mislabeling or adulteration. Notable fraud examples included kebabs with hidden pork and cheeses "filled" with margarine. These revelations have prompted calls for stronger routine screening and transparency. Our findings

have direct relevance in this context. A rapid spectroscopy-based test like ours could be incorporated into official monitoring protocols or quality control by food companies. Because it is non-destructive and quick, inspectors could screen batches of ground meat or finished products en masse, identifying suspicious samples in need of further testing. Early detection of fraudulent meat substitution not only protects consumers (from allergen risk or religious violations) but also helps maintain fair markets. In Türkiye, where the economy of meat products is closely tied to cultural norms, ensuring authenticity is doubly important. Moreover, the introduction of portable, fluorescence-based analyzers (as future work) could empower even small processors or market inspectors to perform on-the-spot checks. For example, a hand-held fluorescence sensor could be used at slaughterhouses or meat processing plants to audit mixtures continuously. Even now, our bench-top results support the feasibility of such approaches. Implementing fluorescence-ML screening would complement Türkiye's regulatory framework (e.g. Law No. 5996 and related communiqués [26]) by providing a technical backbone to the "real-time" adulteration alerts announced by the Ministry [27]. In essence, improved analytical tools would enable the promise of these policies – ensuring that all beef-labeled products truly contain only beef, as mandated by the Codex.

Building on this work, several avenues could enhance detection capability and practical utility. First, expanded spectral techniques are promising. Multispectral or hyperspectral imaging could capture both spectral and spatial information of the meat. For example, Yu et al. [28] developed a portable hyperspectral imager (400-1000 nm) with on-board processing that achieved ≈95% accuracy in classifying meat species and even produced spatial "adulteration maps" of samples. Extending fluorescence to three-dimensional excitation-emission matrices (EEMs) rather than a single emission wavelength might likewise extract more features. Similarly, UV-Vis, combining NIR, and fluorescence measurements could provide complementary fingerprints: Alamprese found that MIR/NIR technique together outperformed UV-Vis technique alone for turkey detection [23], suggesting multi-modal fusion could improve sensitivity. Second, miniaturization and portability are key. Developing handheld fluorescence devices (e.g. LED-based fluorimeters) would allow infield use. Coupling fiber-optic probes with portable spectrometers could enable direct scanning of meat onsite. The Raspberry Pi-controlled HSI system of Yu et al. (2025) demonstrates the feasibility of low-cost portable platforms. Future work should assess such devices in realworld conditions (varying light, temperature, sample presentation) and calibrate them accordingly. Integration smartphone technology (e.g. smartphone spectrometer add-ons) could also democratize testing. Third, real-time monitoring could transform processing lines. For example, an assembly-line fluorescence scanner could continuously check ground meat moving on a conveyor, flagging adulteration instantly. Coupled with cloud-based machine-learning, this would yield a live dashboard of authenticity. Such "smart factory"

integration would greatly aid quality assurance. Research is needed on non-contact or inline sampling approaches (e.g. fluorescence excitation via laser diode, detection through camera-based sensors) to enable non-invasive scanning at speed. Fourth, larger and more diverse datasets will strengthen models. Expanding the sample set to include meats from different breeds, ages, or diet regimens would improve robustness. Including other adulterants (pork, chicken, lamb, offal) either singly or in multi-component mixtures is essential to generalize the method. Multi-species models or variable-selection algorithms could then differentiate among several possible adulterants. Additionally, more replicates at low adulteration levels (5-15% turkey) are needed to push the detection limit lower. Fifth, advanced chemometrics and AI could boost performance. We used kernel-PLSR, but emerging machine-learning techniques (e.g. Random Forests, Support Vector Regression, deep neural networks) may capture complex spectral patterns even better. Deep learning, in particular, has shown promise in spectroscopy: convolutional neural networks can learn features from spectra or images without explicit preprocessing. Future studies might compare PLSR to such methods for quantitation. Data fusion approaches (combining fluorescence with orthogonal sensors) could also be pursued.

4. CONCLUSION

While our fluorescence-PLSR method demonstrates strong potential, its ultimate impact will grow when integrated with these advancements. By extending the spectral range, deploying portable instrumentation, gathering wider datasets, and harnessing modern AI, it should be possible to create real-time, on-site authentication systems. Such tools would not only improve the detection of turkey in beef but could be adapted to a broad array of food fraud challenges, thereby supporting food safety and integrity both in Türkiye and globally.

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