

Summary

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Authentication of selected game species in food products based on nuclear markers and qPCR

The global food industry faces increasing challenges in ensuring the authenticity of high-quality products, particularly within the niche market for game meat. As consumer demand for exotic and sustainably sourced proteins grows, so does the risk of fraudulent practices such as species substitution, mislabelling, and illegal trade. Therefore, this doctoral thesis aimed to establish and validate sensitive, specific, and reproducible quantitative real-time PCR (qPCR) methods for the identification and quantification of three common game species in Poland (red deer, roe deer, and wild boar/pig) which are also popular in the European game meat market.

The study focused on the design of three novel species-specific nuclear DNA markers, carefully selected for their high interspecies variability and short amplicon length (76–127 bp) to ensure stability in processed meat products. The assays targeted the following marker genes: the troponin I gene (TNNI2), the agouti signalling protein gene (ASIP), and the PLAG1 zinc finger gene. These TaqMan probe-based quantitative PCR assays enabled the accurate detection and quantification of red deer, roe deer, and wild boar or pig meat in processed food products.

The methodology encompassed primer design, *in silico* and *in vitro* specificity screening, and optimization of qPCR conditions. All assays demonstrated high analytical sensitivity, with limits of detection (LOD) and quantification (LOQ) as low as 0.01 ng for the species studied. The matrix-adapted quantification approach allowed for relative detection limits as low as 0.5% red deer, 0.1% wild boar, and 0.05% roe deer in simulated laboratory meat mixtures. Cross-reactivity tests using 16 animal species, including uncommon and domestic meats, along with various herbs and spices, confirmed high assay specificity. Validation experiments using laboratory-prepared meat mixtures subjected to various processing conditions (raw meat, oven-cooking, and autoclaving) highlighted the method's robustness.

Application of the validated method to commercially available game meat products from four European countries revealed widespread species misrepresentation. Overall, 64% of roe deer-labelled products and 54% of red deer-labelled products contained undeclared species or lacked the declared species entirely, while four out of 38 products contained undeclared wild boar meat or pork. These findings raise significant concerns regarding regulatory compliance, consumer deception, and food safety risks. The study necessitates the urgent need for effective routine monitoring of game meat authenticity using DNA-based tools. The developed qPCR assays provide a practical solution for industry stakeholders and regulatory authorities, enabling improved enforcement of labelling laws, halal monitoring, protection of consumer rights, and mitigation of economic losses due to fraud. Furthermore, the findings contribute valuable insights into market integrity and highlight the importance of transparent, traceable supply chains in maintaining consumer trust in high-value niche products like game meat.

Keywords: food authentication, meat products, game species quantification, nuclear markers, qPCR.



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