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ORIGINAL PAPER

Quantification of beef, pork, chicken and turkey proportions in sausages: use of matrix-adapted standards and comparison of single versus multiplex PCR in an interlaboratory trial

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Abstract Quantitative PCR methods for the determination of beef, pork, chicken and turkey proportions in sausage were tested in an interlaboratory trial. Twelve different laboratories analysed six meat products each made of different compositions of beef, pork, chicken and turkey. Two kinds of calibrators were used: sausages of known proportions of meat and DNA from muscle tissue. Results generated using calibration sausages were more accurate than those resulting from the use of muscle tissue DNA. Regardless of the method used (either multiplex or single PCR), when using calibration sausages, it was always possible to quantify the proportions of meats in the unknown samples (in the range of 0.5–80%) with high precision and accuracy.

Keywords Species identification · DNA quantification · Multiplex quantitative real-time PCR · Sausage · Interlaboratory trial · Beef · Pork · Chicken · Turkey

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Introduction

Meat products such as sausages, cold cuts and pâté composed of poultry or a combination of pork and beef are consumed in high quantities in Europe. So, the determination of meat proportions in these types of processed meat foods is an important issue for official control laboratories [1]. In case of fraud often declared ingredients deviate from effective ingredients e.g. sausages with, diminished veal contents or other not declared meat combinations, depending on actual market prices. Poultry products advertised as pork-free ("halal") may contain trace amounts of pork as a contamination or in higher amounts to improve the taste. Analytical methods must be able to quantify all expected meat components for a wide range of complex matrices to prosecute producers for fraud or bad production practices.

Precision and accuracy are the crucial performance criteria ensuring comparable results from different control laboratories. PCR-based methods have already proven their applicability for the analysis of mixed processed food in the past [2–17]. The precision and accuracy of PCR methods for food species were usually estimated by comparing results from studies using the same PCR methods [2–7, 9–11]. Relative standard deviations (RSD) of about 30% were reported.

The accurate measurement of meat proportions of samples is a fundamental problem with DNA-based methods. The accuracy of DNA-based methods is impaired when analysing samples with a variety of tissue types. Different tissue types range from fatty bacon to fatless meat and connective tissue. As different tissue types exhibit variable concentrations of DNA, the proportional weight of meat in sausages may not correlate exactly to the proportions of species-specific DNA. This can lead to