DEVELOPMENT OF AN EFFICIENT METHOD FOR THE QUANTIFICATION OF ANIMAL SPECIES IN MEAT BY MEANS OF PCR

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Background

PCR- and ELISA-techniques are of increasing importance for the identification of animal species. A lot of commercially available kits are existing for various animal species. These DNA detection systems are suitable for the qualitative identification of very low amounts of added meat species in food. At present quantitative PCR systems for the determination of animal species are developed (WOLF et al., 2001; KLOTZ et al., 2001; CALVO et al., 2002). Concerning quantification two problems have to be solved. At first all analytical steps like nucleic acid extraction, amplification and detection must be validated. Secondly the obtained results must be correlated to specified standards as the amount of isolated DNA depends on the prevailing matrices like muscle meat, fat, protein, blood or gelatine. A quantitative specification is practicable if there is sufficient knowledge about accuracy of the method and the existing matrices in processed products.

Objectives

A reliable and reproducible method for DNA extraction is most important for quantification independent of the applied PCR technique e.g. competitive PCR or PCR-ELISA. In this report a suitable method for isolation of nucleic acid from animal tissue and processed products is presented. Heat treatment affecting meat products and DNA quality was investigated with respect to its influence on PCR, in particular on quantitative PCR.

Methods

For the extraction of nucleic acid 25 mg to 100 mg muscle meat of various animal species or 50 mg of meat products were used. The isolation was carried out by means of an optimized CTAB method (BINKE et al., 2003).

For determination of the absolute DNA content in pork 12 samples of minced meat were purchased from various butcher shops and the DNA content was determined to get a correlation between DNA and fat content. The analysis concerning fat, moisture, protein and ash was performed by means of NIT (Near Infrared Transmission) technique (FREUDENREICH, 1999).

Different batches of reference sausages were produced containing 50% muscle meat with various amounts of the different animal species, 25% sunflower oil, 23% ice, 2% salt, phosphate, ascorbate and nitrite. Each batch was heat treated at F_C-values of <0.9, 0.9, 3.4, 12.3 and 32.

PCR amplification of a specific fragment of horse DNA was performed applying specific primers purchased by Cibus Biotech GmbH (Gütersloh, Germany). Amplification was carried out in a final volume of 25 µl, containing PCR buffer, 3.5 mM $MgCl_2$ and 1 U HotStarTaq™ DNA Polymerase (Qiagen, Hilden, Germany), 0.1 mM each of dATP, dTTP, dGTP and dCTP (Qbiogen, Heidelberg, Germany), 0.4 µM of the respective primer pairs, 1 µl SYBR-Green I (1:1000), 2µl of sample. DNA was amplified by real time PCR system Rotor Gene 2000 (Corbett Research, Sydney, Australia).

Results and Discussion

Tab. 1 shows the results of the validation of the optimized DNA extraction system for seven different animal species. Nucleic acid can be isolated at good reproducibility (CV less than 10 %) and purity. In accordance with HERBEL and MONTAG (1987) the determined content of nucleic acid in pig, cattle, horse and sheep was similar. In contrast the mean value of the nucleic acid content of poultry appeared 30 % higher than in the other tested animal species.

Tab. 1: Parameters for validation of the optimized DNA extraction system for seven animal species.
Content of nucleic acid (y) in [µg/mL] in dependence upon amount of muscle meat (x) in [mg]

	Pig	Cattle	Horse	Sheep	Turkey	Chicken	Duck
n	12	12	12	12	12	12	12
A	-	2	1	3	-	-	2
b	*5.88*	*6.07*	*5.27*	*5.36*	6.41	7.98	7.41
a	-5.03	30.20	32.0	23.64	20.01	40.07	122.6
r	0.998	0.998	0.998	0.999	0.998	0.997	0.990
r(A)	0.998	0.989	0.993	0.991	0.998	0.997	0.984
CV_{x0}	3.1 %	2.6 %	3.6 %	2.1 %	2.9 %	3.8 %	6.8 %
CV_{x0}(A)	3.1 %	7.2 %	5.9 %	6.5 %	2.9 %	3.8 %	8.8 %
Purity A260 / A280	1.92	1.91	1.91	1.90	1.94	1.95	1.89

n Sample number, **r** Correlation coefficient, **A** Outlier, **CV_{x0}** Coefficient of variation, **(A)** Value with outlier, **b** Slope and **a** Intercept of the linear equation y = bx+a

The absolute DNA content in pork was determinated using 12 different samples of minced meat. The results show a median DNA content of 0.045 % (CV 14 %) which is comparable to the results achieved by HERBEL and MONTAG (1987).

The recoveries of extracted nucleic acid in self made reference meat products were determinated in dependence upon applied heating intensity (Fig. 1). Up to a F_C value of 3.4 recoveries in a calculated range between 70 and 120 % were determinated. At higher heating intensities (F_C-values > 3.4) the nucleic acid yield is obviously decreasing. Nevertheless it can be said that by means of the optimized extraction system the main amount of nucleic acid can be isolated from processed meat products at high purity (A260 / A280).

In addition the influence of heat treatment on PCR was examined. Nucleic acid solutions containing 1 % horse meat were examined by means of real time PCR at different heating intensities (Fig. 2). Up to F_C-values of 3.4 (sample 1-4) DNA amplification appeared similar. A rise of the heating intensity higher than a F_C value of 3.4 lead to a remarkable increase of Ct value. A ΔCt of approximately 6 cycles (sample 2 and 6) corresponds to a variation of the DNA concentration in the sample of about 10 - 100 fold. With respect to these results the heating intensity applied on the processed products seems to be an important parameter influencing quantification.

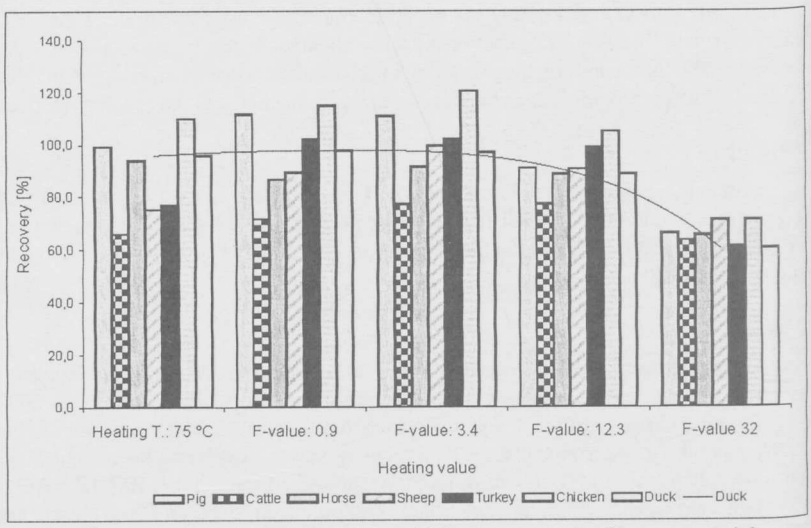

Fig. 1: Recovery of extracted nucleic acid from processed reference materials depending on heating intensity

Conclusion

The presented extraction system is suitable for the isolation of nucleic acid from animal tissue at high reproducibility (CV < 10%) and purity (A260 / A280 > 1.8) for all tested animal species. For not heated minced pork a relatively constant content of DNA of 0.045% with a CV < 14 % was determined. Nucleic acid can also be isolated from processed products at a high yield but the quality of DNA is very much influenced by the applied heat treatment, which is important for the efficiency of DNA amplification. If heat treatment of meat products is very intensive quantification is very difficult or even fails.

Furthermore the amount of isolated DNA depends on the prevailing matrices like muscle meat, fat, protein, blood or gelatine. In this study only nucleic acid from muscle meat was examined. For quantification of animal species in meat products it is necessary to have information about the variation of DNA content of the different matrices. At the moment quantification of animal species in processed products appears to be not very exact for the most applied animal matrices.

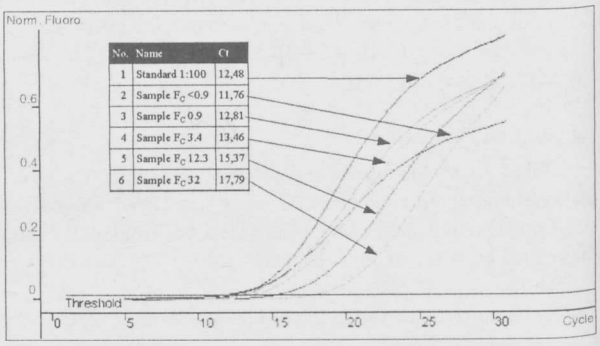

Fig. 2: Real time PCR for the identification of horse in processed products containing 1 % horse meat [1]CT value - PCR cycle number at which fluorescence intensity is reaching the threshold.

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