IDENTIFICATION OF DIFFERENT ANIMAL SPECIES IN HEAT TREATED MEAT HOMOGENATES USING THE POLYMERASE CHAIN REACTION

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1. Introduction

During last several years a number of analytical methods such as ELISA, capillary electrophoresis and polymerase chain reaction (PCR) have been developed in order to enable accurate and reliable identification of species in meat products. Methods based on identification of species-specific protein proved not to be reliable, since number of false-positive results was observed. They were also time-consuming since multiple protein bands extended beyond each other making identification quite ambiguous. It was particularly a case with heat treated products. False-positive results occur due to instability of proteins at the temperatures above 56°C since above this temperature they undergo denaturation. Mitochondrial DNA is much better substrate to be used in species identification since it has been highly conserved during evolution and it is abundant, present at 1000 times higher concentrations then most single-copy nuclear genes, allowing for an extremely low limit of detection (Sawyer et al., 2005.). It is a heat-stable circular DNA molecule which can withstand temperatures of pasteurization and sterilization of meat products. Hopwood et al. (1999) detected chicken meat after cooking at 80°C, 100°C, or 120°C for 30 min by PCR. Arslan, et al. (2006) exposed beef meat to the temperatures ranging from 90°C-200°C (autoclaving, frying and roasting) for a period of up to 150 minutes and reported that they succeeded in DNA identification, although meat had lost its sensory attributes and in cases of frying became charred.

Due to recently adopted food safety and food labeling regulations in Serbia laying down that all food business operator should declare meat species in meat products to consumers we have investigated possibility of species identification in heat treated meat homogenates using PCR. The content of meat homogenates actually represented stuffing of cooked sausages. Meat homogenates were prepared in meat plant and were exposed to the temperatures of pasteurization and sterilization.

2. Material and methods

Meat homogenates were prepared in local mid-sized plant. Overall weight of homogenates yielded 100 kg. Volume consisted of equal amounts of beef, pork and chicken meat (25 kg each), 25 kg of ice, 2 kg of nitrite salt (99.6% NaCl+0.4% $NaNO_2$) and 0.5 kg of polyphosphate. After preparation, homogenates were stuffed into polyamide casings as well as in cans and designated by numbers from 1 to 15.

Meat homogenates which were stuffed into polyamide casings were thermally treated at the temperatures of 80°C, 90°C and 100°C, respectively. Internal temperatures of homogenates during pasteurization were measured by K type thermocouple (HI 9057 KJT thermocouple, Hanna Instruments, Portugal). Pasteurization lasted until desired temperature was attained in the “coldest spot” of homogenate. Canned meat homogenates were subjected to temperatures of 110°C and 121°C, respectively at the pressure of 1.3 bars, during 45 minutes.

Three samples were subjected to each temperature and entire experiment was tripled making a total of 45 (3×5×3) samples investigated.

DNA was isolated from meat homogenates using commercial DNeasy Tissue Kit (Quiagen GmbH, Germany). After heat treatment, meat homogenates were homogenized once more at the 25000 rpm using high speed homogenizer (DI25, Ika Werke GmbH, Germany). Afterwards 25 mg of each homogenate was added to 180 µL of Buffer ATL and 20 µL of Proteinase K. Suspensions were incubated at the temperature of 55°C for 3 hours in order to enable complete. After incubation 200 µL of Buffer AL was added and mixture was subjected to the temperature of 70°C for 10 minutes. Suspension was vortexed and 200 µL of ethanol (96%) was added and entire volume (approx. 500 µL) was pippeted into a special DNeasy Mini Spin columns. Tube was centrifuged at 8000 rpm and flow-through was discarded. Same procedure was repeated using 500 µL of Buffers AW1, AW2 and 200 µL Buffer AE. Last eluate, originating from Buffer AE, was collected in special tube since it contained

DNA. Isolation lasted for 4 hours. Final DNA concentration was measured using spectrophotometer (Anthelie 5, Secomamm, France) by calculating A_{260}/A_{280} ratio. DNA was kept at the 4°C until amplification of target genes.

A total volume of 50 µL of the reaction mixture was prepared in an eppendorf tube. Commercial Biofood Mixed Kit (Biotools, S.A. Spain) was used. Reaction mixture consisted of 25 µL Master Mix (10 mM Tris-HCl pH 9.0, 50 mM KCl, <10% glycerol, < 0.001% dNTP's and primers flanking species-specific regions of a cytochrome b mtDNA), 2 µL of 50 mM $MgCl_2$, 5U of Taq DNA-polymerase and 10 µL of target DNA. Besides target DNA, three controls DNA (chicken, beef, pork) were also put into reaction mixture as a positive control. Thermocycler (Flexigene 412, Techne, GmbH, Germany) was programmed for a 35 cycles. After initial denaturation at the temperature of 94°C for 1 minute and 30 seconds, each cycle consisted of denaturation at the temperature of 94°C for 10 seconds followed by annealing at the temperature of 60°C for 30 seconds and elongation hold at the temperature of 72°C for 40 seconds. At the end of entire process mixture was subjected to the temperature of 72°C for 3 minutes in order to finalize elongation of remaining DNA molecules. PCR products (15 µL) mixed with 5 µL loading buffer (6×Orange Dye, Fermentas, Lithuania) were electrophoresed at 110V for 1 hour through 2% (w/v) agarose (MB Agarose, Biotools, S.A. Spain) gel in 1×TBE, containing 0.5 µg/mL ethidium bromide (Sigma, Germany). Ladder (O'Range 50 bp) was placed (10 µL) to run alongside the samples. Bands were visualized on a UVP transluminator and photographed using digital camera (PowerShot G5, Canon, Japan).

3. Results and discussion

Average temperatures attained in the "coldest spot" of meat homogenates during pasteurization were 75°C, 87.5°C and 98.4°C respectively. PCR amplicons of heat treated muscle homogenates are exhibited on Figures 1 and 2. Chicken DNA positive control generated one product sized 227 bp, beef DNA positive control generated one product sized 278 bp and pork DNA positive control generated one product sized 398 bp. At the temperature of 80°C, all three species were successfully identified; displaying distinct bands for all three species DNA products. Equal results occurred at homogenates processed at the temperatures of 90°C and 100°C, respectively. Canned meat homogenates exhibited clear bands for chicken and pork DNA product while the beef DNA product occurred as a considerably faint band.

Our results are in agreement with those one obtained by Arslan, Ilhak and Calicioglu (2006). It is possible accurately to identify species in pasteurized and sterilized meat products, however faint beef DNA bands obtained at sterilized products indicate that primers used for amplification of beef mtDNA flank smaller DNA fragment, so that high temperatures and pressure brake down already damaged beef DNA.

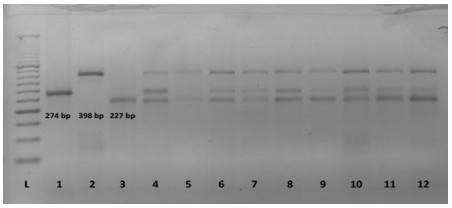

Figure 1. PCR products of pasteurized muscle homogenates. L-Ladder, 1-Beef control, 2-Pork control, 3-Chicken control, 4-6-Muscle homogenates at the 80°C, 7-9- 90°C, 10-12-100°C

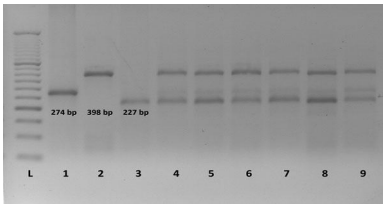

Figure 2. PCR products of sterilized muscle homogenates. L-Ladder, 1-Beef control, 2-Pork control, 3-Chicken control, 4-6-Muscle homogenates 110°C, 7-9- 121°C

4. Conclusion

PCR is fast, more reliable and less labor-intensive way to verify the identity of the amplified product in every sample assayed and can efficiently replace protein-based analytical methods due to low expenses and time savings.

5. Literature

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