5-P12

SPECIES IDENTIFICATION IN MEAT PRODUCTS TREATED UNDER DIFFERENT TEMPERATURES AND HEATING CONDITIONS BY MEANS OF POLYMERASE CHAIN REACTION (PCR) IN COMBINATION WITH RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP)

Rainer Detsch, Karl Otto Honikel and Fredi Schwägele

Institute for Chemistry and Physics, Federal Centre for Meat Research, E.-C.-Baumann-Str. 20, 95326 Kulmbach, Germany

Keywords: Species identification, meat products, DNA, PCR-RFLP

Background:

During the last years species identification has gained more and more importance for consumer protection, due to the open market in Europe and increasing imports of meat and meat products from the rest of the world. Alternatively to methods for animal species identification like separation of proteins by isoelectric focussing techniques (IEF) (Hofmann und Blüchel, 1992) and immunological procedures (ELISA) (Hofmann, 1997a + 1997b) molecular genetic procedures like polymerase chain reaction (PCR) in combination with DNA-sequence analysis (Zimmermann et al.), detection of restriction fragment length polymorphism (RFLP) (Meyer et al., 1995) or single strand conformation polymorphism (SSCP) (Rea et al., 1996) by gel electrophoresis or hybridisation of specific DNA-probes (Meyer et al., 1994) can be applied for the identification of the different animal species in meat and meat products. A fragment of the mitochondrial cytochrome-b gene (359 basepairs) consisting of one variable (307 basepairs) and two conserved domains (26 basepairs each) provides the possibility for identification of the main domestic animal species using specific restriction endonucleases resulting in different DNA-fragment pattern. Up to now there exist only a few detailed informations about molecular genetic methods on species identification in frankfurter type and cooked sausages as well as in canned meat products which were treated under various temperatures for different periods of time.

Objectives:

The aim of the presented studies was to investigate the possibility of application of PCR-RFLP for species identification in dependence upon heat treatment of different meat products e.g. frankfurter type sausages, cooked liver sausages and canned meat. As the fragmentation of DNA corresponds with increasing processing temperatures and DNA-fragments shorter than 200 basepairs cannot be amplified by means of PCR, the amplification should end above certain time / temperature conditions.

Methods:

Frankfurter type sausage was prepared according to a specific "Lyoner" recipe. Ten different samples contained: (1) 96% pork, 1% beef, 1% turkey, 1% chicken and 1% lamb; (2) 1% pork, 99% turkey; (3) 1% pork, 99% chicken; (4) 1% pork, 99% beef; (5) 1% pork, 99% lamb; (6) – (9) 100% of each species; (10) 48% pork, 47% beef, 5% lamb. Several other in composition somewhat different frankfurter type and cooked sausages and also cooked meat (jellied veal) were prepared according to the German "Leitsatz-numbers": 2.212.05 (1. 213); 2.221.05 (1.21); 2.2312.1; 2.2312.1 (1.112); 2.2331.3 with gelatine or gelatine and broth; 2.511.3. Canned meat was manufactured containing a mixture of 45% beef, 45% pork and 10% turkey. The meat was comminuted in a meat grinder and the 200 g cans heated under core temperatures of 117, 118, 120, 122, 123, 126, 127 and 130°C. For characterization of different heat-treatment effects on microorganisms the Fc value was used. The effect of heating 1 minute to 121,1 °C corresponds to a Fc value = 1. Fc values for lower and higher tempertures and longer times are calculated by the addition of Fc values corresponding to different temperatures (table see Tacáks et al. 1969) determined every minute. According to the applied temperatures and heating periods Fc-values between 9,0 to 373 were calculated. Another two cans with beef, pork, poultry, lamb and horse were heated for 30 min at 100°C, respectively for 20 min at 133°C. Species identification was performed either by means of ELISA (Hofmann et al., 1995) or a molecular genetic method using the cytochrome-b gene for amplification by PCR in combination with RFLP (Meyer et al.,

1994). DNA was isolated applying a commercial kit purchased from Macherey and Nagel, Düren, Germany. PCR- or PCR-RFLP-products were detected by Polyacrylamide gelelectrophoresis (PAGE).

Results and discussion:

As shown in table 1 DNA amplification and subsequent species identification by means of RFLP is unrestrictedly possible in the case of frankfurter type and cooked sausages. Up to Fc values of 50 and core temperatures of 127°C DNA-fragments which are long enough (> 200 basepairs) for amplification by PCR can also be isolated from canned meat products. Beginning with core temperatures of 130°C (Fc values ≥ 136) PCR-RFLP for species identification is failing. It must be supposed that after treatment under these conditions the isolated DNA consists already of too small fragments which cannot be amplified and used for further analysis. By means of the meat products manufactured for the here presented studies it could also be demonstrated, that the ELISA test for species identification (pork) is already unsuccessful at core temperatures of 127°C. This means, that the ELISA test specific proteins start to denaturate earlier than mitochondrial DNA. Nevertheless, meat products processed under such extreme conditions are not of importance in practice, because of their poor sensorial characteristics.

Conclusions:

PCR-RFLP is as effective and reliable as the ELISA test for species identification in meat products. In case of doubt it is advisable to apply both procedures.

References: Hofmann, K. (1997) Fleischwirtsch. **77**, 38 – 40. Hofmann, K. (1997) Fleischwirtsch. **77**, 151- 154. Hofmann, K. und Blüchel, E. (1992) Fleischwirtschaft **72**, 85 – 89. Hofmann, K., Fischer, K., Müller, E. und Kemper, V. (1995) Fleischwirtsch. **75**, 1227 - 1230. Meyer, J., Müller, M., Kruse, L., Rüggeberg, H., Ketschau, G. und Hildebrandt, A. (1994) Fleischwirtsch. **74**, 1237 – 1238. Meyer, R., Höfelein, C., Lüthy, J., and Candrian U. (1998) Journal of AOAC 78, 1542 – 1551. Rea, S:, Chikuni, K., and Pavellini, P. (1996) Ital. J. Food Sci. **3**, 211 – 220. Tacáks, J., Wirth F. und Leistner, L. (1969) Fleischwirtschaft **49**, 877 - 883, 1042 - 1047, 1166 - 1172. Zimmermann, S., Zehner, R. und Mebs, D. (1998) Fleischwirtsch. **78**, 530 – 533.

Product:	**Heating temperature/time:**	
Frankfurter type sausages	75 °C /2 h	(+)
Jellied veal	80 °C/ 2 h	(+)
Liver sausage	85 °C/ 1h	(+)
Canned beef	110 °C/ Fc = 0,4	(+)
Canned meat **Autoclave temperature**	**Core temperature / Fc value**	
118 °C	117 °C/ Fc = 9,05	(+)
118 °C	118 °C/ Fc = 13,9	(+)
118 °C	118 °C/ Fc = 17	(+)
121 °C	120 °C/ Fc = 15,4	(+)
126 °C	122 °C/ Fc = 16,8	(+)
130 °C	123 °C/ Fc = 17,4	(+)
130 °C	123 °C/ Fc = 22	(+)
130 °C	126 °C/ Fc = 41	(+)
130 °C	127 °C/ Fc = 50	(+)
130 °C	130 °C/ Fc = 136	(-)
130 °C	130 °C/ Fc = 373	(-)

Table 1:
List of meat products (sausages and canned products) used for species identification performed by means of PCR-RFLP. Frankfurter type and cooked sausages contained beef, pork, lamb, turkey and chicken. Canned meat was manufactured with beef, pork and turkey.
(+) amplification of DNA for all three species used possible. (-) DNA amplification not possible.