Species identification in heat processed meat products

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Introduction

The need for reliable and sensitive methods for meat species identification encompasses many issues including the fraudulent substitution of cheaper/lower quality meats in place of more expensive ones [1]. Besides representing a commercial fraud, incorrect labeling is also a problem for religious issues, since pork meat is forbidden in some religions. Following the European labeling regulations, meat products should be accurately labeled regarding their species content. From several analytical methodologies developed to accomplish meat product authentication, DNA analysis coupled with polymerase chain reaction (PCR) presents a fast, sensitive and highly specific alternative to other methods [2]. The aim of this work was to develop PCR techniques able to identify and quantify different ingredients (pork, poultry and beef) in highly processed meat product, such as Frankfurt sausages.

Metodology

Food samples and standards (binary mixtures with known percentages of pork/poultry) were extracted by two methodologies: the CTAB method, based on liquid-liquid extraction, and the Wizard method, based on silica solid-phase extraction as described by Lipp et al. (1999) [3]. Yield and purity of extracts were assessed by agarose gel electrophoresis and by spectrophotometry.

All PCR amplifications were performed in a iCYCLER BIO-RAD thermocycler using AmpliTaq Gold® DNA polymerase (Applied Biosystems). The oligonucleotide primers used are presented in Table1. The PCR amplification conditions and components used, are presented in Tables 2 and 3. SYBR Green I dye (Applied Biosystems) was used for real-time PCR assays.

Table 1 – Oligonucleotide primers

Species	Primers	Sequence 5'-3'	Amplicon (bp)	Reference
Qualitative PCR				
Pork	*SUS-F*	CTA CAT AAG AAT ATC CAC CAC A	290	Dalmasso *et al* 2004
	SUS-R	ACA TTG TGG GAT CTT CTA GGT		
Poultry	*GAL-F*	TGA GAA CTA CGA GCA CAA C	183	Dalmasso *et al* 2004
	GAL-R	GGG CTA TTG AGC TCA CTG TT		
Cow	*916*	GTA CTA CTA GCA ACA GCT TA	256	Botero *et al* 2003
	1171	GCT TGA TTC TCT TGG TGT AGA G		
Quantitative PCR				
Pork	*Pork-F*	ATG AAA CAT TGG AGT CCT ACT TT TAC C	140	Dooly et al 2004
	Pork-R	CTA CGA GGT CTG TTC CGA TAT AAG G		
Poultry		The same as in qualitative PCR		

Table 2 – PCR Amplification conditions

Target / Primers	Pork Sus-F/Sus-R		Poultry Gal-F/Gal-R		Cow 619/1171		Real-Time PCR	
Steps	**Temp.**	**Time**	**Temp.**	**Time**	**Temp.**	**Time**	**Temp .**	**Time**
Denaturation	94ºC	5 min	94ºC	5 min	94ºC	5 min	94ºC	2.5 min
Amplification	94ºC	30 s	94ºC	30 s	94ºC	30 s	94ºC	30 s
	60ºC	1 min	60ºC	1 min	55ºC	1 min	65ºC	1 min
	72ºC	1 min	72ºC	1 min	72ºC	1 min		
Nº of cycles	35		38		35		45	
Final extension	72ºC	5 min	72ºC	5 min	72ºC	5 min	*	

* Real time-data collection after each cycle; Increse temperature of 0.5ºC (from 65 to 94ºC) and collection of data for melting curve; conditions applied for Pork, Poultry and Soybean PCR-RT tests.

Table 3 – Qualitative PCR components

Component	Reaction volume (µL)		
	Pork	**Poultry**	**Beef**
Ultrapure water	13.8	13.55	14.8
Buffer (10 x)	2.5	2.5	2.5
$MgCl_2$ (25 mM)	2.0	2.25	2.5
dNTPs (2.5 mM each)	2.0	2.0	2.0
Primers (10mM each)	1.25	1.25	0.5
Taq Polimerase (5U/µL)	0.2	0.2	0.2
DNA extract	2.0	2.0	2.0
Total reaction volume	25	25	25

Results

Qualitative PCR

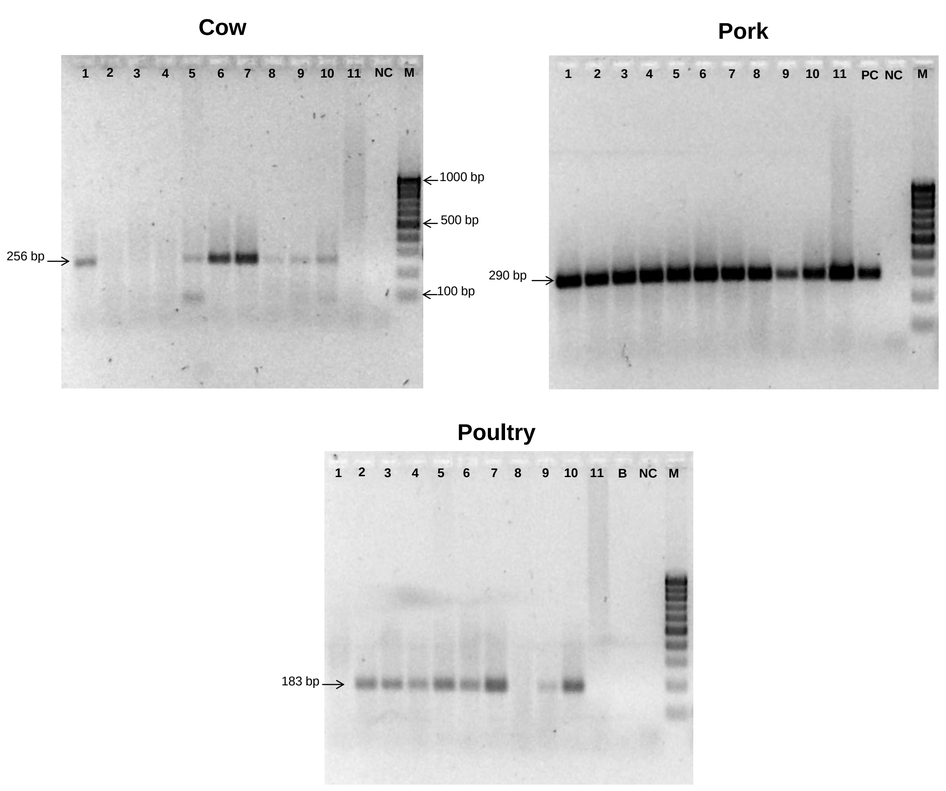

Figure 1 – Agarose gel electrophoresis for PCR products of specific detection of pork, poultry, beef and soybean. M: 100 bp ladder; Samples: 1-11, B: blank, CN: negative control, CP: positive control.

Real-time PCR

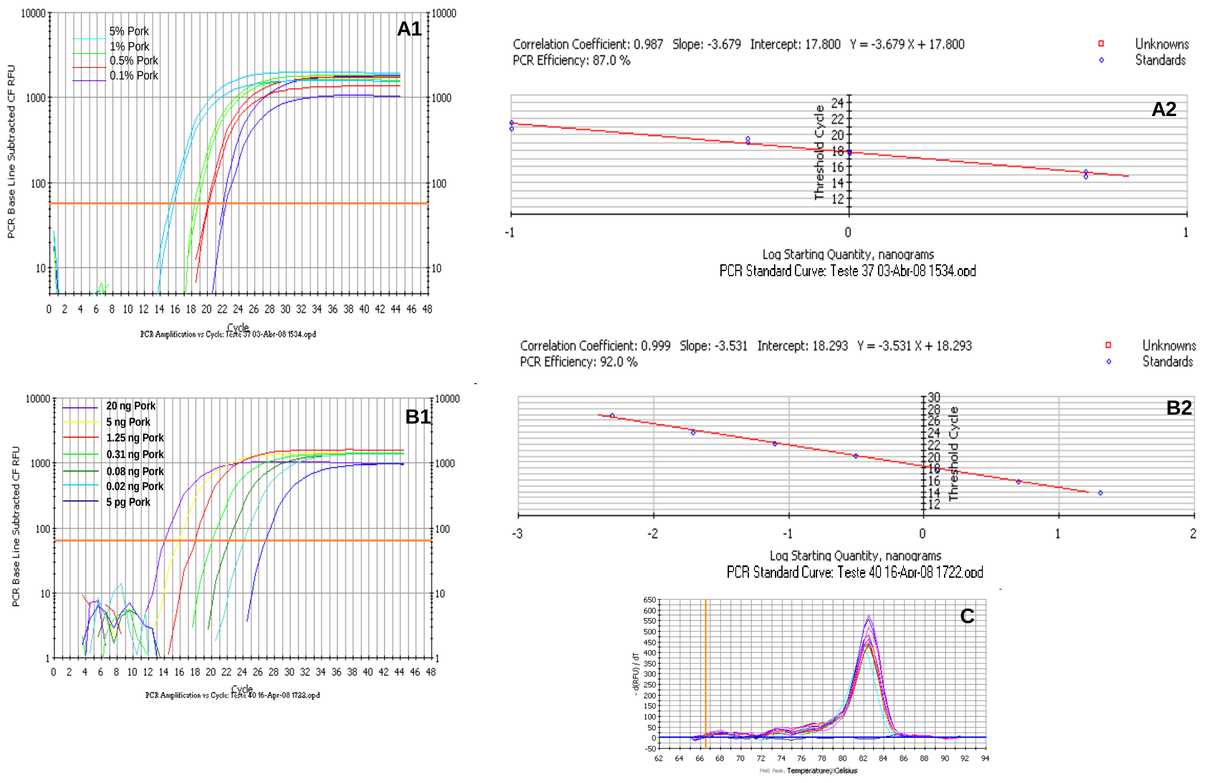

Figure 2 – **A1, B1**: Fluorescence signal with SYBR Green I dye *vs.* Cycle number (A: binary mixtures of pork/soybean; C: 100% pork sample); **A2, B2:** **C:** Melting curves for the 140 bp fragment of pork species.

Table 4 – Agarose gel electrophoresis for PCR products of specific detection of pork, poultry, beef and soybean.

Samples	Pork		Poultry		Milk protein	
	Labelled	Detected	Labelled	Detected	Labelled	Detected
1	✓	✓	✓	X	X	✓
2	✓	✓	✓	✓	✓	X
3	✓	✓	✓	✓	X	X
4	✓	✓	✓	✓	✓	X
5	✓	✓	✓	✓	✓	✓
6	✓	✓	X	✓	✓	✓
7	✓	✓	✓	✓	✓	✓
8	✓	✓	X	X	✓	✓
9	X	✓	✓	✓	✓	✓
10	X	✓	✓	✓	✓	✓
11	✓	✓	X	X	X	X

Conclusion

✓ The developed techniques allowed the detection of the tested species (pork, poultry and cow). The methodologies were successfully applied in commercial samples of frankfurters.

✓ Qualitative PCR assays showed the presence of undeclared pork species in 2 samples, undeclared poultry species in 1 sample and undeclared cow species in 1 sample (Table 4). In one sample, the declared poultry species was not detected. The same happened in 2 samples labelled as containing milk protein.

✓ Real-time PCR assays showed high sensibility (0.1%) for pork (Fig. 2) and poultry (data not shown)

✓ The high real-time PCR efficiency and high correlation coefficients obtained (Fig. A2, B2) suggest that this techniques proved to be adequate for future quantitative assays

References:

[1] Dooley, J. J.; Paine, K. E.; Garrett, S. D.; Brown, H. M. Detection of meat species using TaqMan real-time PCR assays. Meat Sci. 68, 2004, 431-438.
[2] Mafra, I; Ferreira, IMPLVO; Oliveira, MBPP. Food authentication by PCR-based methods. Eur. Food Res.Technol. 22, 2008, 649-665.
[3] Lipp M., Brodmann P., Pietsch K., Pauwels J., Anklam E. IUPAC collaborative trial study to detect genetically modified soy beans and maize in dried powder *JAOAC Int.* 82, 923-928, 1999).