SEMIQUANTITATIVE DETERMINATION OF GOAT TISSUE IN MEAT PRODUCTS BY MEANS OF PCR

Binke, R., Altmann, K., Honikel, K.O., Schwägele, F.
Federal Research Centre for Nutrition and Food (Kulmbach),
E.-C.-Baumann-Straße 20, 95326 Kulmbach, Germany

Background

The "Quantitative Ingredient Declaration" (QUID) for important components in food products was put into force as EU-regulation. Therefore for meat products reliable methods for species identification and quantification gained importance. In a previous paper a species-specific primer system BC290501 for the identification of goat in meat products was presented (Altmann et al., 2004). Both primers bind in the 5´-flanking promotor region of the nuclear single-copy gene *beta-casein.* Twelve different species were tested, but only goat-DNA leads to amplification of a specific product by means of PCR.

At the moment PCR systems for quantification of animal tissue in food or feed are only available for the commercially most important species cattle and pig (Wolf and Lüthy 2001; Calvo et al., 2002; Palisch et al., 2003; Frezza et al., 2003) but not for minor relevant species like goat and sheep.

Objectives

In this paper we report about a PCR assay using two different detection systems based on the primer system BC29051 for semiquantitative determination of goat meat in meat products.

Methods

DNA-extraction was carried out applying the CTAB method optimised for meat and meat products (Binke et al., 2003). Emulsified type sausages with varying goat meat content (Tab. 1) filled into cans with a volume of 50 ml were heated under different temperature regimes: home made cans "KK" (20 min/82 °C; $F_C < 0.9$), "normal" cans "VK" (33 min/116 °C; $F_C = 3.4$), up to cans for use under extreme conditions "TK" (60 min/116 °C; $F_C = 12.3$).

Tab. 1: Composition of emulsified type sausages

Reference product	Ingredients	Goatmeat / muscle meat [%]
Sausage 1	50 % goat meat; 25 % oil; 23 % ice; 1.5 % salt; 0.25 % spices; 0.25 % additives (phosphate; ascorbate)	100
Sausage 2	50 % goat meat; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25% spices; 0.25% additives (phosphate; ascorbate)	100
Sausage 3	25 % goat meat; 25 % pork meat; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25 % spices; 0.25 % additives (phosphate; ascorbate)	50
Sausage 4	10 % goat meat; 40 % pork meat; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25 % spices; 0.25 % additives (phosphate; ascorbate)	20
Sausage 5	1 % goat meat; 49 % pork; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25 % spices; 0.2 5% additives (phosphate; ascorbate)	2.0

The goat specific primers BC290501 F (5´ TCTGGTCCAATTGGTGAGAG 3´) and BC290501 R (5´ AGGCCACAGGTGAAAAAGTC 3´) were commercially synthesized by Qiagen (Hilden, Germany). The amplification products were detected by means of a dual labeled probe BC290501 P (FAM-5´AGGGAAATGTTGAATGGGAAGGATATGC 3´-Tamra) as well as by the intercalating dye SYBR-

Green 1. A 97 bp animal specific DNA fragment based on the myostatin gene, designed by Laube et al., (2002) and a dual labeled probe were used as a reference system for relative quantification.
The amplification of the DNA fragments was performed by Real-Time PCR in a Rotor Gene 2000 with vials containing a final volume of 20 µl: 1x reaction-buffer (Qiagen), 4.5 mM magnesium chloride, 0.05 mM of each dNTP, 0.8 µM of each primer, 0.4 µM dual labeled probe (Qiagen) or 1 µL SYBR-Green 1 diluted 3000 fold, 1.25 unit of HotStarTaq™ DNA-polymerase (Qiagen) and 5 µl DNA solution diluted 40 fold.
The applied PCR-program was the following (40-45 cycles): Initial DNA-denaturation at 95 °C for 15 min; 95 °C for 30 s, 58 °C for 30 s, 65 °C for 30 s for the probe detection system and 72°C for 30 s for SYBR-Green 1 system.
The determination of relative amounts of goat meat per total meat in meat products was calculated according to the equation based on the delta-delta C_T method modified by Pfaffl (2001). Sausage 4 containing 20 % goat meat with a F_C- value at < 0.9 was used as calibrator (tab. 1).

Results and Discussion

For relative quantification it is necessary to have a reference gene which has a constant efficiency for each commercially relevant animal species. DNA from muscle tissue of 12 animal species (Fig. 1) was extracted and diluted under the same conditions.
Figure 1 shows that the most aminal species like goat, pig, cattle have a comparable course of amplification using the myostatin gene. For these animal species the 97 bp fragment of the myostatin gene is suitable as reference gene. In contrast to this the course of amplification in the case of duck, ostrich and kangaroo is different.

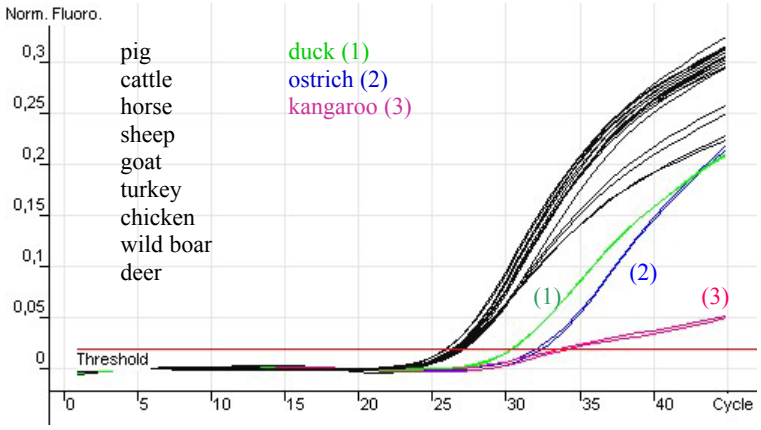

Fig. 1: Course of amplification of the 97 bp myostatin gene fragment of 12 commercially relevant animal muscle tissues

The reaction efficiency is the second critical point for quantification. The efficiency of amplification was calculated for the myostatin and beta-casein systems by means of standard curves (Fig. 2 and 3). A standardized DNA solution (10 µg/ml) obtained from goat meat was 4-fold diluted (2.50, 0.63, 0.16, 0.04 and 0.01 µg/ml). Amplification with a reaction efficiency of 2 means a doubling of amplification product for each cycle resulting in a standard curve graph slope of -3.322. Figure 2 and 3 show a comparable optimum reaction efficiency over 3 magnitudes for the 97 bp myostatin reference gene fragment and the 161 bp goat specific beta-casein gene fragment. A significant difference of efficiency in comparison of the probe and SYBR-Green 1 - systems was not detectable.

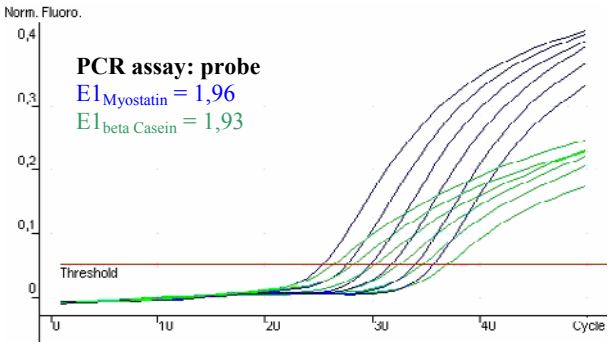

Fig. 2: Determination of reaction efficiencies for the probe assay

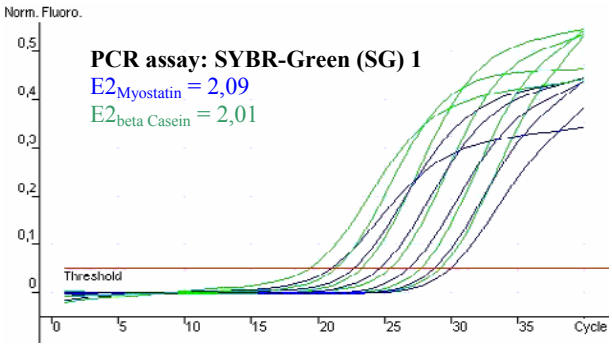

Fig. 3: Determination of reaction efficiencies for SYBR-Green 1 assay

The obtained reaction efficiencies for both assays were applied to determine the content of goat meat in reference products (Tab. 2 and 3). The data show that the probe assay represents a better comparability of the real and calculated content of goat meat than the SYBR-Green 1 assay with a mean coefficient of variation (CV) of 20 %.

Furthermore sausage 2 containing 25 % pork fat shows a lower goat meat content than sausage 1 prepared without pork fat (tab. 1 and 2). Fat tissue contains also DNA like other animal tissues and leads to an increase of total DNA copy number. For this reason the relative content of goat meat is decreasing in comparison to the total meat content. This effect is especially important for the quantification of animal species in liver sausages as liver shows an extremely high DNA content, which is at least tenfold higher than in muscle tissue. In contrast to this the DNA content of fatty tissue is approximately only a quarter in comparison to muscle meat.

With respect to this fact applying this assay a semiquantitative determination of goat meat in meat products is possible for sausages treated at low or medium heating conditions (Tab. 2 and 3).

Tab. 2: Contents of goat meat in reference sausages with standard deviations calculated according to the equation of Pfaffl (2001) in the case of the probe assay (N = 4)

Assay probe	theoretical [% goat]	calculated [%] unheated	calculated [%] KK[1]	calculated [%] VK[2]	calculated [%] TK[3]
Sausage 1	100	103 ± 25	95 ± 25	57 ± 12	29 ± 3
Sausage 2	100	85 ± 7	83 ± 10	60 ± 14	27 ± 7
Sausage 3	50	37 ± 10	43 ± 9	28 ± 6	15 ± 2
Sausage 4	20	20 ± 4	24 ± 2	10 ± 1	8 ± 1
Sausage 5	2	2.3 ± 0.2	2.1 ± 0.1	1.1 ± 0.3	0.3 ± 0.2

Tab. 3: Contents of goat meat in reference sausages with standard deviations calculated according to the equation of Pfaffl (2003) in the case of the SYBR-Green (SG) 1 assay (N = 4)

Assay SG 1	theoretical [% goat]	calculated [%] unheated	calculated [%] KK[1]	calculated [%] VK[2]	calculated [%] TK[3]
Sausage 1	100	129 ± 25	161 ± 22	97 ± 23	63 ± 12
Sausage 2	100	112 ± 27	124 ± 21	98 ± 8	48 ± 4
Sausage 3	50	66 ± 7	73 ± 23	50 ± 12	31± 13
Sausage 4	20	19 ± 2	29 ± 4	14 ± 3	10 ± 2
Sausage 5	2	3.5 ± 1.8	2.6 ± 0.2	1.3 ± 0.2	1.5 ± 1.1

[1]KK = home made cans (F_C-value < 0.9 means 20 min at 82 °C), [2]VK = "normal" cans (F_C-value = 3.4 means 33 min at 116 °C), [3]TK = cans treated for use under extreme conditions (F_C-value = 12.3 means 60 min at 116 °C)

Reference samples heated under extreme conditions are resulting in a significantly reduced content for each sausage. Using the probe assay for samples heated at F_C = 3.4 (VK) a content of about 50 % of the added amounts was determined, whereas samples heated at F_C = 12.3 (TK) showed only about 30 % mean goat meat content (tab. 2). The decreasing content of goat meat is caused by an increase of DNA fragmentation (Binke et al., 2003). Applying a modified myostatin gene fragment with a length of 154 bp (Laube et al., 2002) instead of 97 bp a semiquantitative determination of goat meat in meat products heat treated up to a F_C-value of 3.4 (VK) is also possible. However, the latter system is also not able to quantify the animal species turkey and chicken.

Conclusion

The presented PCR assays are suitable for a semiquantitative determination of goat meat in meat products. A quantitative determination with a small error of determination is only possible if there is sufficient knowledge about the quality and purity of the DNA extracted from processed products. Therefore quantification procedures demand suitable reference standards with a comparable composition and procedure. Further critical points are the specific variations caused by the thermocycler and the DNA extraction as well as the DNA polymerase and the applied dye.
In summary: A quantitative analytical determination of meat (here goat) content in processed food for control of the QUID regulation is not yet possible and needs further studies.

References

Altmann, K., Binke, R. and Schwägele, F. (2004): Qualitative determination of goat in meat products by means of the nuclear single-copy gene beata-casein, Innovations in Food Technology, February 2004, 64-65.
Binke, R., Eichner, R., Zäh, M. and Schwägele, F. (2003): Entwicklung eines leistungsfähigen Extraktionssystems zur Isolierung von Nucleinsäuren aus Fleisch und Fleischerzeugnissen für die PCR, Archiv für Lebensmittelhygiene, 54, 52-55.
Binke, R., Altmann, K. and Schwägele, F. (2003): Influencing factors for the quantification of animal species in meat by means of PCR, Innovations in Food Technology, Issue 21, 130.
Laube, I., Spiegelberg, A., Butschke, A., Zagon, J., Kroh, L.W. and Broll, H. (2002): DNA-analytische Methoden zur Identifizierung der Tierarten Rind und Schwein in Lebensmitteln, BgVV Hefte 03/2002.
Calvo, J. H., Osta, R. and Zaragoza, P. (2002): Quantitative PCR Detection of Pork in Raw and Heated Ground Beef and Pâté, J. Agric. Food Chem., 50, 5265-5267.
Frezza, D., Favaro, M., Vaccai, G., Holst, C., Giambra, V., Anklam, E., Bove, D., Battaglia, P. A., Agrimi, U., Brambilla, G., Ajmone-Marsan, P., and Tartaglia, M. (2003): A Competitive Polymerase Chain Reaction-Based Approach for the Identification and Semiquantification of Mitochondrial DNA in Differently Heat-Treated Bovine Meat and Bone Meal, Journal of Food Protectio, Vol. 66, No. 1, 103-109.
Palisch, A., Mergemeier, S. and Kuhn, M. (2003): Einsatz der Real-Time PCR zur quantitativen Bestimmung des Rinder- und Schweineanteils in Lebensmitteln, Fleischwirtschaft 3, 153-156.
Pfaffl, M.W. (2001): A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acid Research, Vol. 29, No. 9, 2002-2007.
Wolf, C. and Lüthy, J. (2001): Quantitative competitive (QC) PCR for quantification of porcine DNA, Meat Science, 57, 161-168.