Food Chemistry 243 (2018) 82–90

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Development and validation of a fallow deer (*Dama dama*)-specific TaqMan real-time PCR assay for the detection of food adulteration

Maria Kaltenbrunner[a,b], Rupert Hochegger[a], Margit Cichna-Markl[b,*]

[a] Austrian Agency for Health and Food Safety, Institute for Food Safety, Department of Molecular Biology and Microbiology, Spargelfeldstraße 191, 1220 Vienna, Austria
[b] Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Währinger Straße 38, 1090 Vienna, Austria

ARTICLE INFO

Keywords:
Fallow deer
Dama dama
Real-time PCR
Game meat
Food adulteration
Quantification

ABSTRACT

The aim of the present study was to develop a real-time PCR assay for the identification and quantification of fallow deer (*Dama dama*) in food to detect food adulteration. Despite high sequence homology among different deer species, a fallow deer-specific primer/probe system targeting a fragment of the nuclear *MC1-R* gene was designed. This primer/probe system did not amplify DNA from 19 other animals and 50 edible plant species. Moderate cross-reactivity was observed for sika deer, red deer, roe deer, reindeer and wild boar. The LOD and LOQ of the real-time PCR assay were 0.1% and 0.4%, respectively. To validate the assay, DNA mixtures, meat extract mixtures, meat mixtures and model game sausages were analyzed. Satisfactory quantitative results were obtained when the calibration mixture was similar to the analyzed sample in both the composition and concentration of the animal species of interest.

1. Introduction

Some consumers favor game meat over meat from domesticated animals because of its characteristic flavor and tenderness (Hoffman & Wiklund, 2006). Health-conscious individuals might also choose game meat because of its higher content of omega-3 fatty acids compared with, for example, beef (Hoffman & Wiklund, 2006; Poławska, Cooper, Jóźwik, & Pomianowski, 2013). Game meat is popular because it does not contain residues of antibiotics or growth hormones (Fajardo et al., 2006; Hoffman & Wiklund, 2006).

Red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) are the most frequently consumed deer species in Europe. In addition, meat from fallow deer (*Dama dama*) and sika deer (*Cervus nippon*) is commercially available (Fajardo et al., 2006; Hoffman & Wiklund, 2006; Obidziński, Kiełtyk, Borkowski, Bolibok, & Remuszko, 2013). To meet the worldwide increasing demand for game meat, in addition to wild animals living in the forest, deer herds are frequently raised in deer farms (Hoffman & Cawthorn, 2013; Hoffman & Wiklund, 2006).

Since game meat is expensive, it is prone to substitutions with cheaper meat from domesticated animals, such as pigs (Fajardo, González, Rojas, García, & Martín, 2010). According to the Codex Alimentarius Austriacus, 38% (w/w) of the meat in "game" sausage must originate from game species (Codex Alimentarius Austriacus, 2005). If food producers or restaurant owners specify the deer species, e.g., if the product is declared as "fallow deer goulash", then the meat from fallow deer must not be substituted with meat from other deer species, e.g., meat from red deer.

Various methods have previously been developed to detect deer meat adulteration in food, e.g., a PCR-RFLP (restriction-fragment length polymorphism) method (Fajardo et al., 2006), an end-point PCR assay (Fajardo et al., 2007) and a real-time PCR assay (Fajardo et al., 2008). These assays target mitochondrial DNA. Since the copy number of mitochondrial DNA varies between different species and even between different tissue types of the same species, methods targeting mitochondrial DNA are not suitable for quantification (Ballin, Vogensen, & Karlsson, 2009).

To accurately determine the content of deer meat in food, we developed real-time PCR assays targeting single copy genes. Previously, we have presented assays for the quantification of roe deer (Druml, Mayer, Cichna-Markl, & Hochegger, 2015) and the sum of fallow deer, red deer and sika deer (Druml, Grandits, Mayer, Hochegger, & Cichna-Markl, 2015). Since the latter method does not allow distinction between red deer, fallow deer and sika deer, it is not applicable for the verification of the declaration of a certain deer species.

In the present study, we developed a real-time PCR assay for the specific detection and quantification of fallow deer in food. Despite the limited number of gene sequences for fallow deer in the National Center for Biotechnology Information (NCBI) database and the high sequence homology between different deer species, we successfully designed a fallow deer-specific primer/probe system and developed a real-time

* Corresponding author.
E-mail addresses: maria.kaltenbrunner@ages.at (M. Kaltenbrunner), rupert.hochegger@ages.at (R. Hochegger), margit.cichna@univie.ac.at (M. Cichna-Markl).

http://dx.doi.org/10.1016/j.foodchem.2017.09.087
Received 25 February 2017; Received in revised form 25 July 2017; Accepted 17 September 2017
Available online 19 September 2017
0308-8146/

Table 1
Primer and probe sequences tested in this study.

Gene (NCBI acc. no.)	System	Primer/probe sequence (5′–3′)		Length (nt)	Amplicon (nt)
Prion protein PrP (*Prnp*) gene, partial intron 2 sequence and complete cds (AY286007.1)	1a	fw 1	GGTGGCTACATGCTGGGAAGT	21	66
		rev 1	GTCCTCATAGTCATTGCCAAAATG	24	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1b	fw 1	GGTGGCTACATGCTGGGAAGT	21	66
		rev 2	GTCCTCATAGTCATTGCCAAAATG**T**	25	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1c	fw 2	**C**GGTGGCTACATGCTGGG	18	67
		rev 2	GTCCTCATAGTCATTGCCAAAATG**T**	25	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1d	fw 2	**C**GGTGGCTACATGCTGGG	18	67
		rev 1	GTCCTCATAGTCATTGCCAAAATG	24	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1e	fw 3	CT**C**GGTGGCTACATGCTG	18	67
		rev 3	CCTCATAGTCATTGCCAAAATG**T**	23	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1f	fw 5	CT**C**CGTGGCTACATGCTG	18	67
		rev 4	CCTCATAGTCATTGCCAAAATC**T**	23	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1g	fw 4	CT**C**TGTGGCTACATGCTG	18	67
		rev 5	CCTCATAGTCATTGCCAAAATT**T**	23	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1h	fw 3	CT**C**GGTGGCTACATGCTG	18	69
		rev 1	GTCCTCATAGTCATTGCCAAAATG	24	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1i	fw 5	CT**C**CGTGGCTACATGCTG	18	69
		rev 1	GTCCTCATAGTCATTGCCAAAATG	24	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1j	fw 1	GGTGGCTACATGCTGGGAAGT	21	66
		rev 3	CCTCATAGTCATTGCCAAAATG**T**	23	
		probe	CCATGAA**T**AGGCCTCTTA	18	
	1k	fw 1	GGTGGCTACATGCTGGGAAGT	21	66
		rev 4	CCTCATAGTCATTGCCAAAATC**T**	23	
		probe	CCATGAA**T**AGGCCTCTTA	18	
Lactoferrin (Sanger sequencing of a ~200 bp fragment)	2	fw	GACTGGGAGACAGCCTTTGG	20	61
		rev	GCAGTACAGCTCCCAGAAAACA	22	
		probe	CACTGAGGCC**A**CCG	14	
Alpha lactalbumin gene, intron (DQ379356.1)	3a	fw 1	TCAGTAACACCAAATTCCCAGA**A**A	24	76
		rev	ACATCAAGATTCCCAAACAGCTC	23	
		probe	TTTCTTAAAGTTCATGGGTAG	21	
	3b	fw 2	TCAGTAACACCAAATTCCCAGT**A**A	24	76
		rev	ACATCAAGATTCCCAAACAGCTC	23	
		probe	TTTCTTAAAGTTCATGGGTAG	21	
Alpha lactalbumin gene, intron (DQ379356.1)	4a	fw 1	GGCTTATGTGAGAAATCTAATACAGTAATGC	31	74
		rev	GCGCTGC**G**CCAGAGAAG**T**	18	
		probe	CCCTAGGTAAGAACTCCCT	19	
	4b	fw 2	TGAGAAATCTAATACAGTAATGCCTA	26	66
		rev	GCGCTGC**G**CCAGAGAAG**T**	18	
		probe	CCCTAGGTAAGAACTCCCT	19	
MC1-R gene (Y13963.1)	5a	fw	GACACCATGGAGCCACAGATAA	22	65
		rev 1	C**A**GGCAGCTGTGGTGCA**A**	18	
		probe	CGTCGATGACATTGTCCAG	19	
	5b	fw	GACACCATGGAGCCACAGATAA	22	64
		rev 2	**A**GGCAGCTGTGGTGCT**A**C	18	
		probe	CGTCGATGACATTGTCCAG	19	

Bold letters in the primer and probe sequences indicate fallow deer specific bases.
Underlined letters in the primer and probe sequences indicate mismatch bases.

PCR assay for the quantification of the fallow deer content in food.

2. Materials and methods

2.1. Chemicals and food samples

Ethylenediaminetetraacetic acid (EDTA), tris(hydroxymethyl)aminomethane (Tris), sodium chloride (NaCl), hydrochloric acid (HCl), isoamyl alcohol, proteinase K (600 mAnson U/mL) and 2-propanol were purchased from Merck (Darmstadt, Germany). Hexadecyltrimethylammonium bromide (CTAB) was obtained from Sigma Aldrich (St. Louis, Missouri, USA), chloroform was obtained from Ampresco (Solon, Ohio, USA) and ethanol was obtained from VWR Chemicals (Leuven, Belgium). RNase A (85.5 U/mg, 10 mg/mL) and phenol/chloroform/isoamyl alcohol (25:24:1) were purchased from AppliChem (Darmstadt, Germany). Ultrapure water was produced in-house (purity 18.2 MΩ cm at 25 °C).

Game meat samples were provided from Wildpark Ernstbrunn (Ernstbrunn, Austria), the Research Institute of Wildlife Ecology (Vienna, Austria) and the University of Veterinary Medicine Vienna (Vienna, Austria). Meat from domesticated animals, such as pigs, was purchased at local supermarkets and meat markets. The samples were collected from lean muscle meat. Meat was collected between 2015 and 2016 and stored at −20 °C. All animal species were verified through