Food Chemistry 210 (2016) 102–106

Contents lists available at ScienceDirect

Food Chemistry

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

Quantitative detection of pork in commercial meat products by TaqMan® real-time PCR assay targeting the mitochondrial D-loop region

Miju Kim, Insuk Yoo, Shin-Young Lee, Yeun Hong, Hae-Yeong Kim *

Institute of Life Sciences & Resources and Department of Food Science & Biotechnology, Kyung Hee University, Yongin 446-701, Republic of Korea

ARTICLE INFO

Article history:
Received 15 August 2015
Received in revised form 1 April 2016
Accepted 17 April 2016
Available online 19 April 2016

Keywords:
TaqMan real-time PCR
Pork
Mitochondrial D-loop region
Meat products
Quantification

ABSTRACT

The TaqMan® real-time PCR assay using the mitochondrial D-loop region was developed for the quantitative detection of pork in processed meat products. The newly designed primers and probe specifically amplified pork without any cross-reactivity with non-target animal species. The limit of detection of the real-time PCR assay was 0.1 pg of heat-treated pork meat and 0.1% (w/w) pork meat in beef and chicken meat mixtures. The quantitative real-time PCR assay was applied to analyze the pork meat content in 22 commercial processed meat products including jerkies, press hams, sausages, hamburger patties and steaks, grilled short rib patties, and nuggets. The developed real-time PCR method was able to detect pork meat in various types of processed meat products that declared the use of pork meat on their label. All processed meat products that declared no use of pork meat showed a negative result in the assay. The method developed in this study showed sensitivity and specificity in the quantification of pork meat in commercial processed meat products.

1. Introduction

The authenticity of meat products became a major issue for consumers, researchers, and industries after the horse meat scandal in 2013. Meats have been replaced with cheaper meats for economic profit without declaring it on the label of the processed meat products (Fajardo, González, Rojas, García, & Martín, 2010; Mohamad, El Sheikha, Mustafa, & Mokhtar, 2013). The intake of pork causes food allergies in some cases, and eating pork is not allowed for religious reasons in Islamic and Jewish communities. Some countries in the European Union (EU) have established legislation on food labeling to clearly define all raw materials of food products (Ali et al., 2012). The increasing awareness of consumers about the authenticity of meat products requires a highly specific and sensitive quantification method to discriminate pork meat adulterations and verify the honesty of food labeling (Soares, Amaral, Oliveira, & Mafra, 2013).

The methodologies by which species are identified are mainly based either on DNA or protein analyses. Methods using protein analysis have limitations when applied to highly processed meat products due to the aggressive conditions involved in the processing of meat products, such as high heat and pressure. Because DNA has a higher thermal stability than proteins (Lockley & Bardsley, 2000), PCR techniques based on DNA analysis are considered to be specific and sensitive for the identification of animal species in food products (Sentandreu & Sentandreu, 2014). In particular, real-time PCR allows the detection of target animal species in food matrices (Köppel, Zimmerli, & Breitenmoser, 2009) along with the quantitative detection of meat contents in food products (Mohamad et al., 2013). To provide accurate information on the amount of pork meat to consumers, it is important to quantify the content of pork meat in commercial processed meat products. In particular, the probe-based TaqMan® real-time PCR assay can amplify target DNA molecules with specificity, efficiency, and sensitivity. In report by Sakaridis, Ganopoulos, Argiriou, and Tsaftaris (2013), the real-time PCR assay using a dye was performed for discrimination of buffalo meat. The relatively low specificity of real-time PCR assay using a dye compared to the TaqMan® real-time PCR assay was improved by applying high resolution melting (HRM) analysis (Sakaridis et al., 2013). However, the limit of detection of TaqMan® real-time PCR, which would be a key factor for quantitative PCR, was considered to be lower than that of the SYBR Green real-time PCR assay (Fajardo et al., 2010). Thus, the TaqMan® real-time PCR assay is suitable as a powerful quantitative tool to detect pork DNA in processed meat products.

The most important aspect of quantitative PCR is the selection of the target genes used for amplification (Sentandreu & Sentandreu, 2014). Since mitochondrial DNA presents in multiple copies and has a circular shape, analysis using mitochondrial

* Corresponding author.
E-mail address: hykim@khu.ac.kr (H.-Y. Kim).

http://dx.doi.org/10.1016/j.foodchem.2016.04.084
0308-8146/

DNA shows good survival rates in highly processed foods along with a low limit of detection (Mohamad et al., 2013; Sentandreu & Sentandreu, 2014). It is possible to select sequences specific to particular species in mitochondrial DNA because of the hypervariable region in mitochondrial DNA (Fajardo et al., 2008a; Haunshi et al., 2009; Karabasanavar, Singh, Kumar, & Shebannavar, 2014). Due to the higher sensitivity of detection obtained using mitochondrial DNA compared to using nuclear DNA, many studies have used mitochondrial DNA to identify and quantify pork meat in meat products. Martin et al. (2009) selected 12S ribosomal RNA for the detection of pork meat (Martin et al., 2009). Dooley, Paine, Garrett, and Brown (2004), Soares et al. (2013) and Ali et al. (2012) developed pork-specific real-time assays targeting the cytochrome b gene (Ali et al., 2012; Dooley et al., 2004; Soares et al., 2013). The D-loop region has also been frequently chosen for meat discrimination in recent studies (Che Man, Mustafa, Mokhtar, Nordin, & Sazili, 2011; Haunshi et al., 2009; Karabasanavar et al., 2014). However, no study has targeted the D-loop region for the quantitative PCR detection of pork meat.

To assure reproducibility of quantitative PCR measurements, single-copy nuclear DNA has been generally preferred as target gene because of the varied number of mitochondrial DNA among cells (Ballin, Vogensen, & Karlsson, 2009). However, selected fragment from nuclear 18S rRNA gene was co-amplified as an endogenous control for quantitative PCR to obtain reliable measurements in recent reports (Sentandreu & Sentandreu, 2014; Soares et al., 2013). The endogenous control had overcome the limitation of the use of mitochondrial DNA as target gene when the quantification result from species-specific PCR using mitochondria gene was normalized with the result from PCR using nuclear 18S rRNA gene.

Targeting the pork-specific D-loop region of mitochondrial DNA, a TaqMan® probe and primers were developed. A real-time PCR assay was also developed using the pork-specific and universal eukaryotic primers/probes. The assay was also applied to the analysis of commercial processed meat products.

2. Materials and methods

2.1. Samples

A total of 17 authentic muscle samples of pork (*Sus scrofa domestica*), beef (*Bos taurus*), chicken (*Gallus gallus*), turkey (*Meleagris gallopavo*), pheasant (*Phasianus colchicus*), duck (*Anas platyrhynchos*), goose (*Anser anser*), ostrich (*Struthio camelus*), lamb (*Ovis aries*), horse (*Equus caballus*), goat (*Capra hircus*), dog (*Canis lupus familiaris*), deer (*Cervus elephus*), rabbit (*Oryctolagus cuniculus*), cat (*Felis catus*), red kangaroo (*Macropus rufus*), and Korean water deer (*Hydropotes inermis*) were collected from the Conservation Genome Resource Bank (CGRB) and local markets in South Korea. The fresh muscle tissues were cut into small pieces and immediately stored in a freezer at −20 °C until use.

Reference mixtures containing 0.1, 1, 5, 25, 50, and 100% (w/w) of pork in beef and chicken were prepared to create a calibration curve. To apply the quantitative detection method to processed meat products, pork, beef and chicken meats were processed in an autoclave at 121 °C for 15 min at 300 kPa. Then, meat samples were freeze-dried and ground. Each reference mixture was measured to a final weight of 200 mg and used immediately for DNA extraction. Reference mixtures of heat-treated meat were also prepared.

A total of 22 commercial processed meat products were purchased from local markets. Variable types of processed meats including jerkies ($n = 5$), press hams ($n = 5$), sausages ($n = 4$), hamburger patties and steaks ($n = 4$), grilled short rib patties ($n = 1$) and nuggets ($n = 3$) were used. All commercial processed meat samples were ground with liquid nitrogen and stored at −20 °C until use.

2.2. DNA extraction

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions with minor modification. Briefly, 25 mg of each sample was lysed with 360 μL of ATL buffer and 40 μL proteinase K (20 mg/mL) in a water bath at 56 °C for 2 h. After lysis, 4 μL of RNase A (100 mg/μL) was added, and the mixture was incubated at room temperature for 2 min. Subsequently, 400 μL of AL buffer and 400 μL 100% ethanol were mixed. The mixture was eluted into the spin column, and the column was then washed in washing buffers (AW1 and AW2 buffers). Finally, the column-bound DNA was eluted with 50 μL of purified water. The purity and concentration of isolated DNA were measured by a UV spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) and DNA with a 260/280 nm ratio between 1.8 and 2.0 was used as template DNA.

2.3. Primer and probe design

The sequences of the mitochondrial D-loop region for pork and various animal species were downloaded from the GenBank database and were compared with each other by ClustalW alignment analysis (Fig. 1). The pork-specific primers and probe were designed using the Primer Designer program version 3.0 (Scientific and Educational Software, Durham, NC, USA) and synthesized by Bionics (Seoul, Korea) and Bioneer (Daejeon, Korea). The primers and probe used for endogenous control were previously designed targeting the conserved 141-bp fragment in the 18S rRNA gene (Fajardo et al., 2008b). Table 1 shows the nucleotide sequences of the primers and probes used in this study.

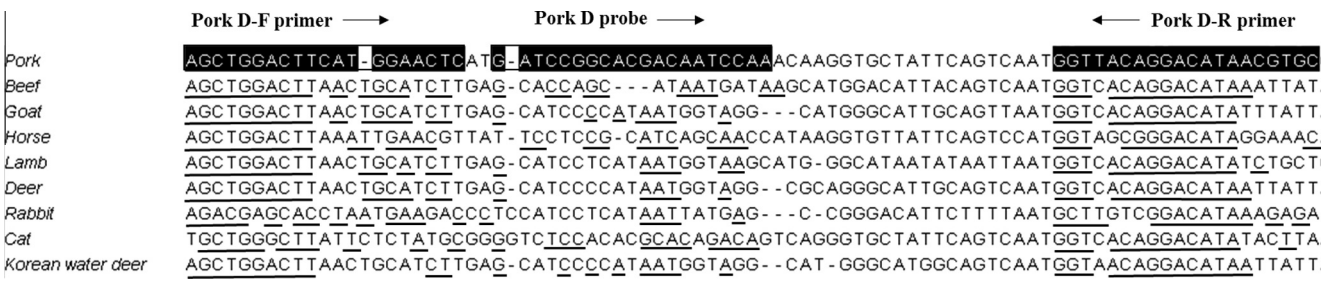

Fig. 1. The sequence alignment of pork specific primers and probe in mitochondrial D-loop gene against other species. Accession numbers of pork, beef, lamb, goat, horse, deer, cat, rabbit, and Korean water deer were AF276931.1, V00564.1, AF089809.1, KP662714.1, NC_001640.1, KP172593.1, NC_001700.1, NC_001913.1 and NC_001821.1 respectively. Target positions for designation of primers and probes were marked with closed boxes and underlines.