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Ultra-fast DNA-based multiplex convection PCR method for meat species identification with possible on-site applications

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ABSTRACT

The aim of this study was to develop an ultra-fast molecular detection method for meat identification using convection Palm polymerase chain reaction (PCR). The mitochondrial cytochrome b (Cyt b) gene was used as a target gene. Amplicon size was designed to be different for beef, lamb, and pork. When these primer sets were used, each species-specific set specifically detected the target meat species in singleplex and multiplex modes in a 24 min PCR run. The detection limit was 1 pg of DNA for each meat species. The convection PCR method could detect as low as 1% of meat adulteration. The stability of the assay was confirmed using thermal processed meats. We also showed that direct PCR can be successfully performed with mixed meats and food samples. These results suggest that the developed assay may be useful in the authentication of meats and meat products in laboratory and rapid on-site applications.

1. Introduction

Food composition and authenticity have become important for safety, religious, and forensic reasons. Moreover, social and/or legal issues regarding the substitution of relatively high-value meats with lower-value meat are becoming more numerous. Hence, routine inspection of meat species is currently attracting increased attention. Therefore, many studies in the meat identification field have been reported and various methods have been developed (reviewed in Kumar et al., 2015).

Earlier methods include chromatography (Ashoor, Monte, & Stiles, 1988) and immunological detection, e.g., enzyme-linked immunosorbent (ELISA) and dot-ELISA assays of particular proteins in the species (Chen & Hsieh, 2000; Haza et al., 1999; Macedo-Silva et al., 2000). However, the biological activity of most proteins is lost upon animal death and some foods are highly processed, while proteins are heat-labile. In addition, immune sera often display cross-reactivity. DNA-based assays are more sensitive and reliable since they are independent of the tissues being compared. DNA is thermo-stable and ubiquitous, present in most cells, and thus it is a better target for meat identification than protein. Hence, the recent development of various molecular methods for meat identification has gained attention. Most of these are polymerase chain reaction (PCR)-based and combined with other molecular biology techniques, such as PCR/sequence analysis (Girish et al., 2004), PCR/restriction fragment length polymorphism (RFLP) (Fajardo et al., 2006; Girish et al., 2005; Partis et al., 2000), PCR-based fingerprinting (Saez, Sanz, & Toldrá, 2004), PCR/terminal RFLP (Wang et al., 2010), and PCR-hybridization (Lin et al., 2014).

Nuclear DNA has been used for species-specific identification of meats (Iwobi et al., 2015; Laube et al., 2007). However, as mitochondrial DNA is more efficient than nuclear DNA (Rastogi et al., 2007), this DNA is a typical target for meat identification using PCR. In most cases, cytochrome b, 12S rRNA, D-loop, or NADH dehydrogenase genes were used as targets (Ali et al., 2015; Bhat et al., 2016; Girish et al., 2004; Hanapi, Desa, Ismail, & Mustafa, 2015; Haunshi et al., 2009; Kesmen, Sahin, & Yetim, 2007; Mane, Mane, Mendiratta, & Tiwari, 2009; Partis et al., 2000; Wang et al., 2010). In this study, we utilized the mitochondrial cytochrome b (Cyt b) gene as the target gene for PCR-based meat identification.

Rapid molecular identification of meat species and direct PCR that does not require prior DNA extraction have recently attracted attention (Lin et al., 2014; Kitpipit, Sittichan, & Thanakiatkrai, 2014). A rapid meat species identification method could be very useful in instances when the number of samples to be tested is large. Such places might include customs houses or large meat processing factories. If the testing speed is increased two times, the number of samples processed is also increased two times. The economic benefit associated with the reduction of processing time

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(or increase in the number of processed samples) would be substantial. In addition, the importance of the reduction of time for forensic sample testing is widely recognized. This also includes forensic animal identification, and thus various attempts have been made to develop rapid on-site testing in this area (Dawnay, Hughes, Court, & Duxbury, 2016; Kitpipit et al., 2014).

Convection PCR uses three heating plates for denaturing, annealing, and polymerization to generate convection in the PCR tube. This method does not require ramping between temperatures employed in conventional thermocyclers. The PCR running time is therefore dramatically reduced in convection PCR. (Hwang, 2011; Hwang, Kim, & Jeong, 2009). Here, we introduce an extremely rapid, simple, and on-site applicable method for the identification of meat species in singleplex and multiplex modes using convection PCR.

2. Materials and methods

2.1. DNA extraction

Meat samples, beef, lamb, and pork, were purchased from commercial sources in Korean markets. To avoid cross-contamination between the meats, each piece of meat was cut with a clean and sterilized scalpel and the inner side or inner surface of the meats was used for sample preparation. DNA was prepared from each meat sample using a DNeasy Blood and Tissue Kit (Qiagen, Germany), as recommended by the supplier. For raw meat samples, 50 mg of each sample was chopped into small pieces before DNA extraction. For thermally processed meat samples, 50 mg of each sample was boiled at 100 °C or autoclaved at 121 °C, for 10 min or 15 min, respectively. After extraction, DNA concentration was measured using a NanoDrop spectrophotometer (Thermo Scientific, USA). DNA samples were serially diluted to prepare samples with designated DNA concentrations. The isolated DNA (1 ng) was used as a template in PCR reactions. Mixed DNA samples were prepared by combining equal amounts of individually isolated DNAs from each meat sample.

2.2. Species-specific primer design and convection PCR

Primers were designed as described by Matsunaga et al. (1999), with modification for convection PCR. Mitochondrial cytochrome b (Cyt b) gene sequences of the three species (beef, lamb, and pork) were obtained from the GenBank database. Accession numbers were as follows: beef, HM045018 (*Bos taurus*, breed: Heck cattle, mitochondrion, complete genome); lamb, NC_001941.1 (*Ovis aries* mitochondrion, complete genome); and pork, AF034253 (*Sus scrofa* mitochondrion, complete genome). These sequences were aligned using Clustal W2 software (http://www.ebi.ac.uk/Tools/clustalw2) (Larkin et al., 2007). One common forward primer (CF) was designed to anneal to the three targets (5′-GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA AA-3′), and three species-specific primers for beef (Beef-R, 5′-CTA GAA AAG TGT AAG ACC CGT AAT ATA AG-3′), lamb (Lamb-R1, 5′-AAA CAT AGC CTA TGA ATG CTG TGG CTA TTG TC-3′), and pork (Pork-R2, 5′-CTG TTC CGA TAT AAG GGA TAG CTG ATA GTA GA-3′) were designed based on detailed analysis and comparison of the aligned Cyt b gene sequences. Detailed primer information is given in Table 1 in Song, Hwang, and Kim (2016).

Convection PCR was performed with a Palm PCR device (G2-12, Ahram Biosystems, Inc., Korea). Unless stated otherwise, DNA amplification was carried out in 20 μL reaction mixtures containing 0.4 U of *PalmTaq* High-Speed DNA polymerase (Ahram Biosystems), 4 μL of 5× *PalmTaq* High-Speed buffer (Ahram Biosystems), 0.2 mM of each dNTP, 1.5 mM $MgCl_2$, 1–0.3 pmol of each forward and reverse primer (IDT, Singapore), and 1 ng of total genomic DNA (gDNA). When different concentrations of primers and template DNA were used, it is stated in the text. The reaction tube was vortex-mixed and centrifuged for 1 min at 8000 rpm. The speed level of convection PCR was set to F3, the annealing temperature was set to 60 °C, and PCR was run over 30 cycles for 24 min, unless stated otherwise. After the completion of PCR, an aliquot of the PCR solution was analyzed by agarose gel electrophoresis. Electrophoresis was performed at 135 V for 20 min in 2% agarose gel (i-My Run electrophoresis system, Cosmo Bio., Japan) in 1× TAE buffer (40 mM Tris, 20 mM acetic acid, and 1 mM EDTA, pH 8.0). PCR products were visualized by fluorescence after ethidium bromide staining, and quantified with a densitometer (Ultra-Lum Imaging System, USA). All experiments were performed at least in triplicate.

2.3. DNA swab sampling procedure

Before swab collection of DNA samples, three solutions were prepared and filtered using 0.1 μm filters: water (Sigma, USA), phosphate buffered saline (PBS; 10 mM Na_2HPO_4, 2.7 mM KH_2PO_4, 137 mM NaCl, and 2.7 mM KCl, pH 7.4), and 1× *PalmTaq* High-Speed buffer containing 1.5 mM $MgCl_2$ (Ahram Biosystems), diluted from a 5× stock provided with the *PalmTaq* High-Speed PCR kit.

Two types of ham, one containing 86.35% of pork (Lotte Food, Korea) and one containing 78.6% of pork with an unspecified amount of beef (Dongwon, Korea), and a sausage (85.6% of pork, Dongwon) were purchased in a Korean market. Single meat pieces, mixed meat samples, or food samples (50–100 mg) were cut into smaller pieces and placed in clean 1.5 mL microfuge tubes. Sterilized cotton swabs were moistened with each solution; wet swabs that were used to repeatedly rub the surface of beef, lamb, or pork pieces were transferred to 1.5 mL microfuge tubes containing 300 μL of each buffer solution. The swabs were shaken well, 0.1 volume of proteinase K (20 mg/mL) was added, and the samples were incubated at 56 °C for 1 h. This was followed by boiling for 5 min, unless indicated otherwise. Sample aliquots (1 μL) were used as DNA templates in subsequent PCR reactions. To reduce the time of the DNA swab preparation procedure, proteinase K incubation and subsequent proteinase K inactivation times were reduced to 10 min and 1 min, respectively. These experiments were performed in a Palm PCR G2-12 device using the isothermal incubation setting.

3. Results and discussion

3.1. Species-specific detection in meat samples using convection PCR in singleplex and multiplex modes

To validate species-specific primers, convection PCR was performed with gDNA isolated from beef, lamb, and pork, and with species-specific primers. As shown in Fig. 1, each primer pair generated species-specific DNA amplicons, 274 bp, 340 bp, and 418 bp, for beef, lamb, and pork, respectively (Fig. 1A). To assess their cross-reactivity, PCR was performed with beef primers using DNA extracted from lamb and pork. No amplification was observed, indicating no cross-reactivity (Fig. 1A, left panel, lanes L and P). Similarly, cross-reactivity of the lamb and pork primers with DNA from other species was tested and no cross-reactivity was observed (Fig. 1A, center panel, lanes B and P; right panel, lanes B and L). The PCR run time was only 24 min, which corresponds to 30 cycles. No unspecific bands were observed when gDNA isolated from other species was used as a template. When combinations of primer sets (multiplex primer sets) were used to identify the meat species, beef, lamb, or pork material was specifically

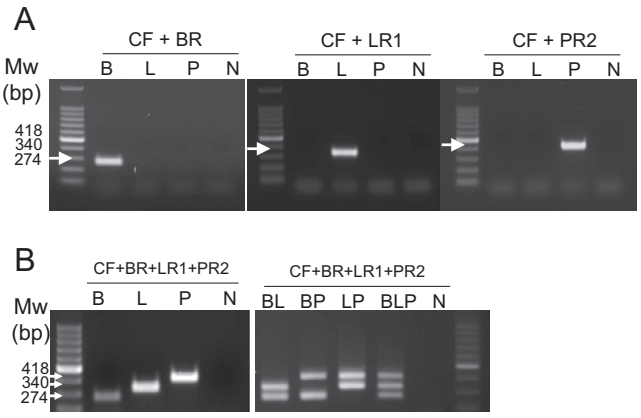

Fig. 1. Species-specific detection of beef, lamb, and pork in singleplex (A) and multiplex (B) PCR. Convection PCR was performed in 24 min, an equivalent of 30 cycles. Isolated gDNA from beef (lane B), lamb (lane L), or pork (lane P) was used as template. Primers were species-specific (A), or a combination of all primer sets was used (B). BL, beef and lamb; BP, beef and pork; LP, lamb and pork; BLP, beef, lamb, and pork; CF, common forward primer; BR, beef reverse primer; LR1, lamb reverse primer; PR2, pork reverse primer; N, no template control; Mw, molecular weight marker.

detected, without background or unspecific bands (Fig. 1B, left). Again, the PCR reaction took 24 min. These observations suggest that convection PCR effectively amplified the signal from every meat species tested and that species-specific primers can be specifically used in 24 min ultra-fast convection PCR. This is the most rapid method of meat species detection that has been reported to date.

It has been reported that multiplex PCR performed using equal amounts of primers does not result in amplicon bands of the same intensity (Ali et al., 2015; Bhat et al., 2016; Matsunaga et al., 1999). Indeed, we observed a similar phenomenon. Hence, we optimized the conditions of multiplex PCR for simultaneous identification of meat species. The primer ratio had to be optimized so that approximately the same signal intensity would be obtained for all amplicons. From the different primer ratios tested (Supplementary Fig. 1), common forward (CF): beef reverse (BR): lamb reverse 1 (LR1): pork reverse 2 (PR2) ratio 8:8:4:3 generated the most comparable intensities of the amplicon bands for beef, lamb, and pork (Fig. 1B, right). Therefore, this primer ratio was used in the multiplex PCR primer set in subsequent experiments. Multiplex PCR has distinct advantages, i.e., lower costs and reagent use than multiple singleplex PCR reactions.

3.2. Meat species detection limit of convection PCR

To determine the meat gDNA detection limit of the developed PCR method, gDNA from each meat species tested was diluted from 1×10^{0} to 1×10^{-4} ng/reaction and singleplex (i.e., with one pair of species-specific primers) or multiplex (with all primer sets) convection PCR was performed, as described in the Materials and methods section (Fig. 2). In the singleplex mode, 1×10^{-3} ng (1 pg) gDNA could be detected for all types of meat used in this study (Fig. 2A). In the multiplex mode, the same amount of gDNA, 1 pg, could be detected for all types of meat used (Fig. 2B). These experiments demonstrated that ultra-fast convection PCR is extremely rapid and very sensitive. The sensitivity is comparable with and/or higher than other reported methods. Detection limits of previously reported end-point PCR methods are approximately 0.25 ng of DNA (Matsunaga et al., 1999), 0.1 ng of DNA (Hanapi et al., 2015), or 0.01–0.02 ng of DNA (Ali et al., 2015; Kesmen et al., 2007). It is generally accepted that real-time PCR is more sensitive than end-point PCR. However, our data revealed that the sensitivity of the assay was higher than or comparable with real-time PCR analyses, where the sensitivity was reported to be 0.15 ng of DNA (Cheng, He, Huang, Huang, & Zhou, 2014).

We also tested the sensitivity of meat detection in mixed mode reactions, to determine the limit of detection in adulterated meats. Genomic DNA from two kinds of meat were mixed at different ratios (100:0, 90:10, 70:30, 50:50, 30:70, 10:90, and 0:100) and tested in combinations: beef with lamb, lamb with pork, and pork with beef (Fig. 3A). As shown in Fig. 3A, a gradual increase of one meat signal and gradual decrease of the other meat signal were observed. We also mixed gDNAs from two kinds of meat in 100:0, 90:10, 99:1, and 0:100 ratios, and demonstrated that as little as 1% adulteration can be detected in beef/lamb, lamb/pork, and pork/beef mixtures (Fig. 3B). However, it is difficult to detect 1% of meat species in a mixture; it may be concluded that our method can detect as little as 1–5% of meat species in the mixture.

A previously reported meat identification technique gave unsatisfactory results with meat mixtures (Girish et al., 2005). Most approaches detected meat adulteration or contamination at the 1% level (Bhat et al., 2016; Calvo, Rodellar, Zaragoza, & Osta, 2002; Hsieh & Ofori, 2014; Köppel, Eugster, Ruf, & Rentsch, 2012; Mane et al., 2009). These data are comparable with those from the present work.

3.3. Detection of meat species in processed meat samples

Detection of meat species in thermally processed meat samples was next performed. Each meat sample was cooked at 100 °C for 10 min or autoclaved at 121 °C for 15 min, and gDNA was isolated and subjected to convection PCR (Fig. 4). Each type of meat was unambiguously detected with species-specific primer sets. Our data demonstrated that, although DNA might be degraded and/or altered by a high temperature treatment, different meat species were still unequivocally detected by convection PCR. When multiplex primer sets were used to identify processed meat species (treated at 100 °C for 10 min), specific DNA amplicons were detected without background or unspecific bands (Fig. 4B, lanes B, L, and P). Multiplex PCR with a combination primer set and multiple templates also resulted in a specific and clear detection of processed meats (Fig. 4B, lanes BL, BP, LP, and BLP). These observations suggest that convection PCR effectively amplified the signal from each processed meat species tested.

Each cell contains large numbers of mitochondria, and each mitochondrion possesses multiple copies of the mitochondrial genome. Hence, the odds of mitochondrial DNA surviving an extreme heating treatment or other processing method are high (Girish et al., 2004). Thus, our mitochondrial gene target was sufficiently robust to be used as a marker after thermal meat processing. Meat mitochondrial DNA serves as a more efficient marker than nuclear DNA because of its high copy number and heat stability (Rastogi et al., 2007).

DNA bands of weaker intensity were previously observed for heat-processed meats because of DNA fragmentation during the processing (Bhat et al., 2016). Another report stated the difficulty of amplifying DNA from cooked meats (Matsunaga et al., 1999). The authors speculated that, when meat is heat-processed, >439 bp DNA fragments would be more affected than smaller DNA fragments (Matsunaga et al., 1999). However, our data showed that the results of DNA amplification by convection PCR were not significantly affected by thermal processing of the samples. Even large DNA fragments (418 bp, a size similar to 439 bp that was reported as recalcitrant to amplification) were successfully amplified using convection PCR after thermal meat processing.

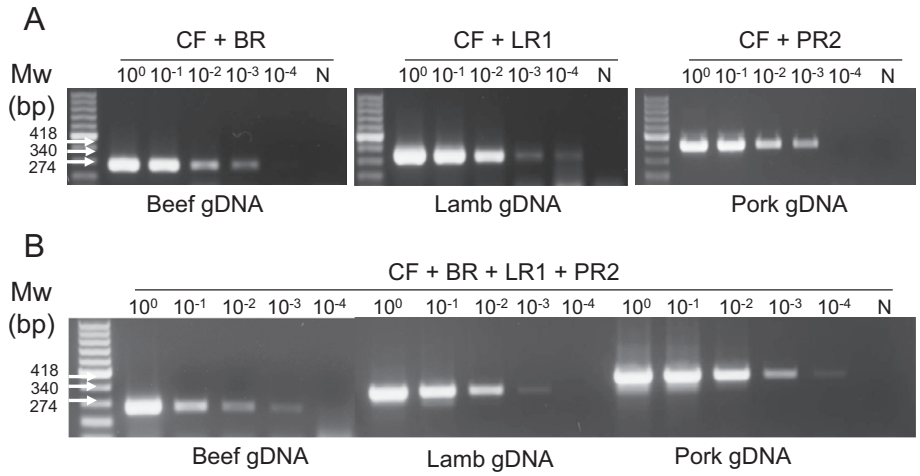

Fig. 2. Determination of gDNA detection limits of singleplex (A) and multiplex (B) convection PCR modes. Genomic DNA of each meat species was serially diluted and used as template. Species-specific primer sets (CF and BR, LR1, or PR2) were used in singleplex convection PCR, and a mixture of all primers (CF, BR, LR1, and PR2) was used for multiplex convection PCR. N, no template control; Mw, molecular weight marker.

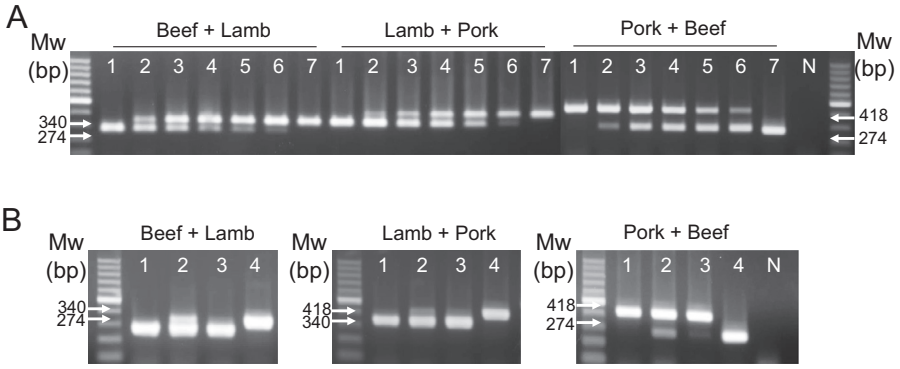

Fig. 3. Detection of meat species in mixtures. (A) Different gDNA ratios of the two indicated meat species were used, as follows: 100:0 (lane 1), 90:10 (lane 2), 70:30 (lane 3), 50:50 (lane 4), 30:70 (lane 5), 10:90 (lane 6), and 0:100 (lane 7). Gradual converse changes of band intensities were observed. (B) The ratios of the two indicated meat species were varied, as follows: 100:0 (lane 1), 90:10 (lane 2), 99:1 (lane 3), and 0:100 (lane 4). Convection PCR was run for 24 min (30 cycles). N, no template control; Mw, molecular weight marker.

3.4. Direct-convection PCR for on-site applications

If the meat identification methods developed in this study could be used in on-site applications, their meat identification usage would greatly increase. One of the obstacles when translating a laboratory method into an on-site application is the complexity of the DNA purification process. Hence, we used cotton swabs to collect DNA from meat samples. The meat surface was scrubbed several times with a wet swab, the swab was rinsed with PBS, *Taq* DNA polymerase buffer, or distilled water, and the sample was then treated with proteinase K and used for convection PCR. DNA amplicons were produced from samples with PBS or *Taq* DNA polymerase buffer as rinsing buffers. When distilled water was used as the rinsing buffer, only faint or weak bands were obtained (Fig. 1 in Song et al., 2016).

Speed is another important factor to consider in on-site applications. Thus, we attempted to reduce the times of proteinase K treatment and enzyme heat-inactivation. Proteinase K was used at 2 μg/μL, and the proteinase K treatment time was reduced from 30 to 20 and 10 min. The tested heat-inactivation time was 1 or 3 min. The reduction of proteinase K treatment and heat-inactivation times did not significantly affect DNA amplicon yields. When the amount of proteinase K was reduced from 2 to 1 and 0.2 μg/μL, again, the DNA amplicon yield did not significantly change (Fig. 2 in Song et al., 2016). These suggest that the DNA purification time can be reduced to 11 min. If we include the time of swab scrubbing (0.5 min), centrifugation after heat-inactivation (1 min), and convection PCR (24 min), the time from DNA isolation to PCR amplification takes ~36.5 min. If we include the time of agarose gel electrophoresis (~20 min), the total meat identification procedure (sampling, DNA isolation, PCR amplification, and detection) takes less than 60 min.

Since proteinase K treatment is optimized for convection PCR, we used a Palm PCR device for the entire heating process. The isothermal step 2 protocol of 56 °C for 10 min and 95 °C for 1 min was used for pretreatment, followed by subsequent convection PCR amplification (Fig. 5).

To check the specificity and cross-reactivity of swabbed samples, PCR was performed with each meat primer set using swabbed DNA from beef, lamb, and pork pieces. Species-specific DNA amplification was observed with species-specific primers (Fig. 5A, left panel, lane B; center panel, lane L; and right panel, lane P), which revealed that the reaction specificity is maintained in the modified on-site applicable method. Beef-specific primers led to no DNA amplification products in lamb or pork samples, showing that there is no cross-reactivity with the modified method (Fig. 5A, left panel, lanes L and P). Similarly, the cross-reactivity of lamb- and pork-specific primers with DNA from other species was tested

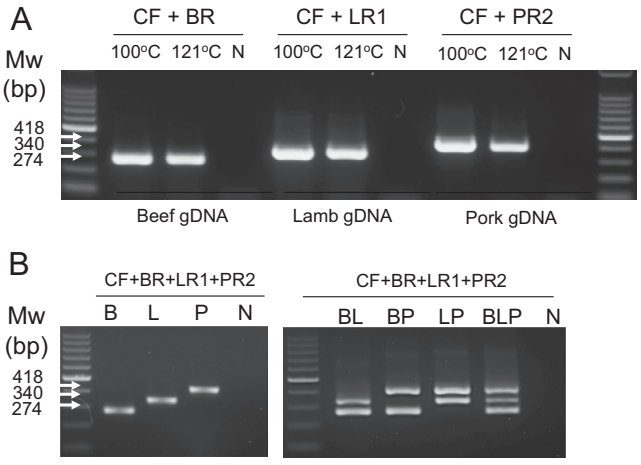

Fig. 4. Identification of thermally processed meats using convection PCR with species-specific primers in singleplex (A) and multiplex (B) PCR modes. A small piece of each meat was boiled at 100 °C for 10 min or autoclaved (at 121 °C for 15 min). Isolated gDNA from beef (lane B), lamb (lane L), or pork (lane P) was used as template. Either species-specific primers (A) or a combination of all primer sets (B) was used. BL, beef and lamb; BP, beef and pork; LP, lamb and pork; BLP, beef, lamb, and pork; CF, common forward primer; BR, beef reverse primer; LR1, lamb reverse primer; PR2, pork reverse primer; N, no template control; Mw, molecular weight marker.

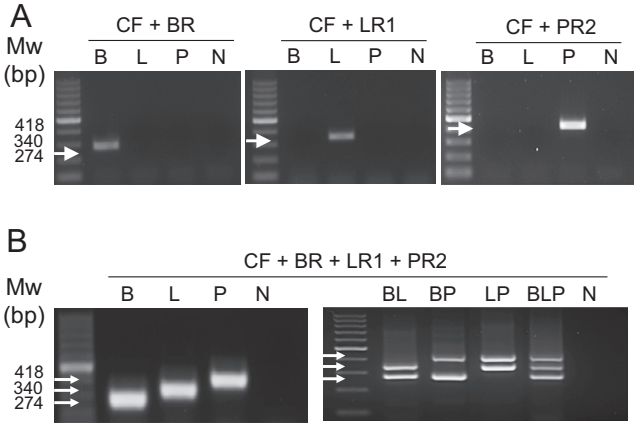

Fig. 5. Direct-convection PCR for meat identification for on-site applications. Following swab sampling, convection PCR was performed in a single device, in singleplex (A) and multiplex (B) modes. Swabbed samples of beef (lane B), lamb (lane L), or pork (lane P) were used as templates. Species-specific primer sets (CF and BR, LR1, or PR2) were used in singleplex convection PCR, and a mixture of all primers (CF, BR, LR1, and PR2) was used for multiplex convection PCR. N, no template control; Mw, molecular weight marker.

and no cross-reactivity was observed (Fig. 5A, center panel, lanes B and P; right panel, lanes B and L). When multiplex primer sets with single meat swab samples were used, every meat species (beef, lamb, and pork) was specifically identified, without non-specific band formation (Fig. 5B, left panel). Again, when multiplex primer sets with mixed meat swab samples were used, duplex or triplex amplification products were clearly detected (Fig. 5B, right panel). We also tested the sensitivity of meat detection in swab samples of mixed meats. Swab samples were acquired from beef and lamb samples that were mixed at different ratios, and convection PCR was performed. A gradual increase of the lamb signal and a gradual decrease of the beef signal were observed (Fig. 3A in Song et al., 2016). When multiplex convection PCR was performed with serial dilutions of swab samples, DNA amplification was observed with 1000 times diluted swab samples (Fig. 3B in Song et al., 2016).

We then attempted to detect meat species in the actual cooked food samples. Three cooked food samples were used: ham and sausage containing only pork (sample 1: 86.35% pork and sample 2: 85.6% of pork) and ham containing pork and beef (sample 3: 78.6% comminuted pork and unspecified amount of beef). The content of beef was not stated; however, considering that the total meat content was approximately 86 ± 5% in the other two food samples, the content of beef could be <8% in sample 3. DNA from each cooked food sample was prepared as described in the Materials and methods section. Direct-convection PCR was performed with multiplex primer sets (Fig. 6). As expected, only pork was detected in samples containing pork (lanes 1 and 2 in Fig. 6). When the food sample contained pork and beef, two bands of expected sizes indicating pork and beef were detected (lane 3 in Fig. 6). The band intensity of pork beef was approximately 10:1 which corresponds to the expected contents of pork and beef in sample 3 (78.6:8 ≅ 10:1). These data suggest that the modified protocol works well with the actual cooked food samples where the matrix is more complex than in raw meat samples.

One of the most important requirements when developing DNA-based meat identification methods for on-site applications is short operation time. This includes the time of DNA preparation, PCR, and detection. The direct-convection PCR method developed in this study could be performed in 1 h. This is the fastest DNA-based meat identification assay reported to date. DNA-based multiplex PCR assays performed with conventional PCR devices take ca. 1.5–3 h of PCR runtime, depending on parameter settings (Ali et al., 2015; Karabasanavar, Singh, Kumar, & Shebannavar, 2014; Kesmen et al., 2007). If DNA extraction time and gel electrophoresis are included in the calculation, the process would take longer, ca. 3–4 h. A direct multiplex PCR assay was reported previously and took ca. 90 min (Kitpipit et al., 2014). The direct-convection PCR method reported here does not require expensive equipment, e.g., a real-time PCR instrument, and reduces the time and cost of DNA preparation. Another beneficial aspect stems from the fact that the convection PCR machine used in this study is a battery-operated, hand-held portable device. Thus, it can be used for on-site meat identification when a regular electricity supply is not available.

Our assay requires a very small amount of the sample (simply scrubbing the meat surface with a swab is sufficient) to achieve successful DNA amplification. This suggests that a small amount of DNA is sufficient for meat identification. The small sample requirement is advantageous during meat detection and identification when, typically, trace amounts of contamination or adulteration are to be detected.

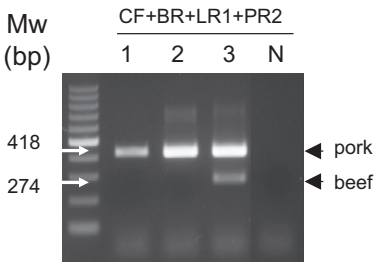

Fig. 6. Direct-convection PCR with cooked food samples. For direct-convection PCR, swab samples were prepared as described in Materials and methods, and convection PCR was performed in the multiplex mode. The samples used were ham containing 86.35% of pork (lane 1), a sausage containing 85.6% of pork (lane 2), and ham containing 78.6% of pork and <8% of beef (lane 3). N, no template control; Mw, molecular weight marker.

4. Conclusions

In the present study, we demonstrated that a detection of meat species is achievable using convection PCR at ultra-fast speed, in 24 min. The detection can be achieved using both singleplex and multiplex regimes. The detection sensitivity was as low as 1 pg of gDNA for each meat species used in this study. This method can detect 1% adulteration in raw meats and also in thermally processed meats. To examine on-site application of the method developed in this study, we tested direct-convection PCR with a PCR buffer provided by the manufacturer and raw meats and actual cooked food samples. Obvious DNA amplification was observed in samples acquired by scrubbing the meat surface with cotton swabs. The total time required for sample preparation and PCR amplification was reduced to 36.5 min. If we include the time of agarose gel electrophoresis, the whole process can be performed within 1 h.

We believe that the ultra-fast speed and sensitivity of the molecular detection method presented in this study offer a reliable detection strategy for meat species identification. The method can be easily extended to other commercial meat products of varying matrix and composition. In addition, this method may be used for ultra-fast detection and identification of meats in laboratory and on-site applications.

Conflict of interest

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2017.02.085.

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