Rapid detection of porcine DNA in meatball using recombinase polymerase amplification couple with lateral flow immunoassay for halal authentication

ABSTRACT

Point-of-care diagnostic methods for animal species determination are critical for rapid, simple, and accurate enforcement of food labelling. PCR is the most common method for species identification. However, the requirement of using a thermal cycler created drawbacks for the PCR application, particularly in low-resource settings. Hence, in this study, a method for porcine DNA detection using recombinase polymerase amplification (RPA), coupled with nucleic acid lateral flow immunoassay (NALFIA), was developed. Porcine-specific primers targeting pig (Sus scrofa) cytochrome b gene fragments specifically amplify a 197 bp fragment of the mitochondrial gene as being visualized by 2% agarose gel and PCRD NALFIA. The reaction temperature and time were 39 °C and 20 min, respectively. Herein, the specificity of the primers to porcine was confirmed after being assayed against six animal species, namely cow, goat, chicken, duck, dog, and rabbit. The porcine-specific RPA assay shows a high limit of detection of 0.01 ng/μL pork DNA. Based on the preliminary performance data obtained from this study, the potential of this method as a rapid and sensitive tool for porcine DNA detection in meat-based products is foreseen.