

Duplex Real-Time Loop-Mediated Isothermal Amplification Assay for Simultaneous Detection of Beef and Pork

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Abstract : Product mislabeling and adulteration have been increasing the concerns in processed meat products. Relatively inexpensive pork meat compared to meat such as beef was adulterated for economic benefit. These food fraud incidents related to pork were concerned due to economic, religious and health reasons. In this study, a rapid on-site detection method using loop-mediated isothermal amplification (LAMP) was developed for the simultaneous identification of beef and pork. Each specific LAMP primer for beef and pork was designed targeting on mitochondrial D-loop region. The LAMP assay reaction was performed at 65 °C for 40 min. The specificity of each primer for beef and pork was evaluated using DNAs extracted from 13 animal species including beef and pork. The sensitivity of duplex LAMP assay was examined by serial dilution of beef and pork DNAs, and reference binary mixtures. This assay was applied to processed meat products including beef and pork meat for monitoring. Each set of primers amplified only the targeted species with no cross-reactivity with animal species. The limit of detection of duplex real-time LAMP was 1 pg for each DNA of beef and pork and 1% pork in a beef-meat mixture. Commercial meat products that declared the presence of beef and/or pork meat on the label showed positive results for those species. This method was successfully applied to detect simultaneous beef and pork meats in processed meat products. The optimized duplex LAMP assay can identify simultaneously beef and pork meat within less than 40 min. A portable real-time fluorescence device used in this study is applicable for on-site detection of beef and pork in processed meat products. Thus, this developed assay was considered to be an efficient tool for monitoring meat products.

Keywords : beef, duplex real-time LAMP, meat identification, pork

Conference Title : ICFARD 2017 : International Conference on Food Adulteration and Rapid Detection

Conference Location : Copenhagen, Denmark

Conference Dates : October 02-03, 2017