Development of a New Generation Quantitative Multiplex PCR Kit for Detection of Adulteration in Meat Products

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ABSTRACT

Meat is one of the basic foods that has a very important place in human nutrition. Today, mostly due to the increase in food prices, imitation and adulteration in food has increased considerably. In this respect, determination of meat types used in a meat product is very important in terms of protecting consumers and preventing food adulteration. Quantitative PCR (qPCR) is one of the laboratory methods that give high precision results with high sensitivity. The aim of this study is to produce a multiplex meat species-specific qPCR kit that can detect horse, donkey, bovine, pork species in one reaction tube using species-specific probes. Initially, the primers to be used in this study were designed from conserved gene sequences common to the species. Taqman probes were designed to distinguish between horse-donkey, bovine and pork species. DNA was isolated from the species to be detected. After singleplex qPCR studies performed for each primer-probe set, multiplex qPCR studies were performed for simultaneous detection of species. In multiplex reactions, DNA from each target species has been successfully amplified. No peaks were observed in no-template negative control reactions, indicating that the kit is free of false positive results. In addition, species-specific probes did not amplify other species meaning that there is no cross-species amplification. In conclusion, a multiplex kit was produced that simultaneously detects four different species with high sensitivity. This kit can be used to increase food safety and prevent adulteration in meat products and saves time and cost.

INTRODUCTION

The determination of meat types used in a meat product is very important in terms of protecting consumers and preventing food adulteration [2]. The significant majority of the qPCR kits available for detecting meat species on the market consist of lyophilized primer-probe mixtures [5], and usually only one species can be detected in a tube [1]. In multiplex reactions, at most three species can be detected at the same time [5]. This study aims to produce a multiplex qPCR meat species imitation and adulteration detection kit that can detect horse, donkey, bovine and pork species in a single reaction tube using species-specific probes.

MATERIALS AND METHODS

The primers used in this study were designed from conserved gene sequences common to the species (PrimerBioSoft program and NCBI-Blast method). Taqman probes, which are preferred due to their improved specificity, higher signal rate and ability to perform multiplex reactions, are species-specific to distinguish horse-donkey, bovine and pork species using different dyes (Figure 1) [1,3,4]. The Taqman probe sequence was designed to hybridize with the amplified gene region and was selected to be closer to one of the primers to be approximately 20-25 base pairs [4].

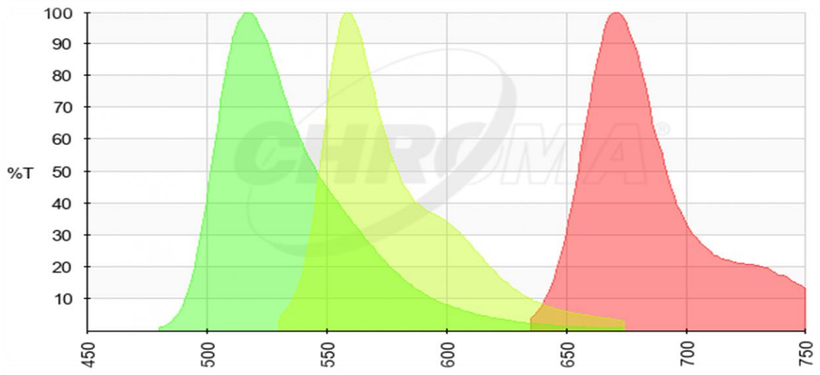

Figure 1: Wavelength (nm) for FAM, HEX and CY5 dyes [8]

Initially, DNA was isolated from the species to be detected in the study (EcoPURE Genomic DNA Kit). DNA was isolated from the tissue of a pork, from the blood of a horse and a bovine, respectively, according to the manufacturer's recommendations. For the donkey, synthetic DNA obtained with the Gibson Assembly Protocol was used [6]. The qPCR reactions were carried out in 20 µl volume. 100 ng of DNA was added to the qPCR reaction for all species [4]. QPCR studies were performed in the Biorad CFX96 instrument using primers and specific probes specific for each species, dNTP, MgCl2, and Vazyme Taq polymerase(Cat no:Q611-01:100 rxn) [7]. Double, triple and quadruple multiplex qPCR studies were performed for the simultaneous detection of species. In multiplex reactions, the DNA of each target species was successfully amplified. It shows that the kit does not contain false positive results as no peaks were observed in the negative control reactions.

RESULTS

The results of the qPCR study to detect the species are as follows on the right side: Figures 2-8

FAM: HORSE AND DONKEY
HEX: BOVINE
CY5: PORK
FOR QPCR REACTIONS

DISCUSSIONS AND CONCLUSIONS

This study showed that horse, donkey, bovine and pork species can be distinguished from each other with high specificity and in a short time in a multiplex approach. Thus, it can be used successfully in routine control experiments to prevent adulteration and imitations in meat products and to detect unwanted meat types in food products around the world. As a result, it can be ensured that unfair gains are prevented and consumer health is protected.

REFERENCES

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[8] https://www.chroma.com/spectra-viewer

SINGLEPLEX STUDIES

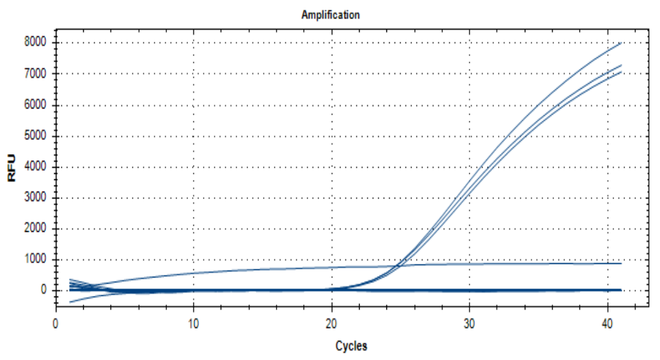

Figure 2: Singleplex qPCR experiment in horse species is FAM dye which is blue

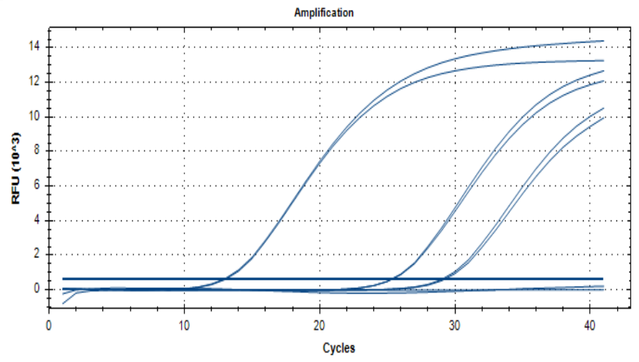

Figure 3: Singleplex qPCR experiment in donkey species is FAM dye which is blue

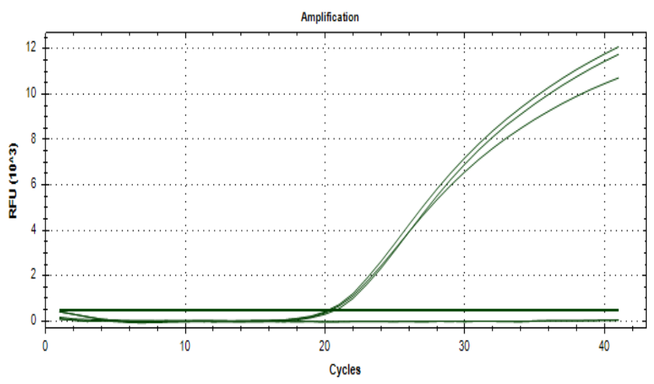

Figure 4: Singleplex qPCR experiment in bovine species is HEX dye which is green

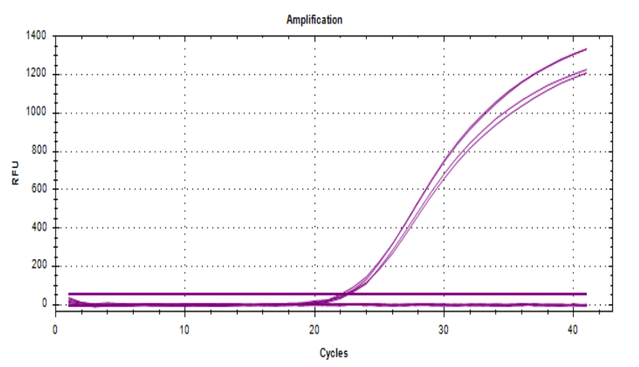

Figure 5: Singleplex qPCR experiment in pork species is CY5 dye which is purple

MULTIPLEX STUDIES

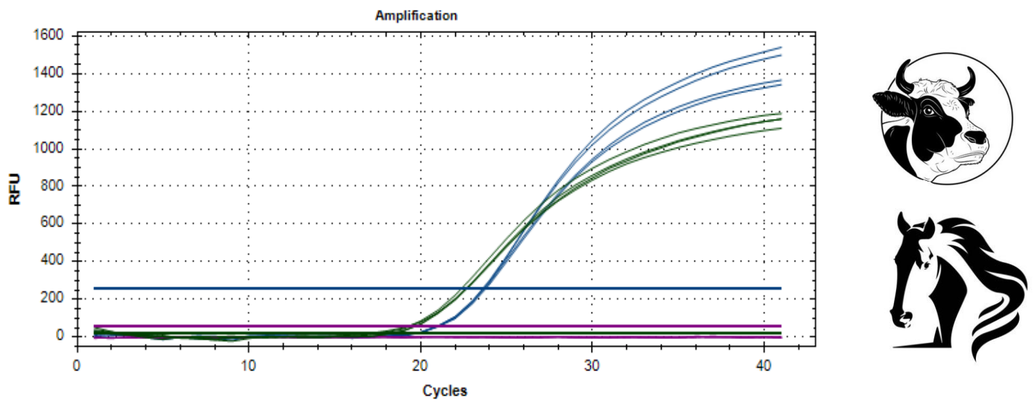

Figure 6: Multiplex qPCR experiment in horse and bovine species are FAM and HEX dyes which are blue and green

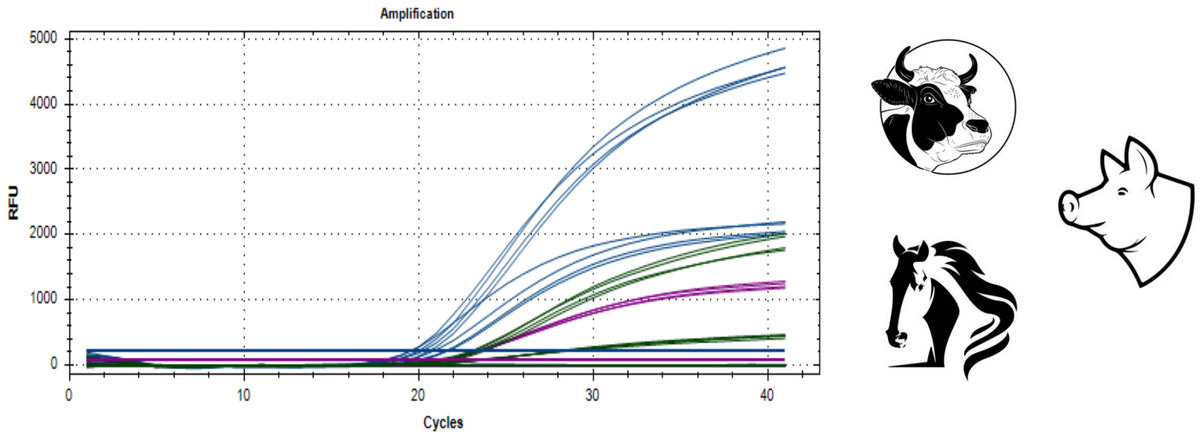

Figure 7: Multiplex qPCR experiment in horse, bovine and pork species are FAM, HEX and CY5 dyes which are blue, green and purple

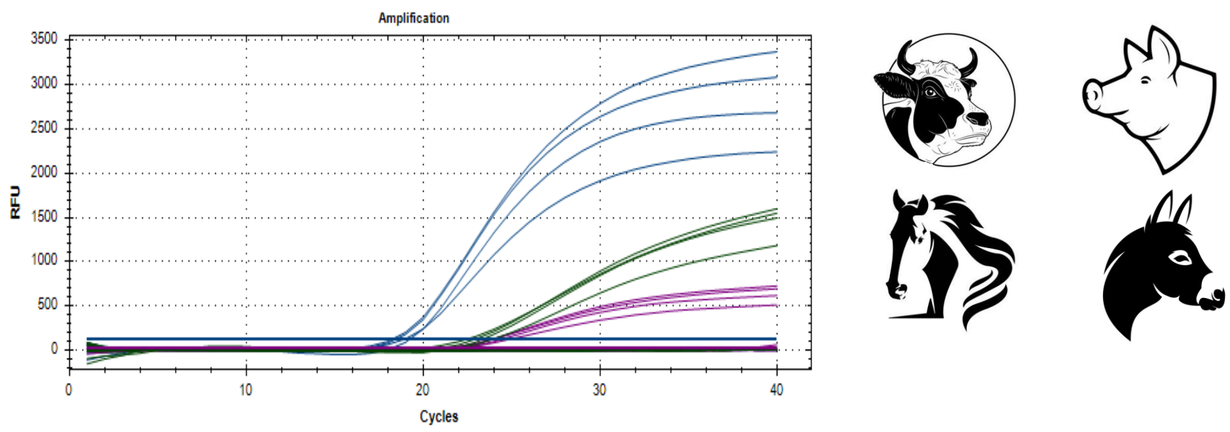

Figure 8: Multiplex qPCR experiment in all species are FAM, HEX, CY5 dyes which are blue, green and purple