Duplex real-time PCR assay using SYBR Green to detect and quantify Malayan box turtle (Cuora amboinensis) materials in meatsballs, burgers, frankfurters and traditional Chinese herbal jelly powder

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Abstract

The Malayan box turtle (Cuora amboinensis) (MBT) is a vulnerable and protected species widely used in exotic foods and traditional medicines. Currently available polymerase chain reaction (PCR) assays to identify MBT lack automation and involve long targets which break down in processed or denatured tissue. This SYBR Green duplex real-time PCR assay has addressed this research gap for the first time through the combination of 120- and 141-bp targets from MBT and eukaryotes for the quantitative detection of MBT DNA in food chain and herbal medicinal preparations. This authentication ensures better security through automation, internal control and short targets that were stable under the processing treatments of foods and medicines. A melting curve clearly demonstrated two peaks at 74.63 +/- 0.22 and 78.40 +/- 0.31 degrees C for the MBT and eukaryotic products, respectively, under the combination of 120- and 141-bp targets from MBT and eukaryotes. The assay was used to screen 100 commercial samples of traditional Chinese herbal jelly powder from eight different brands; 22% of them were found to be MBT-positive. Detection of MBT DNA in food chain and herbal medicinal preparations is a growing concern as MBT materials are widely used in Chinese herbal desserts, exotic dishes consumed with the hope of prolonging life and youth.

Keywords

Author Keywords: Malayan box turtle; SYBR Green real-time PCR; limits of detection and quantification; protected species; herbal jelly powder

KeyWords Plus: POLYMERASE-CHAIN-REACTION; DEER CAPREOLUS-CAPREOLUS; CYTOCHROME-B GENE; MEAT-PRODUCTS; SPECIES IDENTIFICATION; POTENTIAL FRAUD; SUITABLE METHOD; FALLOW-DEER; FOOD-CHAIN; RED-DEER

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