Pig Species Identification in Meatballs Using Polymerase Chain Reaction Restriction Fragment Length Polymorphism

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ABSTRACT

The information given to consumers is essential for them to choose one food product over another. The falsification of food contents on product labels is a widespread problem, especially with products related with pig or others prohibited food in Islam. Proving conclusively that fraud has occurred requires the detection and quantification of food constituents. Falsifications of meat or food are often biochemically similar to the materials they replace, consequently the identification and measurement extremely difficult. The DNA based methods have now been successfully adapted for detection of food substitution. In this research, Polymerase Chain Reaction (PCR) products of cytochrome b mitochondrial DNA gene were applied to identify the existence of pig in meatball product. Genomic DNA of pig, bovine, and chicken were isolated and subjected to PCR amplification targeting the mitochondrial cytochrome b gene. Pig species differentiation was determined by digestion of obtained 359 bp amplified product with BseDI restriction enzymes, which generated pig species electrophoresis pattern. PCR-Restriction Fragment Length Polymorphism (RFLP) revealed the presence of the pig meat in meatball product and distinguished between bovine, chicken, and pig sample. Pig mitochondrial cytochrome DNA gene was cleaved into 228 bp and 131 bp fragments but the bovine, and chicken cytochrome b gene were not digested by BseDI enzyme. The digestion was conducted at 55^{o}C for 3 h and visualization of the digest product was performed in 2% agarose gel. PCR-RFLP technique using BseDI restriction enzymes is reliable for the detection of the pig meat in meatball for the *Halal* authentication.

Key words: pig species, identification, PCR-RFLP, halal authentication

INTRODUCTION

Indonesian traditional meatballs is one of the comminuted meat products and its popularity in all classes of Indonesian society. The products are served in hot soup with others stuff such as tofu, noodle, cabbage and chili or tomato sauce and the popular name in Indonesia is called bakso. Meat are processed to make bakso originally from beef but nowadays some others such as chicken, fish, and pork commonly also been mixed in some meatball products. The wide variety of meatball products availabe on the market in Indonesia seems favourable but leads to several fears, where almost population are moslem who prohibited to consume pork. This is an important challenge for the people in charge of the official control of food, that have to verify the species of meat ingridients that are not always easily identifiable.

Strategies utilized to detect an adulterated product have traditionally relied on wet chemistry to determine the amount of a marker compound or compounds in a test material followed by a comparison of the value(s). Obtained with those previously documented for authentic material of the same type. This approach is often time-consuming and therefore expensive; it also has the shortcoming that food adulterers are becoming increasingly sophis-ticated at masking their efforts and the range of analytes which must be quanticed to ensure authenticity is continuously increasing (Downey, 1998).

Many various methods based on DNA techniques have developed such as multiplex PCR assay (Matsunaga et al., 1999), *PCR-based finger printing* (Saez et al., 2004). Colgan et al. (2001) analyzed meat bone meal using real time PCR to investigate the meat source origin and to verify the quantity of meat in DNA mixture