CHAPTER V

CONCLUSION AND REECOMMENDATION

A real-time PCR assay with SYBR green method is suitable for the determination of adulterated pork in gelatin formulations. Two species-specific qPCR assays were optimized based upon repetitive elements of the porcine and bovine genomes and they allowed sensitive detection of porcine and bovine at concentrations as low as 10^{-5} ng/µl. In addition, the lack of cross reactivity when the sets were used to amplify DNA from the other species indicates high specificity of the qPCR assay for its own species. When binary gelatin blends containing various amounts of porcine and bovine gelatin were prepared and analyzed by the qPCR assays, the determined ratios of porcine material to bovine material were very close to their theoretical values, and a contamination level of as low as 1% of the other species in the gelatin blends could be determined. When evaluated in gelatin capsules, although significantly less DNA was detected, determination of porcine and bovine species identities and estimation of the relative abundance of each species was possible. The data reported in this study are from DNA samples that were potentially degraded during manufacturing of gelatin and gelatin capsules, which are highly processed products. Therefore, the porcine and bovine species-specific qPCR assays described here represent a simple, reliable and sensitive DNA-based test for determining the species of origin of highly processed products.

Using a commercial DNA extraction kit from Agilent porcine detection kit has been demonstrated that porcine DNA can be reliably detected in gelatin capsule. The amounts of DNA present in isolated DNA using Agilent were higher than in Qiagen and Kogene kits. Therefore, the Agilent kit had successfully isolated DNA from gelatin capsules. However, the additional protocols on the purification is required in order to further reduce the amount of interfering protein.

The validation method of this research expects broad applicability of the assay with minor modifications to other food matrices as well. However, since the extent of DNA degradation that occurs during gelatin and gelatin capsule manufacture may vary and the copy number of repetitive elements between different animal subjects within the same species may vary (although the data generated from this study suggested, with careful qPCR assay design, there were minimal impact from amplifying repetitive elements on DNA quantization), the DNA quantity determined may not always represent the amount of porcine and bovine species-specific materials in gelatin and gelatin capsules. Thus the quantitative data obtained would only be an approximation.

The FTIR spectroscopic techniques can be used for rapid classification of gelatin. In order to ensure the result of real-time PCR, the formation of Amide A, I, II and III were analyzed using PCA analysis. These regions were found to give information about the origin of the gelatin. Two independent PCA models were calculated defining three separate classes of samples. However, PCA was not successful for classification of adulteration gelatin in capsule shells due to the similar chemical structure.

Since PCR based techniques are effective in identifying small pieces of DNA, they have received significant attention in recent years. The ideal PCR is the one with high specificity, sensitivity and efficiency and are influenced by the nature of target sequence, as well as by each component of PCR. Often, the conditions that would permit maximum yield are not compatible with high specificity and conditions optimized in regard to specificity may adversely affect the efficiency. Thus, in setting up a PCR it is important to determine beforehand to attain the specificity, efficiency and sensitivity of the PCR that is required for the intended application.