Introduction: A growing need for reliable meat species identification

- In order to protect customers’ interests, it has been necessary to develop effective methods that enable verification of the species composition of different food products.
- In EU countries, producers must ensure that production processes comply with European Council (EC) Regulation No. 510/2006 and that their products are correctly labeled in compliance with EC Regulation No. 13/2000.
- Reliable meat species identification is important to prevent incorrect handling, including fraud and unintentional traces, which may lead to health problems and/or violation of religious beliefs.
- Most methods for meat species identification are PCR-based, since identification is possible in raw and processed foods.
- The purpose of this study was to optimize the well-known method of PCR-RFLP. The process was optimized in conjunction with the QIAxcel Advanced System.
- The optimized method delivers results in under 8 hours and is highly suited to routine analyses.

Currently available methods

- The most commonly used methods are as follows.
- Most PCR methods require mitochondrial DNA. Hundreds to thousands of copies of mtDNA are present in each animal cell. These numerous copies increase the chance of detecting the target animal DNA, even in processed food (Pascoal, et al. 2011, Maede, 2006).
- DNA detection in processed food can be very difficult because intensive treatment of food can degrade and fragment DNA. It is therefore crucial to use short amplicons (<1000 bp).

The QIAxcel based PCR-RFLP protocol

- We have optimized and validated an efficient PCR-RFLP protocol for semi-automated meat species identification.

Protocol in brief
1. Homogenization and lys of meat samples from various animal species of interest (cattle, pig, sheep, deer, rabbit, chicken, duck, turkey, and goose).
2. DNA purification using QIAxylury and the QIAxylury DSP DNA Max Kit.
3. PCR amplification of the DNA samples.
4. Digestion of the PCR products with 4 restriction enzymes (Hae III, Hinf I, Rsa I, and Dnl I).
5. Analysis of digested samples by native capillary electrophoresis using the QIAxcel Advanced System.
6. Interpretation of the results using a programmed Excel® sheet.
7. Validation using QIAxcel ScreenGel software.

Conclusion

- Compared with other methods, the PCR-RFLP protocol in combination with the QIAxcel Advanced System is a powerful tool; it enables discrimination of a large number of meat species on a large scale and with high sensitivity in one working day (8 hours).
- QIAxcel Advanced eliminates exposure to hazardous chemicals, such as ethidium bromide, providing a safer method for the user.
- The semi-automated method is reliable and affordable, therefore a good candidate for use in the food industry.
- Potential procedural or interpretation errors are reduced through semi-automation, provided that the database is complete and handled correctly.

References

- Wolf, et al. 1999
- Colombo, et al. 2002
- Pascoal, et al. 2011

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