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SYBR Green I Real-time polymerase chain reaction as a tool to detect poultry's meat adulteration with pork's meat

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Abstract

Nowadays, meat species adulteration in ground and comminuted products is being considered as a widespread problem in retail markets [1]. This problem encompasses many issues, such as adulteration by substitution with lower value meats, the presence of undeclared species and the fraudulent substitution of meat by lower price vegetable proteins. Another issue to be considered is related to religious practices since pork's meat consumption is sometimes forbidden. Several techniques are currently used for meat species identification in complex mixtures, including different protein-based methods such as HPLC, ELISA and electrophoretic techniques. Nevertheless, these methods can be significantly less sensitive and difficulties can arise in the case of thermally processed foods. Due to the higher stability of DNA molecules compared to proteins, and to its ubiquity in every type of cell, they are currently preferred as target compounds for meat species identification. Moreover, the analysis of DNA coupled with polymerase chain reaction (PCR) presents a fast, sensitive and highly specific alternative to protein-based methods [2].

In the present work, the development of a real-time PCR technique for pork's meat detection in complex matrices is reported. To achieve this objective, DNA was extracted from reference binary meat mixtures containing known percentages of pork's meat. The real-time PCR approach was based on the specific amplification targeting the 18S rRNA mitochondrial gene for pork species detection and targeting a eukaryotic DNA fragment as a reference gene for quantification. The amplification products were monitored by using the fluorescent dye SYBR Green I associated with melting curve analysis to verify the specificity of obtained fragments. Under our experimental conditions, pork and eukaryotic detection systems produced fragments with 149 bp and 140 bp, and with melting temperatures of 83.5ºC and 87.5º, respectively. Calibration curves were obtained with the cycle threshold (Ct) values by using the DDCt method. The detection and quantification of pork's meat was achieved in the range of 0.1% to 25%, with a high correlation coefficient (R^2=0.9943) and a PCR efficiency of 88.7%. The developed methodology was successfully validated using blind samples and applied to the quantitative evaluation of pork's meat in different poultry processed meat products, including sausages, hamburgers and nuggets.

[1] Asensio, L., González, I., García, T., Martín, R. (2008). Food Control, 19, 1–8.
[2] Mafra, I., Ferreira, I.M.P.L.V.O., Oliveira, M.B.P.P. (2008). Eur. Food Res. Technol. 227:649-665.