

## Species identification and authentication of hare (*Lepus*) meat by the use of the mitochondrial *cytb* gene

## C.G. Santos<sup>1,2</sup>, V.S. Melo<sup>1,2</sup>, J. Costa<sup>1</sup>, J.S. Amaral<sup>1,2</sup>, M.B.P.P. Oliveira<sup>1</sup>, I. Mafra<sup>1\*</sup>

<sup>1</sup>REQUIMTE, Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, <sup>2</sup>Polytechnic Institute of Bragança <sup>\*</sup>E-mail: <u>isabel.mafra@ff.up.pt</u>

Nowadays, consumers are increasingly concerned with issues of food safety and authenticity. In particular, game meat has been much appreciated by consumers for their exotic flavor and texture, low in fat and cholesterol as well as by the absence of steroids or other drugs. Food authenticity assessment is important in that it avoids unfair competition among producers and allows consumers to have accurate information about the products they purchase. Therefore, it is important to ensure that species of high commercial value declared are not replaced by other species of lesser value [1].

The present work was part of a project aiming to assess the authenticity of "Alheiras de caça" based on the development of species-specific polymerase chain reaction (PCR) techniques to detect game meat species, including hare meat. Since there were no reports in the literature concerning hare meat identification, this work aimed to propose for the first time a PCR technique able to specifically detect this species.

Mitochondrial *cytochrome b* (*cytb*) gene was used to design species-specific primers for hare detection [2]. The new primers were assessed for their specificity to *Lepus* species and the PCR amplification was optimized to detect 0.01% of hare meat in pork meat. To confirm the identity of the PCR products obtained, the fragments were sequenced. Performing a BLAST for the obtained sequences, the results showed a 100% of homology for *Lepus granatensis*, while primers matched also *L. europaeus* and *L. capensis*. Additionally, with the proposed new primers, we developed a novel methodology based on real-time PCR with the new intercalating EvaGreen dye. This technique proved to be fast and specific for the identification of hare with the sensitivity of 1 pg of hare DNA. The application of the developed species-specific PCR to 18 commercial samples of "Alheiras de caça" showed that hare meat was absent in all samples, even in one that was labeled as containing this meat. It can be concluded that the proposed new primers can be used by both species-specific end-point PCR or real-time PCR to accurately authenticate hare meat in food products.

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