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Analytical Methods

Survey of the authenticity of prawn and shrimp species in commercial food products by PCR-RFLP analysis of a 16S rRNA/tRNA^{Val} mitochondrial region ☆

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Abstract

A novel PCR-RFLP method was evaluated as a tool to assess the incidence of incorrect labelling of prawns and shrimps in commercial food products. The whole method can be performed in less than 8 h in only one day of work. PCR amplification with primers 16Scru4/16Scru3, targeted to the amplification of a *ca.* 530 bp region of 16S rRNA and tRNA^{Val} mitochondrial genes, was coupled to restriction analysis with *Alu*I, *Taq*I or *Hin*fI. Forty-one commercial food products were considered. The molecular method considered allowed the identification of up to 17 different prawn and shrimp species in all the processed products considered. Seven (28%) of the 25 food products declaring one or more species in their labels were incorrectly labelled. Authentication was successfully assessed in commercial peeled products subjected to industrial processing, in which none of the products displayed labelling at species level. Overall, incorrect labelling was detected in 10 (24.4%) of the 41 commercial products tested, while another 16 samples (39%) exhibited incomplete labelling. The molecular method evaluated in this study proved to be a rapid and easy-to-perform two-step analytical approach to achieve species identification of commercial whole specimens of frozen prawns and shrimps and in peeled processed products where such raw materials are included as added-value ingredients.
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1. Introduction

Food authenticity is an issue of major concern for food authorities, since incorrect food labelling represents commercial fraud to the consumers, in particular when this implies the replacement of one ingredient by another of lower commercial value (Lockley & Bardsley, 2000). Incorrect labelling may also have negative sanitary implications – such as allergy or toxic syndromes – derived from the inadvertent introduction of any food ingredient that might be harmful to human health (Mermelstein, 1993; Patterson & Jones, 2000; Sotelo, Piñeiro, Gallardo, & Pérez-Martín, 1993).

The fish products sector is characterized by the high commercial value of many of its products, this being specially relevant in the case of prawn and penaeid shrimp species, both when they are sold as whole specimens and when they are included as high-value ingredients in pre-cooked food products. Prawns and penaeid shrimps are Decapoda crustaceans belonging to the superfamily *Penaeoidae,* and

☆ The PCR-RFLP protocol evaluated in the present study for prawn and penaeid shrimp species identification is freely available for research purposes but protected for industrial exploitation by Spanish Patent No.

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they are important resources from both commercial fisheries and for aquaculture in many countries, accounting for more than 30% of the global consumption of this type of crustacean worldwide (Pérez-Farfante & Kensley, 1997; Rosenberry, 2001). Among prawn and shrimp species, morphological characters are particularly difficult to use for species differentiation due to their phenotypic similarities and to the fact that their industrial processing often removes their external carapace (Vondruska, Otwell, & Martin, 1988).

To overcome these problems, molecular methods based on protein and DNA analysis have been developed. Thus, electrophoretic and immunological methods have been proposed for the detection and differentiation of Decapoda crustaceans (An, Marshall, Otwell, & Wei, 1989; Shanti, Martin, Nagpal, Metcalfe, & Subba Rao, 1993). However, such methods are laborious, time-consuming, and their application may be hampered by the lack of stability of the polypeptide targets as a result of industrial processing (Piñeiro, Vázquez, Figueras, Barros-Velázquez, & Gallardo, 2003; Piñeiro et al., 1999). Such limitations have been solved with the introduction of methods based on DNA amplification and DNA hybridization (Gutiérrez-Millan, Peregrino-Uriarte, Sotelo-Mundo, Vargas-Albores, & Yépiz-Plasencia, 2002; Khamnamtong, Klinbunga, & Menasveta, 2005; Klinbunga et al., 2001; Lavery, Chan, Tam, & Chu, 2004; Quan, Zhuang, Deng, Dai, & Zhang, 2004). Among the DNA targets, mitochondrial DNA (mtDNA) has been used in PCR-based studies, the 16S rRNA gene and, to a lesser degree, the cytochrome oxidase I (COI) gene, having been reported as good molecular markers for some crustacean species in phylogenetic studies (Baldwin, Bass, Bowen, & Clark, 1998; Bellis, Ashton, Freney, Blair, & Griffiths, 2003; Brzezinski, 2005; Bucklin, Frost, & Köcher, 1992; Maggioni, Rogers, Maclean, & D'Incao, 2001).

Nonetheless, a limited number of reports have focused their interest on the development of PCR methodologies for the identification of prawn and shrimp species in foodstuffs. Although only focused on three Eastern Pacific species, a previous study described the usefulness of a 1.38 kb mitochondrial region that comprised fragments of the 16S rRNA and 12S rRNA genes and the entire $tRNA^{Val}$ region for phylogenetic analysis of penaeid shrimp species (Gutiérrez-Millan et al., 2002). These species were: *Farfantepenaeus californiensis*, *Litopenaeus vannamei* and *Litopenaeus stylirostris*. Another previous study provided a molecular method – targeted to COI, cytochrome oxidase II (COII) and 16S rRNA mitochondrial genes – for the identification of species using restriction analysis of a 312 bp fragment (Khamnamtong et al., 2005). Likewise, that study considered the identification of only five species: *Penaeus monodon*, *Penaeus semisulcatus*, *L. vannamei*, *Fenneropenaeus merguiensis* and *Marsupenaeus japonicus*. More recently, a method for the detection of crustacean DNA based on a PCR-RFLP approach has been proposed (Brzezinski, 2005). The method, aimed at the detection of potentially allergenic proteins, did not allow prawn and shrimp species identification, although it did permit their generic detection and differentiation with respect to crab, lobster and crawfish species (Brzezinski, 2005).

Accordingly, the main goal of the present work was to evaluate the usefulness of a novel PCR-RFLP method based on the generic primers 16ScruC4/16ScruC3, targeted to a 16S $rRNA/tRNA^{Val}$ 530 bp mitochondrial region, for the identification of a broad number of prawn and crustacean species in commercial food products subjected to different technological processes.

2. Materials and methods

2.1. Commercial food products

Forty-one commercial food products containing or consisting of prawns or shrimps were considered. The products were purchased from supermarkets in Northwestern Spain or directly from Spanish companies involved in the commercialization of aquatic food products. Thirty-two samples concerned frozen penaeid shrimps while the remaining nine products were more processed commercial products containing prawns or shrimps as food ingredients. Reference samples consisting of nearly twenty penaeid species were used for purposes of comparison (Table 1).

2.2. DNA extraction and purification

Samples from the prawns and shrimps were scraped from the food products with sterile surgical blades. Representative portions of 0.2 g of each sample were placed in sterile 2 ml tubes and subjected to DNA extraction. A commercial DNA extraction kit (DNeasy tissue minikit, QIAGEN, Valencia, CA, USA) based on the use of purification micro-columns was used. The concentration of the purified DNA extracts was determined by measuring the fluorescence developed after mixing with Hoechst 33258 reagent (Sigma, St. Louis, MO, USA) in a LS 50 fluorimeter (Perkin Elmer, Wellesley, MA, USA).

2.3. Evaluation of a PCR-RFLP method for prawn and shrimp species identification

Primers 16ScruC4 (5́-AATATGGCTGTTTTTAAGCCTAATTCA-3') and 16ScruC3 (5́-CGTTGAGAAGTTCGTTGTGCA-3́), constructed in two well-conserved regions of the 16S $rRNA/tRNA^{Val}$ mitochondrial genes of prawns and penaeid shrimp species, were evaluated. The GenBank accession numbers of the penaeid species considered for the development of such primers are detailed in Table 1. Such primers allowed the amplification of a *ca.* 530 bp fragment of the 16S $rRNA/tRNA^{Val}$ mtDNA genes in the case of prawns and penaeid shrimps (Fig. 1A). The small size of this molecular target facilitates amplification from fresh, frozen or pre-cooked samples,

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