



IMPACT OF HIGH-PRESSURE PROCESSING ON THE INACTIVATION OF ANISAKIS LARVAE AND QUALITY OF EUROPEAN HAKE (MERLUCCIOUS MERLUCCIOUS) HEAVILY INFESTED OF ANISAKIS

Sara Balestra¹, Ilaria Ercole², Marika Di Paolo¹, Marica Egidio¹, Loriana Casalino³, Veronica Rodríguez-Fernandez⁴, Ivana Orlando², Raffaele Marrone¹

¹Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli, Italy; ²Fondazione di Ricerca SSICA, Stazione Sperimentale per l'Industria delle Conserve Alimentari, Parma, Italy; ³Dipartimento di Economia, Statistica e Commercio, Universitas Mercatorum, Roma, Italy; ⁴Dipartimento Interdisciplinare di Benessere, Salute e Sostenibilità Ambientale, Laboratorio di Parassitologia presso la UOC Microbiologia e Virologia dell'Ospedale Universitario Policlinico Umberto I, Sapienza-Università di Roma, Italy.

Aim. The increasing demand for raw and minimally processed fish products has raised concerns regarding the risk of anisakiasis associated with the consumption of *Anisakis* genus larvae. Although freezing remains the standard control measure, alternative non-thermal technologies are being investigated to ensure product safety while maintaining quality. High-Pressure Processing (HPP) represents a promising approach for inactivating foodborne hazards with minimal effects on the sensory and nutritional properties of food products. This study evaluates the efficacy of HPP in the inactivation of *Anisakis* spp. larvae in heavily infested European hake (*Merluccius merluccius*) fillets and examines its effects on specific physicochemical quality parameters.

Methods. The study was conducted on heavily infested European hake (*Merluccius merluccius*) specimens (n = 63), originating from FAO Fishing Area 27 (Subarea VIIIb, Bay of Biscay). To evaluate the presence of *Anisakis* larvae, a first screening of the coelomic cavity and viscera of each specimen was conducted. Whole fish were vacuum-packaged and treated by High-Pressure Processing (HPP) using a QUINTUS QFP35L-600 system. Six pressure-time combinations (200, 250, and 300 MPa for either 3 or 5 min) were evaluated for their efficacy in inactivating *Anisakis* spp. larvae and compared with an untreated control group (CTR). After HPP treatment, the fillets were analysed for physicochemical quality parameters (pH, water activity and color). To detect larvae which could be migrated in fish muscles, fillets were tested by a chlorine-peptic digestion according to ISO 23036-2:2021 and the larvae were classified as viable, viable after mechanical stimulation, or non-viable. Viability was monitored for 24 and 48 h, after which larval viability was determined. Genetic identification was performed on a

representative subset of 64 nematodes using sequence analysis of the mitochondrial (mtDNA cox2) and the nuclear (nas10 nDNA) gene loci.

Results. HPP treatment at 300 MPa for 5 min resulted in the complete inactivation of *Anisakis* spp. Larvae, emerging as the most effective condition. Treatment at 300 MPa for 3 min also showed high efficacy, reducing larval viability by 99%. In contrast, treatments at 200 MPa, regardless of processing time, did not ensure complete inactivation; however, the proportion of non-viable larvae was higher after 5 min (49,99%) than after 3 min (32,35 %). No significant differences in pH or water activity were observed between HPP-treated and CTR samples. Colour analysis revealed an increase in lightness in fillets treated at 300 MPa for both 3 and 5 min compared with the CTR. The redness parameter remained unchanged across treatments, whereas yellowness decreased in samples processed at 200 MPa. Notably, fillets treated at 300 MPa for 3 and 5 min exhibited a whitish cooked-like appearance.

Conclusions. This study confirms the efficacy of HPP in the inactivation of *Anisakis* spp. larvae in fishery products, identifying 300 MPa for 5 min as the optimal condition among those tested, in agreement with evidence reported by EFSA. Although this treatment achieved complete larval inactivation, it also induced noticeable changes in the appearance of the fish fillets, resulting in a whitish, slightly cooked-like aspect. Despite these promising findings, the study was limited to a single fish species and specific processing conditions. Further research is needed to validate these results in other commercially relevant fish species, assess sensory acceptance, and optimize processing parameters.