Effect of heat treatments on mobility and *in vitro* infectivity of *Anisakis* L3 in hake muscle infected under controlled conditions

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The importance of Anisakis infection of fish is well recognized by the fisheries sector and food safety authorities, since consumers may be accidentally infected by larvae in the third stage (L3) after consumption of raw or undercooked fish or cephalopods carrying the parasite, causing anisakiasis and allergic sensitization. Anisakis L3 is moderately tolerant to heat stress and in order to mitigate the risk of the intake of live larvae in cooked seafood products is important to define with precision at which point the parasites are no longer infective after a heat treatment, since overcooking may decrease sensory acceptance of fish muscle whereas undercooking may lead to health problems. The aim of this work was to study the effects of heating rate and final temperature to find minimal thermal treatments to inactivate Anisakis simplex.

Live A. simplex L3 were obtained from heavily parasitized hake ovaries and viscera, washed with 0.85% NaCl and stored at 4 ºC until use. Hake muscle was infected by placing 15 L3 Anisakis on steaks of aprox. 1 cm thickness and covered with another steak of the same size (200g). Each sandwich was wrapped in aluminium foil and larvae allowed to migrate (4 ºC, 24 h). Sandwiches were introduced in plastic bags and heat treatments were performed (oven or water bath) in the range of 40-80 ºC, at different heating and cooling rates. Anisakis viability (motility and fluorescence) and in vitro infectivity (agar penetration test) were monitored.

In the oven (set at 200 ºC) none of the larvae were viable after 20 min heating and those which survived after 10 min were not able to penetrate into agar. When sandwiches were heated in a water bath (set at 95 ºC), no larvae were viable beyond 70 ºC; from those which survived in the range 55-65 ºC, none of them penetrated into agar whereas at temperatures close to 50 ºC, half of those showing mobility were able to penetrate into agar.

Although more studies are needed to define with precision the minimal thermal treatments, these results stress the importance of using not only mobility as a measurement of viability but also measurements such as the agar penetration test as an indicator of the larval penetrability, since this ability may play an important role for the invasion of the gastrointestinal mucosa.

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