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Note

Anisakids Survival after Microwaving, Freezing and Salting Fish from Argentina

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Some studies support the effectiveness in controlling nematodes in fishes for human consumption by freezing at –20°C and by cooking at 74°C by microwave process. The aim of this work was to analyse the effect of different treatments over anisakids from Argentinean fishes. The known anisakids in fishes from Argentina belong to genera *Hysterothylacium*, *Terranova*, *Anisakis*, *Contracaecum* and *Pseudoterranova*, being the three latest recognised as pathogens for human. Living larvae of anisakids obtained from fishes were used for survival assessment. Some parasites were kept in NaCl (0.85%) at 4-5.5°C until death. *Anisakis*, *Terranova*, *Pseudoterranova*, *Contracaecum* and *Hysterothylacium* survived during 330, 75, 75, 210 and 90 days, respectively. For freezing, microwaving and salting treatments, infected fillets were exposed at –20°C until 24 hours, 1 minute at 64.05°C and 75.56°C and to salt during 24 hours, respectively. No surviving anisakids were observed neither after freezing nor salting. *Anisakis* sp. survived at 64.05°C.

Keywords: anisakids, survival, microwave, freezing, salting, fishes, Argentina

Introduction

The presence of parasites can affect the aesthetic of fish products and its commercial value. Some parasites may also represent a hazard to human health. Nematodes of the familiy Anisakidae are world wide recognised within this group.

Anisakids are parasites whose life cycles occur in water. Definitive hosts are fishes, reptiles, birds and mammals. Intermediate and/or paratenic hosts are fishes and cephalopods. In these hosts, parasites can reach high densities (Anderson, 1992). Humans may become infested if viable larvae are consumed in raw seafood, such as sushi, sashimi, ceviche and lomi-lomi, or poorly cooked fish or seafood. Anisakidosis is the human disease caused by ingestion of larval nematodes belonging to the family Anisakidae (Adams *et al.*, 1997; Adams and DeVlieger, 2000). Although the larvae can not develop in humans, they may live and cause severe symptoms. The larvae invade the stomach and intestine walls producing eosinophilic granuloma surrounding the worm, characterized by infiltration and proliferation of neutrophils (Young and Lowe, 1969; Oshima, 1972). Adding to the infection with anisakid nematodes, the exposition to larvae antigens can cause allergic reactions (Ardusso Lovera *et al.*; 1996, Fernández de Corres *et al.*, 2001; Audicana *et al.*, 2002).

In spite of the importance of anisakidosis for human health, there are few references about survival and resistance of anisakids in fish products under different treatments and all of them proceeded from the North hemisphere. Some studies support the effectiveness in controlling parasites by freezing at –20°C (–4°F) in all parts of the products for at least 24 hs (FDA/CFSAN 2001). Commercial blast freezing in salmon industry has demonstrated to be effective to kill nematodes, although general public uses domestic freezers. Adams *et al.* (1999) found that a temperature of 63°C is not enough to kill *Anisakis simplex* larvae in fillets cooked in a microwave oven. The U.S. Food and Drug Administration (FDA) (2001) recommended reaching internal temperatures for fishery products: 63°C (145°F) by conventional methods and 74°C (165°F) by microwave process. Nematodes are also resistant to brining solutions. However in a 22% salt solution (saturated solution) the worms dead in 10 d (Smith and Wootten, 1978).

There are not previous studies on survival and/or resistance of anisakids in fish products from Argentinean waters. Nevertheless the most common anisakids in fishes from Argentina belong to the genera *Anisakis*, *Hysterothylacium*, *Contracaecum*, *Terranova* and *Pseudoterranova* (Navone *et al.*, 1998; Timi *et al.*, 2001). *Anisakis*, *Contracaecum* and

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Pseudoterranova are recognised as pathogens and contain sibling species complexes, i.e.: *Anisakis simplex* complex and *Pseudoterranova decipiens* complex (Paggi *et al.*, 2000), whose members are not distinguishable based on morphology, mainly for larval stages.

The aim of this work was to analyse the effect of different treatments over anisakid nematodes obtained from edible fishes from Argentinean waters and the results will to contribute to alimentary industry.

Materials and Methods

Fishes and viscera were obtained from local fish markets. For isolation, anisakid nematodes belonging to the genera *Anisakis*, *Contracaecum*, *Hysterothylacium*, *Terranova* and *Pseudoterranova* were removed manually from 98 viscera and 149 fishes belonging to 22 different species of teleosts: *Genypterus blacodes*, *Pomatomus saltatrix*, *Engraulis anchoita*, *Urophycis brasiliensis*, *Cheilodactylus bergi*, *Pinguipes brasilianus*, *Conger orbignyanus*, *Micropogonias furnieri*, *Paralichthys* sp., *Merluccius hubbsi*, *Acanthistius brasilianus*, *Stromateus brasiliensis*, *Umbrina canosai*, *Cynoscion guatucupa*, *Macrodon ancylodon*, *Percophis brasiliensis*, *Raneya brasiliensis*, *Helicolenus dactylopterus lahillei*, *Pseudopercis semifasciata*, *Trachurus lathami*, *Prionotus nudigula* and *Mullus argentinae*.

Nematodes were washed in NaCl solution (0.85%). Encapsulated larvae were immersed in beakers containing pepsin (EC 3.4.23.1, Merck KgaA, Germany) and HCl during 1 hour and then transferred to Petri dishes containing NaCl (0.85%). Larvae were identified under a light microscopy and then exposed to survival, freezing, microwaving and salting treatments.

Survival A total of 1249 anisakids were arranged in Petri dishes containing NaCl (0.85%) at 4 – 5.5°C in the following way: *Anisakis* sp., 42 larvae in 8 dishes; *Contracaecum* sp. 1, 126 larvae in 12 dishes; *Contracaecum* sp. 2, 25 larvae in 3 dishes; *Hysterothylacium* sp., 747 larvae in 23 dishes, *Hysterothylacium aduncum*, 276 larvae in 9 dishes; *Terranova* sp., 23 larvae in 5 dishes and *Pseudoterranova* sp., 10 larvae in 2 dishes. Temperature was registered with a maximum-minimum thermometer. Dishes were examined and saline solution renewed weekly. Dead parasites were recorded and removed from dish.

Freezing treatment Living nematodes were placed in sandwiches made of fillets. These fillets were prepared as sandwiches. Average weight and thickness of each fillet was 23.17 ± 3.56 g and 1 cm respectively. Fillets were trimmed and the tail portion was used. A total of 205 living larvae was used. Inside of each sandwich 5 live nematodes belonging to genera *Anisakis*, *Contracaecum*, *Hysterothylacium* and *Pseudoterranova* were added. 16, 8, 16 and 1 sandwiches with larvae of each genus respectively were prepared. Sandwiches were exposed to temperatures in the range −18°C to −22°C during 3, 5, 15 and 24 hs. After treatment sandwiches were allowed to room temperature and each larva was removed from fish and transferred to saline solution. Survival was assessed by whether nematodes moved in response to stimulation with a dissection needle.

Microwave treatment Fillets were prepared for microwave processing as sandwiches. Two groups of 4 and 5 sandwiches were prepared. Average weight of each fillet was 48.00 ± 14.6 g and 30.00 ± 3.90 g respectively and thickness 1 cm each one. Fillets were trimmed and the tail portion was used. Inside each sandwich, 5 living larvae of *Anisakis* sp. were added. Nine sandwiches were placed in an uncovered Petri dish. Microwave treatment was performed with a commercial microwave oven (BGH Quick Chef, Compact 16150). Temperature was tested with a LCD Digital Multithermometer (Model Nº ST- 9285 B) with stainless steel sensor probe. Samples were neither covered nor rotated. After 1 min with microwave operating on 75% of power (600 watts), temperature sensor was inserted into the sample without removing it from the oven. Once temperature was registered, sample was removed and maintained at room temperature during 15 min. After that, the sandwiches were carefully opened. Nematodes were extracted with a needle and placed in a Petri dish containing NaCl solution. Survival was assessed by whether nematodes moved in response to stimulation with a dissection needle.

Salting Anchovies, *Engraulis anchoita*, pre-salted by immersion in saturated solution by NaCl during 4 d, were headed and gutted and trimmed along the body in the laboratory. Four or five living larvae of *Anisakis* sp., *Contracaecum* sp., *Hysterothylacium* sp. and *Pseudoterranova* sp. were located inside each fish. A total of 442 living larvae (*Hysterothylacium* sp.: 365; *Anisakis* sp.: 64; *Contracaecum* sp. and *Pseudoterranova* sp.: 5) was used for this treatment. Anchovies were salted following local commercial salting techniques (1 layer of fish – 1 layer of salt, successively, into a recipient) from 1 hour to 24 hs. After that, nematodes were recovered from fish with a needle and placed in a Petri dish containing NaCl solution (0.85%).

Results

Larvae of different genera survived differentially in NaCl solution at 4°C – 5.5°C: 50% of larval *Anisakis* lived approximately 120 d. Only 13.6% reached 330 d alive (Fig. 1). Thirty and 50% of larval *Terranova* and *Pseudoterranova*, respectively, survived 45 d, and only 11% and 20% respectively reached 75 d alive (Fig. 1). There were at least two

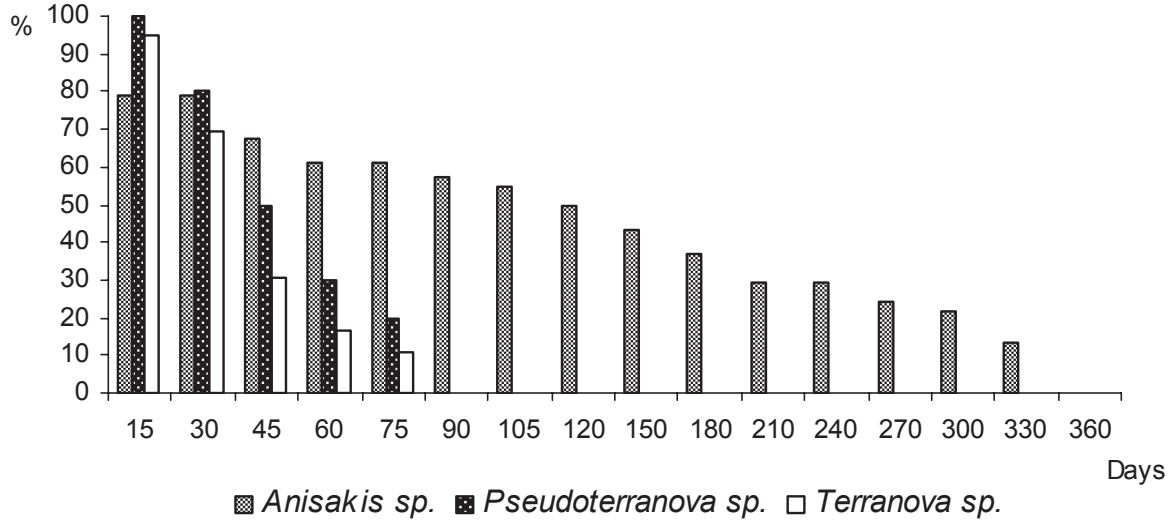

Fig. 1. Survival of anisakid larvae belonging to genera *Anisakis*, *Terranova* and *Pseudoterranova* in Petri dishes containing NaCl (0.85%) NaCl solution at 4°C – 5.5°C.

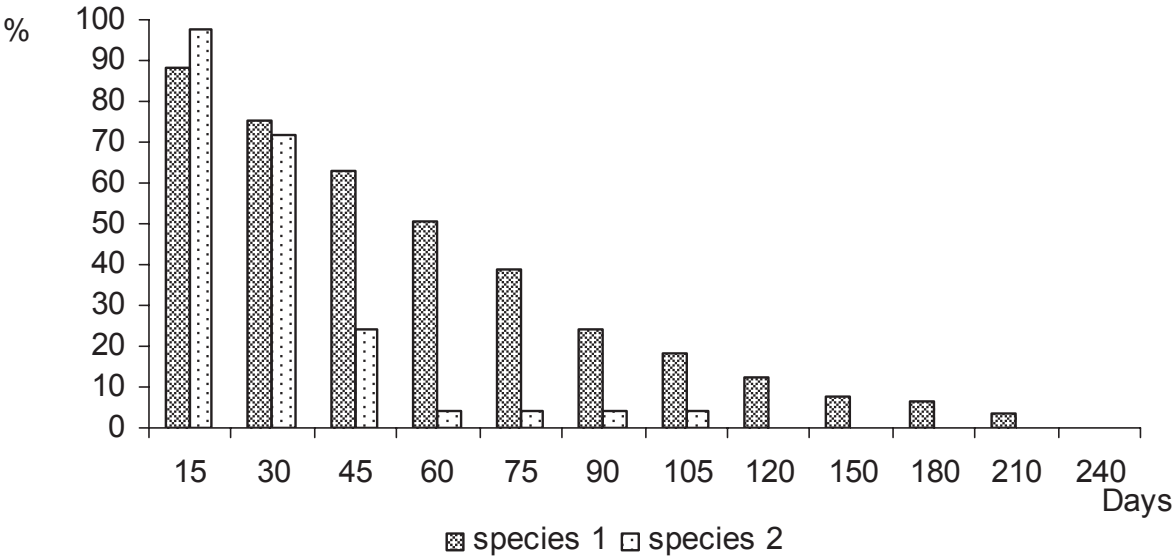

Fig. 2. Survival of anisakid larvae belonging to genus *Contracaecum*, species 1 and 2 in Petri dishes containing NaCl (0.85%) NaCl solution at 4°C – 5.5°C.

species of *Contracaecum* in examined fishes: species 1 (or species complex) from *G. blacodes*, *E. anchoita*, *A. brasilianus*, *S. brasiliensis*, *M. hubbsi* and *C. guatucupa* and species 2 only from *M. hubbsi*. In this way, 50.9% of species 1 survived 60 days whereas only 4.2% of species 2 survived this period (Fig 2). Two types of larvae of genera *Hysterothylacium* were also found: *Hysterothylacium* sp. (from *A. brasilianus*, *S. brasiliensis* and *C. guatucupa*) and *Hysterothylacium aduncum* (from *E. anchoita* and *M. hubbsi*). Some differences of survival between *Hysterothylacium* sp. and *H. aduncum* from *M. hubbsi* were found: 45.4% of *Hysterothylacium* sp. lived 30 d whereas 36% of *H. aduncum* survived this period. The 1.2% of both species reached 90 d alive (Fig 3).

A decreasing percentage of survival of Anisakidae larvae under freezing treatment from 3 h to 24 h was observed. The internal temperature of fillets (1 cm of thickness, 50 g weight) reached −20°C after 5 h at −23°C. No surviving nematodes were registered after 24 h. 100% of larvae belonging to genera *Anisakis* sp.and *Contracaecum* sp. survived until 5 hs at temperature in the range −18°C to −22°C, whereas

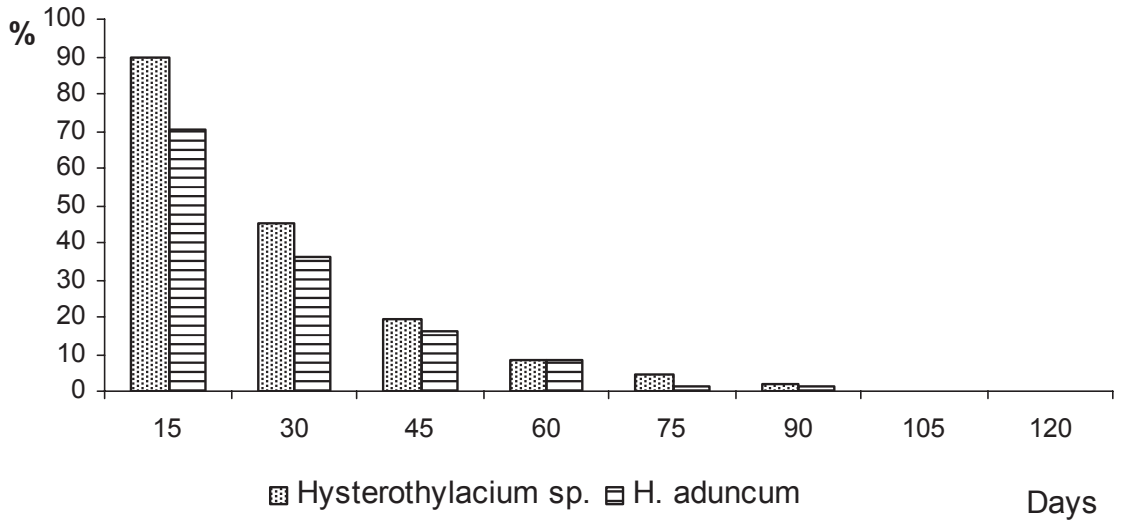

Fig. 3. Survival of anisakid larvae of *Hysterothylacium* sp. and *Hysterothylacium aduncum* in Petri dishes containing NaCl (0.85%) NaCl solution at 4°C – 5.5°C.

larvae of *Hysterothylacium* sp. and *Pseudoterranova* sp. died during the first 3 h.

A survival of 10% of larvae of *Anisakis* at internal temperature of 64.05°C during 1 minute under microwave treatment was observed. No surviving larvae at 75.56°C were registered.

No surviving larvae of genera *Hysterothylacium*, *Contracaecum* and *Pseudoterranova* were observed after 1 hour of salt treatment. *Anisakis* larvae survived 7 h of salt exposition but no surviving larvae were observed after 16 h. *Anisakis* which survived 5 h were removed and exposed to NaCl (0.85%) at 4 − 5°C. The 75% of these larvae survived 5 months more.

Discussion

Any nematode species possesses a suite of adaptations which enables it to survive in the environments in which it lives (Wharton, 2002). These organisms have resistance adaptations that allow them to survive the stress until conditions become favourable again such as cold tolerance mechanisms (Wharton *et al.*, 2003; Wharton and Aalders, 2002). In spite of this knowledge, there were not found studies about time of larvae survival in domestic refrigerator in Petri dish with NaCl solution and without nutrients nor antibiotic. The fact that the most of larvae belonging to genus *Anisakis*, *Hysterothylacium*, *Contracaecum*, *Terranova* and *Pseudoterranova* survived more than 90 d demonstrated the high resistance of this group, mainly *Anisakis* larvae which lived almost a year under these conditions.

Anisakis larvae (L3) have a moderate ability to survive freezing and perhaps they may have any adaptive significance (Wharton and Aalders, 2002). Death by freezing may result from the mechanical disruption during ice formation of the plasmatic membrane of cells or the membrane of organelles (Wharton, 1999). In U.S.A. it is estimated that up to 60% of sushi is frozen at some point before its consumption. According to the FDA it is actually illegal to serve any form of raw fish that has not been previously frozen to kill parasites. In Japan, nearly 50% of sushi and sashimi is frozen. Temperature reached in the fish depends on the freezer, the mass of the fish and the time of exposure. Butt *et al.* (2004) proposed –10º for 24 h, but Huss (1993) and FDA (2001) recommended –20°C for 24 h in all parts of the product. In the present study 100% of *Anisakis* larvae supported 7 h at this temperature, but it was corroborated that larvae not survived after 24 h at –20°C. The 7 h of survival may be due to the 5 h that takes for the internal temperature of the fillet to reach temperatures under −20°C.

According to Huss (1993) all nematodes were killed when heated to 55°C for 1 minute and Butt *et al.* (2004) suggest an adequate cooking at 60°C to kill the anisakids. Nevertheless, Hauk (1977) reported that 14% of larvae of *Anisakis* present in the flesh were viable after 1 minute during smoking (57°C). Furthermore, the cold smoked whole (temperatures from 18°C to 41°C for 24 h.) had viability of approximately 87.5%. In the same way, Adams *et al.* (1999) and Adams and DeVlieger (2000) found that cooking in a microwave requires a higher temperature to kill anisakids due to the uneven heating. They report that a temperature of 77°C in the thickest part of the product is recommended and

that heavier fillets evidence higher percentage of survival of the larvae at 60°C. In the present study, 10% of larvae of *Anisakis* survived within sandwiches at internal temperature of 64.05°C during 1 minute, whereas in the smaller sandwiches, the temperature reached 75.56°C, at which none of larvae of *Anisakis* survived.

In other countries, studies of survival of anisakids are referred to marinated fish. The primary components of this method are salt and acid, but lethality of parasites is caused by the concentration of salt (Adams *et al.*, 1997). In saturated solution the worms will be dead in 10 d (Smith and Wootten, 1978; Hauck, 1977). Depending on the percentage of NaCl it was registered a survival of 7 weeks and 5 to 6 weeks with 4.3% and 8 − 9% of salt, respectively (Karl *et al.*, 1995; Huss, 1993). In the present study no surviving larva was found after 24 h of exposition of infected fish at dry salt. Furthermore, time of storage of salted anchovies in Argentina is very long (6 − 8 months), so they have to be free of alive nematode hazard. Taking into account that in this work were used nematodes from fishes of commercial significance, the results should be considered an important contribution to alimentary industry.

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