How effective is freezing at killing anisakid nematodes? An experimental evaluation of time-temperature conditions

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The presence of parasites in marine fish is a serious problem for the fishing industry in many countries. Some species of parasite may pose a risk to consumers. The consumption of raw or inadequately cooked marine fish can lead to several disorders caused by the ingestion of viable anisakid nematodes. *Anisakis simplex* and *A. pegreffii* have been reported as causative agents of human infection, but other anisakids (*Contracaecum osculatum* and *Pseudoterranova* sp.) are also known to represent a potential hazard. Human health may also be compromised by allergic reactions to parasite antigens.

Treatment to kill viable parasites in fishery products for human consumption is mandatory in many countries. For parasites other than trematodes the freezing treatment must consist of lowering the temperature in all parts of the product to at least -20°C for not less than 24 h, or to -35°C for not less than 15 h (EU Regulation No. 1276/2011).

The question arises: how long should the product be held at the set freezing temperature (from the moment of placement to removal from the freezer) to meet the EU criteria?

The objective of the study

- to experimentally evaluate the time-temperature conditions necessary to kill anisakid larvae (*A. simplex* and *Pseudoterranova* sp.).

MATERIAL AND METHODS

Two species of fish were selected for the study:

- cod *Gadus morhua* - obtained from catches in the North Atlantic (Division 27.2.a.1 and 27.2.a.2);
- herring *Clupea harengus* - caught in the southern Baltic Sea (Division 27.3.d, ICES Subdivision 24).

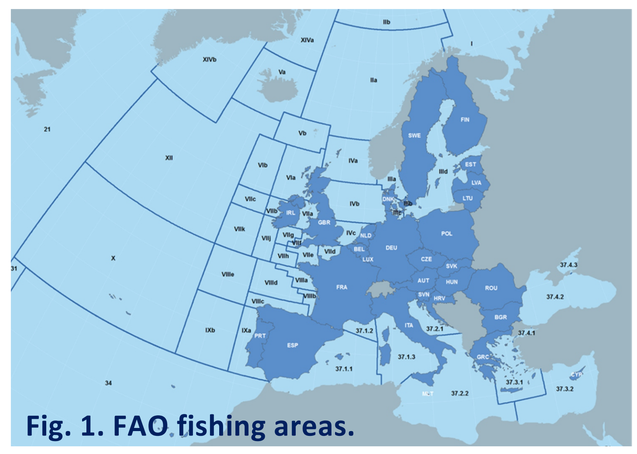

Fig. 1. FAO fishing areas.

Two freezers with different rates of temperature change were used for the experiment:

- single-compressor freezer (model LGT-4725, Liebherr) with a conventional (static) cooling system (compressors with power of 433 W), without air circulation inside the refrigeration compartment;
- two-compressor freezer (model MDF-U443-PE, Panasonic) with a cascade cooling system (compressors with power of 400W and 750W) and fan-forced air circulation in the refrigerator for precise temperature uniformity.

FREEZING EXPERIMENT

Skinless fillets of cod (n = 40) with visible parasites and whole (ungutted) herring (n = 240) were exposed to temperature of -15°C, -18°C, -20°C in the single-compressor freezer (freezing rate 1.1°C/h) and -20°C, -25°C and -35°C in the two-compressor freezer (freezing rate 5.6°C/h).

During the entire freezing process, internal temperature of samples was recorded with wireless data loggers Track Sense Pro, Ellab.

Fig. 2. Fish samples in single-compressor (A) and two-compressor freezers (B).

RESULTS

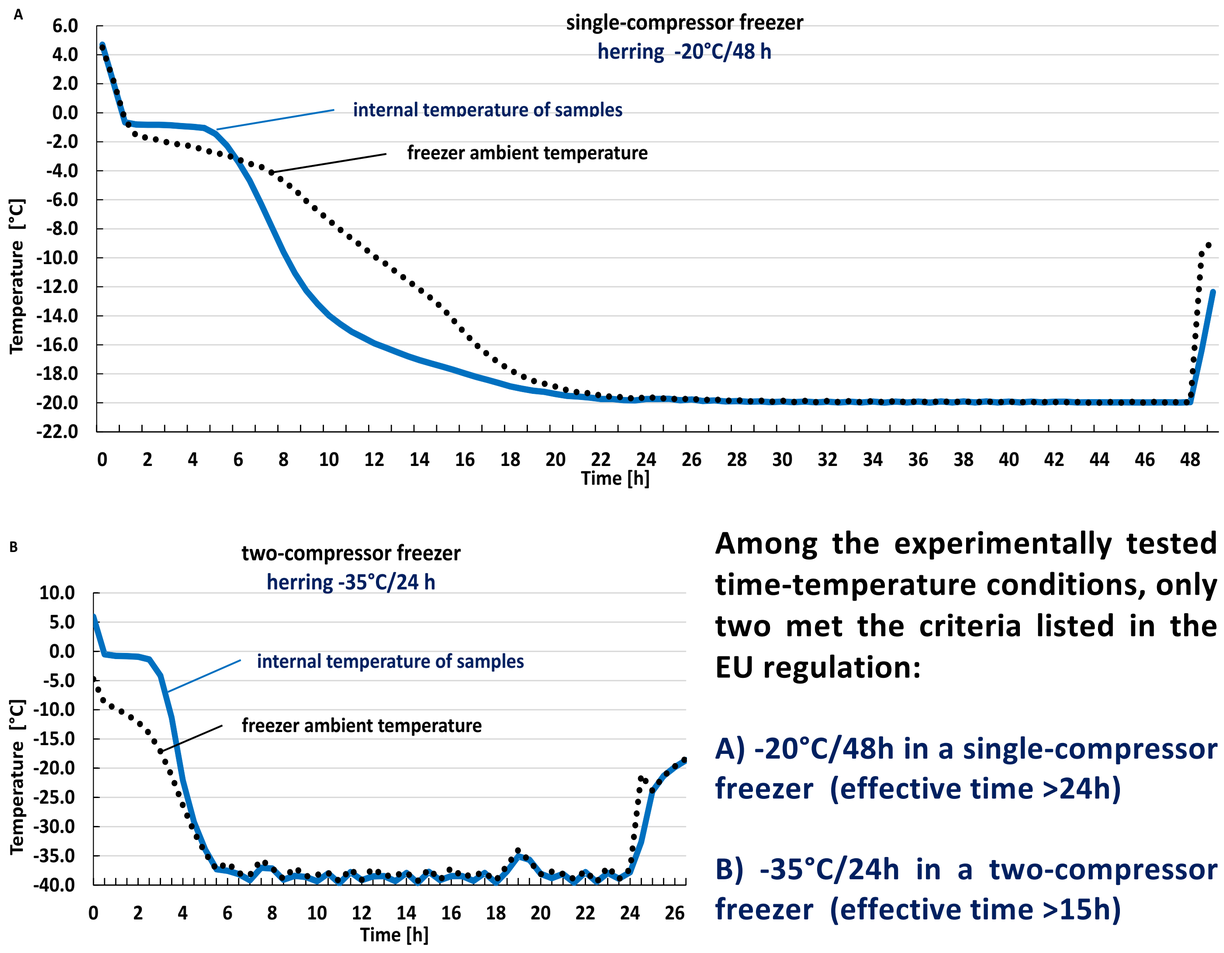

Fig. 3. Freezing curves for herring samples.

Among the experimentally tested time-temperature conditions, only two met the criteria listed in the EU regulation:

A) -20°C/48h in a single-compressor freezer (effective time >24h)

B) -35°C/24h in a two-compressor freezer (effective time >15h)

The viability of anisakid larvae after freezing was assessed based on their mobility (EFSA 2010) and susceptibility to staining with malachite green (Leinemann and Karl 1988). Application of this dye allows dead and viable nematodes to be distinguished.

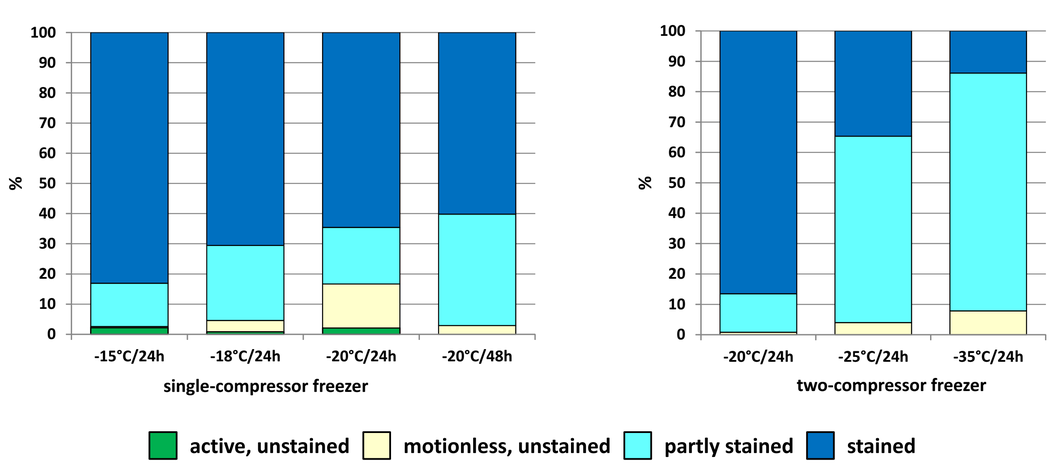

Fig. 4. Mobility and coloration of *A. simplex* larvae obtained from herring samples.

In total, the cod fillets contained 990 *Pseudoterranova* sp. larvae, while *A. simplex* larvae, which were also found in fillets, were less numerous (n = 72). All *A. simplex* and *Pseudoterranova* sp. larvae in cod fillets died at a temperature of -15°C or lower.

A total number of 1267 *A. simplex* larvae were found in herring body cavities, but not all were dead after freezing. Spontaneous movement was observed in 12 parasites held in the single-compressor freezer for 24 h. Eight larvae kept at -15°C were active immediately after thawing. A few larvae survived at -18°C (two individuals) and -20°C (two individuals) and showed spontaneous mobility after thawing. All parasites stored in the single-compressor freezer at -20°C for 48 h and in the two-compressor device at ≤ -20°C/24 h were motionless after thawing. Stimulation with tweezers and incubation at 37°C did not provoke any activity.

Obtained results clearly demonstrate that the appropriate freezing process is essential to ensure the safety of fish products.

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Leinemann M, Karl H (1988) Untersuchungen zur Diferenzierung lebender und toter Nematodenlarven (*Anisakis* sp.) in Heringen und Heringenzeugnissen. Arch Lebensmittelhyg 39: 147-150.