FREEZING TO KILL NEMATODE PARASITES IN FISH PRODUCTS:

IMPLICATIONS FOR HACCP

Peter Howgate, May 1998

BACKGROUND

On 25 March 1998, a contributor to the seafood@ucdavis.edu listserve wrote:

'I was looking at the HACCP information on inactivation of parasites given under:

http:/www-seafood.ucdavis.edu/haccp/plans

and was surprise to see that freezing at -20°C for 7 days (and not 24 hr as often seen in the literature) was recommended as a preventive measure to kill the parasites.

As there is a big difference between 24hr and 7 days, I was wondering if anybody could give me comments on that recommendation.'

This recommendation for -20°C for 7 days is not in the plan at the URL cited. What the file PARASITE.HTM at that site shows is a portion of a HACCP plan relating to control of parasites, and the table shows in the Critical Limit column a recommendation to 'Freeze at - 35°F for 15 hours'. This recommendation, as cited, contains a minor error compared with the FDA recommendation to be discussed below; the -35°F should be -31°F or -35°C. The -20°C for 7 days regime is given in the ucdavis compendium site in the PARASITE.HTM file, http:/www-seafood.ucdavis.edu/haccp/Compendium/Biological/PARASITE.HTM. The two regimes, -20°C for 7 days, and -35°C for 15 hours, derive from the U.S. FDA Fish and Fishery Products Hazards and Controls Guide, Chapter 5, Parasites. The recommendations are in both the first and second editions, and predate the legislation on HACCP; Sakanari and McKerrow (1989) in a review of anisakiasis quote the same recommendations from an FDA 'code interpretaion' .

The correspondent draws attention to the difference between the -20°C for 7 days recommendation and that of -20°C for 24 hours recommended elsewhere; both are quite different from the -35°C for 15 hours recommended as an alternative to the -20°C for 7 days in the FDA Guide. Freezing to kill parasites is one of the few examples in fish processing in which a processing step will control a hazard and is a critical control point with all that designation entails. In my opinion the recommendations in the FDA Guide are defective in that the critical limits advocated, and the monitoring procedures, are confusing and do not accord with Good Manufacturing Practices for the freezing of fish. In the following notes I will review the literature on the effects of freezing on nematode parasites, and propose an alternative HACCP plan, but before this I will comment on the EU legislation on the requirement for freezing to control hazards from parasites in fishery products.

EU REGULATION ON FREEZING TO KILL NEMATODE PARASITES

The -20°C for 24 hours regime alluded to by the original correspondent as being often seen in the literature refers to the practice in Europe and Scandinavia of freezing and storing in the frozen state fish that will later be converted to lightly preserved products. The specific conditions referred to are requirements of current EU legislation, which, in turn, derive from earlier national regulations in force in several European and Scandinavian countries for the control of nematodes as a health hazard in fish. The EU regulations are contained in Council Directive 91/493/EEC. Chapter IV, Article V.2 of the Annex

to the Directive requires:

'The fish and fish products referred to in point 3 which are to be consumed as they are must, in addition, be subjected to freezing at a temperature of not more than -20°C in all parts of the product for not less than 24 hours.'

(I do not like the wording of this section, but I will discuss this aspect later). Point 3 lists types of fish or products which must be subjected to these conditions; they are products which are lightly cured and intended to be consumed without prior cooking.

This requirement is incorporated into regulations in member states of the EU. As I have already mentioned, the Directive embodies the equivalent national legislation already applied in some member states when the Directive was published, and, in those states, there has been no change in the requirements to freeze the relevant fish and fish products following the entry into force of the 91/943/EEC Directive. The original national legislations when enacted in the late 1960's was based on experimental data, later confirmed by studies published in 1989, and as far as I am aware, the efficacy of this regime for the control of human health hazards from parasites in fishery products has not been questioned. The provisions of the Directive apply to products imported into the EU so a manufacturer of lightly preserved fishery products wishing to export to the EU would have to freeze and store the raw materials or products as described.

HACCP plans are concerned only with human health hazards; compliance with regulations is a function of the quality assurance programme. In the case of relevant products prepared in, or exported to, the EU the manufacturer would have to treat the raw material or products as described in the Directive even if there were no requirements for HACCP plans. In fact, the Directive does require the processor to establish a HACCP plan, and it would obviously be sensible for the HACCP team to consider the mandatory freezing step as a critical control point with all that that implies. However, the team might not consider the minimum temperatures and times specified in the regulations to be adequate to ensure safety and could impose stricter conditions.

If the manufacturer also exports to the USA, he will note that incorporation of the conditions of EU regulations in his HACCP plan would not meet the recommendations of the FDA Guidelines. As I understand the situation on these matters in the USA, the legislation does not specify the freezing and frozen storage conditions for control of hazards from parasites in fishery products, and the FDA guidance is just that, guidance, not mandatory requirements. A processor could adopt other conditions of temperature and time if those conditions are effective in controlling the hazard. These subtleties might not be appreciated by a would-be exporter of lightly preserved fishery products to both EU and USA markets who might believe he would have to process under two different sets of conditions for the different markets, or would have to adopt the -20 C for 7 days regime which would satisfy both the EU regulations and the FDA Guidelines.

As far as production in, or for, the USA is concerned the three sets of freezing and storage condition already referred to can be seen as alternatives and the processor could select any, or others, for his HACCP plan for lightly preserved products provided the conditions can be shown to be effective for controlling the hazard. As far as production in, or for, the EU market is concerned, the processor has to comply with the -20 C for 24 hours condition, and the question for the HACCP team is whether or not these conditions are adequate to control the hazard.

REVIEW OF THE LITERATURE ON LETHALITY OF FREEZING AND FROZEN STORAGE TO NEMATODES

Anisakiasis was first recognised - at least in published papers - as a disease of humans caused by the larval stage of nematode parasites in fishery products in the late 1950's, firstly in The Netherlands but subsequently in many other parts of the world (Bier et al, 1987; Sakarni & McKerrow, 1989). before this recognition, Gustafson (1953) published a paper on the effects of freezing on Anisakis larvae. His investigations were initiated by enquiries from a zoo, but he claimed there was interest in the problem of nematodes in products on the part of public health authorities. According to a comment in his paper, this interest was in the aesthetic aspects.

Gustafson used heavily infested portions of Sebastodes flesh and froze them in 120g packs in still air in freezer cabinets at several temperatures. Thermometers were placed in the packs to record their internal temperatures. Samples were removed at intervals, the nematodes recovered by enzyme digestion of the samples, and the number of dead and live nematodes determined. Freezing and storage at -5°C or -10°C, even for several days, did not kill all the larvae. After 12 days at -10°C, 38 out of 1029 larvae recovered, 4%, were still alive. On storage at -17°C, one pack taken out at 6 hours had reached an internal temperature of -14°C and 33 out 600, 5.5%, of the recovered nematodes were alive. The next sampling time was at 24 hours and at this time, and longer, at -17°C, the core temperatures had reached the freezer temperature and no live nematodes were recovered.

Gustafson also froze samples to -20°C or below under different conditions of freezing - in still air or by immersion in a methyl cellusolve/CO2 bath. No live nematodes were recovered from any of the samples frozen to below an internal temperature of -21°C. One of the seven samples frozen to an internal temperature of -20°C or -21°C, that frozen in still air at -42°C for 2 hours, to an internal temperature of -21°C, had 15 live nematodes out 850 recovered, 2%; no live nematodes were recovered from the other 6 samples.

He also froze 45.4kg (100lbs) blocks of herring by placing them in still air at -30 to -33°C for 16 hours then transferring them to a store at -12°C. He does not report the internal temperature reached. Two samples taken at 24 hours of this sequence did not yield any live nematodes.

Regulations concerning treatment of herring used for the production of marinated and lightly preserved products came into force in The Netherlands in 1968. Prior to that the TNO Institute for Fishery Products, Ijmuiden, carried out experiments on the lethality of processing treatments, including freezing, to nematode larvae in herrings. The results are summarised by Houwing (1969). The paper does not give details of the investigations, but states: 'If the herring is frozen until a product temperature of -20°C is reached, and this herring is kept during 24 hours at this temperature, the product is safe. We also found that, if herring is frozen cryogenically, the nematodes are inactivated immediately after the product temperature is decreased to -30°C, so that a further storage is not necessary in this case.'. From information elsewhere in the paper, cryogenically means freezing in a tunnel by liquid air. The other freezing processes referred to are freezing of 10cm thick blocks in 5 hours in a plate freezer, and freezing of 5kg drums of herring in brine in 11 hours in a blast freezer.

The Houwing (1969) paper does not provide experimental details, but I have an extract from an internal report of the TNO Institute which summarises that work. The herring were frozen under conditions used in the Dutch fish processing industry at that time, and referred to in Houwing (1969). They were frozen under different conditions to -20°C in 3 to 10 hours, and subsequently stored at -20°C. The products were thawed immediately after freezing and after periods of storage, and the nematode larvae recovered and tested for vitality. Twenty eight out of 355 larvae, about 8%, recovered from the product frozen to -20°C in three hours and thawed immediately showed vitality, but none in the samples frozen over 5 or 10 hours. However, 23 of the 407 larvae recovered from the material frozen in 5 hours were comatose, that is, showed some movement when stimulated. Samples of the product frozen in 5 hours were sampled after 15 and 50 hours of storage and no live or comatose larvae were recovered. The extract I have does not refer to the cryogenic freezing.

Again the experiments show that a small proportion of larvae can survive the freezing processes described in the internal report, but subsequent storage in the frozen state, in this case at -20°C, for several hours is lethal.

Deardorff and Throm (1988) tested the lethality of what they referred to as 'commercial blast- freezing' to nematode larvae. Salmon and rockfish were frozen singly on trays in a blast freezer at -35°C. They were in the freezer for 15 hours. This protocol 'closely simulated' the usual freezing procedures of the fish processing plant. The paper does not give an internal temperature of the fish on completion of freezing, but given the size of the fish, 1.8-3.6kg, and the conditions of freezing, I would expect the core temperature to be at -35°C after 15 hours. The frozen fish were subsequently stored at -18°C, and samples were taken after 1, 24, 48, and 48 hours of storage, thawed, and digested to recover the parasites. They found six viable, but comatose, larvae out of 1 671 larvae recovered from eight fish of each sort after 1 hour of storage, but no viable larvae were recovered from fish stored for the longer periods.

Karl and Leinemann (1989) froze 7kg blocks of round herring and 7.5kg blocks of herring fillets in a horizontal plate freezer to internal temperatures between -30°C to -34°C in 2.5 hours. Samples were thawed immediately; otherwise the blocks were stored at -18°C. The authors found that 5 out of 207 larvae recovered from a block of round herring thawed immediately after freezing showed very weak movement on stimulation, the remainder being dead. No viable larvae were found in 565 larvae revered from blocks of round and of fillets stored for 24 hours before thawing.

Round herring were also frozen singly in an air blast freezer to core temperatures of -18 or - 20°C and subsequently stored for 24 hours. There were no viable larvae found in 497 larvae recovered from samples of these fish.

The workers also studied the effect of slow freezing as might be practised in the home or in small enterprises. They packed herring in containers holding 5, 10 or 20kg and held in the containers in still air in a cold store operating between -17 to -21°C until the core temperature reached that of the cold store. The packs were then thawed in still air. The paper records the time taken for the packs to cool to the store temperature, between 23 and 62 hours, and to thaw, up to 48 hours at room temperature. The authors record that the herring showed a distinct lowering of quality after passing through this process. No living nematodes were found in the 5kg pack. Some living nematodes were recovered from the 10kg pack, but these lost their motility within 24 hours (held in 1% acteic acid). 1 000 larvae were isolated from the 20 kg packs of which 15 showed spontaneous movement.

Hauck (1977) studied the survival of nematode larvae during certain processing operations, mostly associated with smoking, but including freezing. The text does not describe how the herring were frozen other than they were frozen whole. A table in the paper shows the number of larvae examined after the treatments and the proportion of viable larvae. The column for frozen fish shows 2 099 larvae being recovered from the frozen product, but the cell for the proportion of viable larvae has a dash which I interpret to mean no viable larvae were found.

These references seem to be the total of published papers on the effect of freezing on the viability of nematode larvae in fish. I also have the extract already referred to from the unpublished report of the TNO Laboratory, IJmuiden, The Netherlands, but its conclusions do not add to the results in Houwing (1969). Though there only a few publications, they are sufficient to draw conclusions to form a basis for HACCP plans.

It is clear that freezing and frozen storage are effective for killing nematodes and thus eliminating the hazards from the presence of these parasites in fish. It is to be expected that the lethality of freezing to nematode larvae would have parallels with the lethality of heat to microorganisms in that lethality will be a function of temperature and dwell time. Even -5°C shows some lethality, but the results of the Gustafson experiments suggests the critical temperature to ensure a high proportion of nematodes are killed within

several hours is -17°C, and a temperature of -20°C or below is required for a 100% kill within a few hours. It is also apparent from the results of the investigations reported in these papers that freezing of itself, even down to-35°C may not kill all larvae. A very small proportion of larvae survive, but they are moribund, that is, the larvae do not show spontaneous movement, but will move when stimulated. It is likely that such moribund larvae would not be capable of infecting humans. For example, van Mameren & Houwing (1969) in a study of the effects of irradiation on Anisakis larvae, dosed rabbits with larvae irradiated at 0.6Mrad. The paper records that 82% of the larvae survive this degree of irradiation, but none of the dosed rabbits showed any primary infection or inflammation. Presumably the irradiation had attenuated the infectivity of the parasites even though the in vitro tests indicated viability. However it would be safer in a HACCP analysis of risk to assume that motile larvae could be infectious. Freezing, then, should be followed by a period of storage in the frozen state to ensure complete elimination of the hazard.

It will be part of my argument - developed below - that the EU Directive and the FDA Guidelines do not distinguish adequately between freezing, that is the process of reducing the temperature of the product, and storage in the frozen state.

IMPLICATIONS FOR HACCP, AND FOR THE EU REGULATIONS AND THE FDA GUIDELINES

There is no doubt that the presence of nematode parasites in fishery products is a hazard to human health. The risk factor is the consumption of raw products, including lightly preserved products that are not be cooked before consumption. There is experimental evidence that freezing and frozen storage can eliminate the hazard, and will be an effective control measure in a HACCP plan. It may well be the case in many fish processing operations that the risky products will be manufactured from raw material that has been frozen as a means of preservation. For example, pelagic fish, being the product of seasonal fisheries, are often frozen in bulk to provide year-round supplies. In these cases the freezing step is adventitiously the control measure to eliminate the hazard, and the HACCP team will take this into account in preparing the HACCP plan. However, there will be situations in which the raw material is unfrozen fish and a freezing step must be introduced in the processes solely as a control measure in the HACCP sense. There are various combinations of freezing conditions and storage times that can achieve the necessary control and it a question for management is to select one that is convenient for the processing systems in place in the plant. The HACCP team will then evaluate the step for its efficacy in effecting control.

Before discussing the requirements of the EU Directive 91/493 and the recommendations of the FDA Guidelines, let me return to the distinction between freezing and frozen storage. 'Freezing' is the operation of applying refrigeration to a product so that its temperature is reduced below its freeing point. Subsequent to this process the product might be stored in the frozen state. In my view, these operations, freezing and frozen storage, should be distinguished as separate operations in a HACCP plan, and each subjected to critical limits and to monitoring as required. I have discussed above the separate actions of freezing and frozen storage in literature as controls for the parasite hazards in fishery products.

Both the EU Directive and the FDA Guidelines are loosely worded in this respect. I have quoted the wording of the EU Directive above, and to me this requirement means that the processor must freeze the products for 24 hours, that is leave them in a freezer for 24 hours. The wording would be technologically correct if the relevant passage went something along the lines of '... be subjected to a freezing process in equipment intended for the freezing of fishery products, and subsequently stored at a temperature of not more than -20o C in all parts of the product for not less than 24 hours.'. I note from a transcript, in English, of the Dutch regulation of 1968 that was the forerunner of the EU requirement that the freezing and frozen storage stages were separately regulated. It required that the herring be frozen in such a way that the herring reached a temperature of at least -20°C within 12 hours, and that the herring then be stored for 24 hours at -20°C. This combined process is in accord with the results of investigations of the effect of freezing on the viability of nematode larvae in fish products.

The recommendations in the FDA Guidelines are confusing, are not in accord with good freezing practice, or are not necessary. In Chapter 5, PARASITES, in the Controlling parasites section, second paragraph, is the statement:

> 'Freezing (-4°F (-20°C) or below (internal or external) for 7 days or -31°F(- 35°C) or below (internal) for 15 hours) of fish intended for raw consumption also kills parasites. FDA's Food Code recommends these freezing conditions to retailers who provide fish intended for raw consumption.'

I do not believe these are reasonable recommendations for a retailer. I think it unlikely that a retailer would have equipment capable of freezing products to an internal temperature of -35°C, and the experimental evidence shows that storage at -20°C for, at most, one day is quite adequate to kill parasites. The option of internal or external for the -20°C regime in not clear. I have not come across any studies that led to this protocol of -20°C for 7 days, but I assume the reference is to freezing of products by exposing them to an external temperature of -20°C. This reads like just putting the product in a cold store or freezer cabinet at this temperature. Freezing in this way is not good practice and is to be deplored. Slow freezing like this is deleterious to the quality of the product, and, judging from the results reported by Karl and Leinemann (1989), might not be effective in killing all the parasites.

The -35°C for 15 hours reads like the conditions described by Deardorff and Throm (1988). The paper does not record that the internal temperatures of the fish were measured, though the operating temperature of the freezer was noted. The wording in the FDA Guidelines gives the impression that the internal temperature should be at -35°C for 15 hours, which cannot be implied from the Deardorff and Throm paper. Indeed that cannot have been the case; the product was presumably put in at factory ambient temperature. Deardorff and Throm describe the process as commercial blast-freezing, but make no comment at to whether they consider the freezing process to be typical of commercial practice. They describe it only as being typical of the process at the plant they used. Good freezing practices require that fishery products be frozen rapidly in equipment designed for the purpose to an average temperature below that of the intended storage. As the temperature of the refrigerating medium, typically -35 to -40°C, will almost certainly be below that of the cold store, it is necessary only to freeze to a core temperature close to the storage temperature. In the Deardorff and Throm study the storage temperature was -18°C and this core temperature would be achieved in an air-blast freezer for the size of fish they were using in 3 or 4 hours. The company was tying up an air blast freezer for far longer than was necessary. Again, it is not a practice I would recommend to a processor.

I do not like the example of Table 5-1; I believe it is not a practicable process. The plan is intended for a process for freezing salmon fillets. The control measure and its critical limits are given in column (3) as 'Freeze at -35°F or below for 15 hours'. I have already pointed out the defects of this in the case of freezing whole fish; the criticisms are even more valid for a process for freezing fillets. Salmon fillets would freeze to a core temperature of -30°C in about two hours; freezing for 15 hours is an even more inefficient use of an air-blast freezer. But what is even mor impracticable is the requirement to monitor the internal temperature of each fillet on a recording thermometer. Are the drafters of this recommendation really serious. An air-blast freezer might hold a tonne or more of product. Assuming each fillet weighs 1kg, a reasonable yield from a 3.5kg salmon, the load would comprise about 1 000 fillets. Quite an undertaking, and an unnecessary one at that, to measure and monitor the temperature of each fillet.

PROPOSALS FOR A MODEL HACCP PLAN FOR FREEZING TO CONTROL PARASITE HAZARD

My proposal for a model HACCP for freezing to control the hazard from parasites in fishery products is as follows.

Column (3), control measure and critical limits. This should be in two parts: freezing, and cold storage. For example:

a) Freeze according to good freezing practices to below -20°C at the thermal centre;
b) Store at or below -20°C for at least 24 hours.

Requirement a) is consistent with the HACCP Principles and Application Guidelines of the National Advisory Committee on Microbiological Criteria for Foods adopted August 14, 1997. These recommend that HACCP systems be built ' ... upon a solid foundation of prerequisite programs.'. A plan for a particular process, a prerequisite program, will refer to the process specifications for the freezing step described in the plant's quality assurance manual.

Requirement b) has been shown experimentally to be effective for eliminating the parasite hazard and is a process than is convenient to fit into the operating procedures of a fish processing plant.

It is possible that just freezing to a low temperature without any subsequent storage would effect elimination, but I do not believe there is adequate experimental data available to specify a critical control point. The Houwing (1969) paper suggests that freezing to -30°C is adequate, but I have reservations about that conclusion. The paper provides no details, but the fish were frozen in a cryogenic tunnel with liquid air. The operating temperature of the atmosphere in a cryogenic tunnel is much lower than -30°C, more like -80°C, and it occurs to me that the products could have been for a time below the stated temperature. The Deardorff and Throm study showed a very low level of vitality of parasites in products that would have been below -30°C at the core for a few hours.

Column (4), What is monitored:

a) Compliance with process specification;
b) Batch codes and cold store stock records.

Column (5), How:
a) Supervision of operation of the freezer and checking of freezer log;
b) Examination of batch codes and stock records on removal of material from the store.

Column (6), Frequency:
a) Each freezing operation;
b) On removal of a batch from store.

Column (7), Who:
a) Shift supervisor, quality controller, or freezer operator;
b) Warehouse man, shift supervisor, or quality controller.

Column (8), Corrective actions:
a) Keep in the freezer until completion of freezing, or return to freezer;
b) Retain in cold store for the minimum dwell.

Column (9), Records:
a) Freezer and refrigeration room logs;
b) Cold store stock records.

Column (9), Verification:
a & b) Review monitoring records and corrective actions a least weekly. Confirm from temperature records that the cold store is maintaining a temperature below -20°C.

Column (10), Validation:
Review the process specification for the freezing step to ensure it is adequate to achieve a core temperature in the product of -20°C. Measure the core temperatures of sample packs. Review the literature on control methods for parasites in fishery products.

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