CLOSTRIDIUM BOTULINUM

THE ORGANISM/TOXIN

Clostridium botulinum will only grow at low Eh values which are normally correlated with the absence of oxygen. There are a number of neurotoxin types which differ markedly in their characteristics and ability to cause disease in humans.

Two groups are important in food:

Group I-Types A, B and F (proteolytic strains)

Group II-Types B, E and F (nonproteolytic strains)

Because group I organisms are proteolytic their growth generally causes spoilage of the contaminated food.

There are two manifestations of disease relevant to food; classic foodborne botulism and infant botulism.

GROWTH AND ITS CONTROL

Growth:

Temperature: Group I Minimum 10^{o}C, optimum 35-40^{o}C, maximum 45-50^{o}C.

Group II Minimum 3.3^{o}C, optimum 18-25^{o}C, maximum 40-45^{o}C.

pH: Group I Minimum 4.6, group II Minimum 5.0. Toxin is produced by either group at pH values down to 5.2, but in some instances toxin may be produced at pH values lower than this; for example in potatoes adjusted to pH 4.83 and temperature abused.

Atmosphere: Normally grows in the absence of oxygen. However this may be deceptive as there have been outbreaks caused, for example, by spores being trapped between food and aluminium foil wrapping, which is not an obvious low oxygen environment. The presence of even 20% O_2 in packaged foods is not necessarily enough to prevent growth.

CO_2 concentrations above 75% have been shown to retard the growth of *C. botulinum*. However, 100% CO_2 is not sufficient to prevent toxin production by group II organisms in temperature abused fish. Modified atmospheres used in conjunction with adequate refrigeration did not prevent outgrowth and toxin production by group II organisms in cooked turkey.

Water activity: Group I inhibitory NaCl = 10%, group II 5%. Minimum a_w for growth group I 0.94, group II 0.97. Toxin can be produced at a_w values permitting growth.

The minimum a_w for growth decreases as the pH increases towards pH 7 in vacuum-packed potatoes.

Survival:

Temperature: *Spores and toxin:* Resistant to freezing.

Vegetative cells: Undefined

pH: *Toxin*: Stable at low pH, inactivates quickly at pH 11.

Water Activity: Spores survive drying.

Inactivation (CCPs and Hurdles):

Temperature: *For spores:* D_{100} group I 25 min, group II <0.1. D_{121} group I 0.1-0.2 min, group II <0.001 min. A 12 D process, controlling group I spores, has been adopted for the canning of low-acid (pH> 4.6) foods. This is the equivalent of heating to 121^{o}C for 3 min.

Toxin: Inactivated by treatment at 85^{o}C for 1 min, 80^{o}C for 6 min or 65^{o}C for 1.5 hours. Toxins may be slightly more heat stable at lower pH values.

Resistant to freezing.

Vegetative cell: Killed by a few minutes exposure to 60^{o}C.

pH: *Spore:* Thermal death is accelerated at extremes of pH (<5.0 and >9.0) for type A spores. Citric acid is more inhibitory than HCl.

Toxin: Stable at low pH, inactivates quickly at pH 11.

Preservatives: (NB: Some of the preservatives discussed here may not be permitted in New Zealand). Nitrite is an important preservative for the control of *C. botulinum*. In addition sorbates, parabens, nisin, phenolic antioxidants, polyphosphates, ascorbates, EDTA, metabisulphite, *n*-mono alkyl maleates and fumarates and lactate salts are useful preservatives if used as hurdles.

Nitrite and nitrate curing do not completely inhibit growth in temperature abused vacuum-packed trout. Nitrite in combination with other factors can control growth in meat products. Inhibition by nitrite is affected by pH (greater inhibition at lower pH values).

Sorbic or ascorbic acids may be used in combination with a low concentration of nitrite to inhibit *C. botulinum*.

Liquid smoke appears to be effective in fish but not in meat products.

Methyl and propyl paraben inhibit growth at concentrations of the order of 0.1%.

Diphosphates were shown to be more inhibitory than tri- or polyphosphates.

Nisin is used as an anti-clostridial compound in dairy products. Concentrations used can be as high as 500µg/g.

Lactic acid bacteria added in starter cultures, inhibit growth of *C. botulinum* in meat products.

Sulphites do not inhibit growth in modified atmosphere packed (30% N_2:70% CO_2) potatoes stored at room temperature.

Bacteriocins produced by some lactic acid bacteria are inhibitory to *C. botulinum*.

The interactions of various preservatives, as would be used in hurdle technology, are too complex to allow generalisation and particular combinations intended for food use will need to be validated.

Sanitisers/Disinfectants: (These products must be used as advised by the manufacturer). Spores are inactivated by ozone or chlorine dioxide. Chlorine used at low pH is more effective than at neutral or alkaline pH.

Disinfectants used in the food industry, i.e. hydrogen peroxide, chlorine and iodophors are effective at inactivating spores. Hydrogen peroxide is used at levels up to 35% in combination with heat.

Ethylene oxide is effective at inactivating spores although other factors such as temperature impact on its efficacy.

Normally chlorinated water should effectively inactivate toxin.

(N.B. The absence of a sanitiser/disinfectant from this section does not imply that it is ineffective).

Radiation: Spores of *C. botulinum* are the most resistant of those from bacteria of public health concern. In frozen foods the D values range from 2.0 to 4.5 kGy. D values may be lowered by the presence of O_2, preservatives or temperatures above 20^{o}C. Doses used in food preservation do not effectively eliminate the organism. Toxins are not destroyed at doses used for foods.

Radiation is ineffective at destroying the toxin.

THE ILLNESS

Incubation: Typically 12 to 36 hours, sometimes several days. For infant botulism 3 to 30 days.

Symptoms: Vary from mild disease to illness that can be fatal within 24 hours. Initial symptoms are nausea and vomiting followed by neurological symptoms including problems with vision, fatigue, lack of co-ordination, problems with throat and mouth.

In infant botulism the first sign is constipation. A number of signs may then follow including lethargy, inability to feed properly, floppiness and respiratory arrest. Infant botulism has been reported to account for around 5% of sudden infant death syndrome cases in the USA and Europe.

Condition: In botulism food containing pre-formed toxin is eaten. In infant botulism spores are consumed, germinate, colonise the gut and produce toxin. Infant botulism is the most common form of botulism in the USA.

Toxins: It is the toxins produced that are responsible for the injurious effects.

At Risk Groups: All people are at risk from botulism. Adults are more susceptible if they have radically altered intestinal microflora as might be produced by the effects of antibiotic or chemotherapies.

As the name suggests, infant botulism occurs in children less than 1 year in age.

Long Term Effects: Botulism has a high case fatality rate (8%). 80% of cases will be hospitalised and require intensive supportive therapy.

Dose: In mouse bioassays the LD_{50} is <0.1 ng/kg. Estimates of the dose required to kill humans varies from 0.1 to 1.0 μg.

In infant botulism implicated honey samples have contained approximately 10^4-10^5 spores /kg.

NZ Incidence: Only three cases are known to have occurred in New Zealand. No cases of infant botulism are known.

Treatment: Initial steps are to remove toxin from the body. These efforts include treatment with an antiserum and clearing contents from the stomach and intestines. Subsequent treatment is supportive and involves artificial ventilation.

Treatment for infant botulism is similar except that the use of antiserum does not seem to be effective. The treatment is therefore primarily supportive.

SOURCES

Human: Not known to be carried asymptomatically.

Animal: Animals can be affected by botulism.

Food: Type E is associated with seafood, while type A is strongly associated with vegetable products. *C. botulinum* is frequently found in seafood from a number of parts of the world. The numbers of spores present are very low in all foods, except in those cases where the foods are associated with food poisoning. The organism can also be found in fruits and vegetables that have close contact with the ground, e.g. asparagus, potatoes, cabbage etc. The main problem lies with outgrowth of the few spores that may be present in a food.

Honey has been implicated as the vehicle in a proportion of cases of infant botulism.

Associations of the organism with foods in New Zealand are not known.

Environment: Types A, B and F are generally found in soil or sediment. Type E is a normal inhabitant of the marine environment, but may also be isolated from soil. The organism has been isolated from dust, including vacuum cleaner dust. In New Zealand type C/D have been isolated from sediments.

Transmission Routes: Considered to be primarily foodborne, although wound botulism is known (especially among intravenous drug users).

OUTBREAKS AND INCIDENTS

Outbreaks:

New Zealand:

Puha and Mussels (Tiroi): 2 cases, both hospitalised. Control point failure: Incomplete lactic acid fermentation.

Overseas:

Roasted Egg Plant in Oil: 7 cases, 4 hospitalised. Control point failure: Failure to inactivate spores, storage conditions allowed growth.

Beef Stew: 1 case, 1 hospitalisation. Control point failure: Inadequate heat treatment or post cooking contamination, temperature abuse, anaerobic conditions.

Hazelnut Yoghurt: 27 cases, 1 died. Control point failure: unsafe change in product formulation, under processing.

Marscapone Cheese: 7 cases, all hospitalised, 1 death. Control point failure: Temperature abuse, contamination of raw ingredients.

Cheese Sauce: 8 cases, 4 hospitalised, 1 death. Control point failure: Cross contamination, temperature abuse.

Salted Fish: 8 cases, 2 hospitalisations, 1 death. Control point failure: Temperature abuse.

Chopped Garlic in Oil: 34 cases, seven required ventilator support. Control point failure: contaminated raw ingredient, temperature abuse.

Tinned Salmon: 4 cases, 2 died. Control point failure: not identified but presumably under-processing.

Epidemiological studies: Several studies into non-food risk factors for infant botulism have been conducted but there is no clear consensus on risk factors, although exposure to dust does seem to recur as a factor. The role of breast feeding remains controversial.

ADEQUATE PROCESSING GUIDELINES

N.B. These guidelines have been derived from published information. Industry is advised to ensure that processing steps they are using are adequate to meet their particular food safety objectives.

Cook meats to:	**Internal temperature reached**	**Time**
Minced meats (beef, veal, lamb, pork) + pork cuts Minced poultry Meat cuts (beef, veal, lamb), fish, seafood Poultry, breast Poultry, whole	71°C 74°C 63°C 77°C 82°C	15 sec ″ ″ ″ ″
Cook low-acid canned foods under pressure	121°C	3 min
Cool cooked foods at room temperature until: Continuously cool cooked foods under refrigeration to achieve a reduction of: Followed by:	≥55°C 55ºC to 25ºC 25ºC to 5ºC	 In less than 5 hrs In less than 10 hrs
Hold foods at	≤ 3.3°C or ≥ 60°C	
Reheat cooked foods (to inactivate toxin)	85°C 80°C 65°C	1 min 6 min 1.5 hours
Reduce pH of food to ≤ 4.6		
Add appropriate level of preservative to store foods safely		

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