Thermal and Non-thermal processing of fishes

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Introduction

'Thermal processing' or 'preservation by heat' is a widely used method for storage life enhancement of food due to its high safety level and convenience. The basic purpose of thermal processing of foods is to reduce or destroy microbial activity, reduce or destroy enzyme activity and produce physical or chemical changes to make the food meet a certain quality standard. e.g., gelatinization of starch & denaturation of proteins to produce edible food. Thermal processing is generally referred to as canning, which helps in realizing long-term microbiological stability for non-dried foods without the use of refrigeration, by prolonged heating in hermetically sealed containers, such as cans or retortable pouches, to render the contents of the container sterile. But there are a number of heat processing methods employed by the food industry. Mild processes are Blanching and Pasteurisation. More severe processes include Canning, Baking, Roasting and Frying.

Blanching

Blanching is a mild heat treatment, which primarily destroys enzymes and also reduces microbial load. It is not intended as a sole method of preservation, but as a pre-treatment prior to freezing, drying and canning. Blanching is carried out at up to 100 °C using hot water or steam at or near atmospheric pressure. Microwave is also used for blanching, which has the advantages such as rapid heating and reduced loss of water-soluble components.

Pasteurization

Pasteurization is a relatively mild heat treatment in which food is heated to <100°C. This can be used to destroy enzymes and relatively heat-sensitive microorganisms. Pasteurization is intended to inactivate vegetative cells not the spores of all pathogenic bacteria and is used to extend the shelf life of food by several days, e.g., milk. There are different methods of pasteurization such as low-temperature long time, LTLT/batch/holding method (63°C for 30 min), high-temperature short time, HTST/flash method (72°C For 15 sec) and ultra-heat treatment (UHT) at higher temperatures and shorter times, e.g., 1 s at 135 °C.

Sterilization/Canning

Sterilization is heat processing at high temperatures (above 100 °C) with the objective of destroying all forms of microorganisms including spores. Canning is a method that sterilizes food by heat in airtight containers to achieve a commercially sterilized product. This allows food to be stored at room temperature while maintaining food safety and organoleptic quality for months or even years. It is invented by the Frenchman Nicholas Appert and is sometimes referred to as 'appertization'. There are two typical forms of canning: in-container sterilization (i.e., retort processing) and out-of-container sterilization (i.e., aseptic processing).

Classification of foods based on pH

From a thermal-processing standpoint, foods are divided into three distinct pH groups, which are given below.

1. High-acid foods (pH < 3.7)
2. Acid or Medium-acid foods (pH 3.7 - 4.5)
3. Low-acid foods (pH > 4.5).

Canned seafoods are characterized by a pH > 4.6 and aw > 0.98.

Clostridium botulinum

Foods with a pH greater than 4.6 are called 'low acid canned foods' (LACF), for which the micro-organism of major concern is *Clostridium botulinum*. *C. botulinum* is a highly heat resistant mesophilic, Gram-positive, rod-shaped spore-forming anaerobic pathogen, which produces the toxin botulin. It has been generally accepted that *C. botulinum* and other spore-forming human pathogens do not grow and produce toxins below a pH of 4.6. Growth of *C. botulinum* is a risk in low-acid foods having a pH above 4.6 including fishery products, where it is necessary to apply a time–temperature regime sufficient to inactivate spores of *C. botulinum*.

Botulinum cook or the 12D concept

Experience has shown that the minimum heat process necessary to preserve low-acid canned foods (LACF) should enable the reduction of the most heat-resistant *C. botulinum* spores to 10^{-12} of its initial count. This is known as the botulinum cook or the 12D concept.

D value/Decimal reduction time/Thermal reduction time

The D value is the time required to reduce the number of spores of *C. botulinum* (or any other micro-organism) by a factor of 10 at a specific reference temperature (121.1°C) or it is the time necessary to inactivate 90 % of a given microbial population by heating at a constant temperature. The unit of measurement for D is 'minute'. The D value for bacterial spores is independent of initial numbers, but it is affected by the temperature of the heating medium. The higher the temperature, the faster the rate of thermal destruction and the lower the D value. A thermal process based on the 12D concept should achieve a probability of survival of one spore in one of one trillion containers. In other words, the probability of one container being non-sterile is equal to 10^{-12}, i.e., one can in one trillion cans is not sterile.

The thermal death time (TDT)

TDT is the heating time required to cause complete destruction of a microbial population. It may be defined as the time required at any specified temperature to inactivate an arbitrarily chosen proportion of the spores. TDT value depends on the initial microbial load (while D value does not). TDT is always measured with reference to a standard initial load or load reduction, it simply represents a certain multiple of D value. For example, if TDT represents the time to reduce the population from 10^{0} to 10^{-12}, then TDT is a measure of 12 D values. i.e., TDT = nD, where n is the number of decimal reductions. The extent of inactivation in the case of pathogenic microorganisms (*C. botulinum*) is equivalent to a 12 D process. For the purpose of heat process determination with respect to their lethality towards specific micro-

organisms, the reciprocal of the thermal death time (TDT value) called the lethal rate, L is used.

'z' value

The slope of the TDT curve is defined as 'z' value, which is the number of degrees for the TDT curve to traverse one log cycle. The 'z' value is also known as the temperature sensitivity indicator. It represents a temperature range resulting in a ten-fold change in D values or, on a semi log graph, it represents the temperature range between which the D value curve passes through one logarithmic cycle. The 'z' value is usually taken as 10 °C in the case of *C. botulinum*.

F_o value

Thermal processes are calibrated in terms of the equivalent time the thermal centre of the product, i.e., the point of the product in the container most distant from the heat source or cold spot, spends at 121.1 °C. This thermal process lethality time is termed as process value or F_0 value. F_0 is the sterilization process equivalent time, defined as the number of equivalent minutes at T = 121.1 ºC delivered to a food container calculated using Z value (the temperature increase required for a tenfold decrease in the D value) of 10 ºC. Lower F_os yield microbially safe and shelf-stable products without undue impairment of flavour, consistency, colour or nutrient content.

Commercial sterility

Commercial sterility is the condition achieved by the application of heat, sufficient alone or in combination with other appropriate treatments, to render the food free from microorganisms capable of growing in the food at normal non-refrigerated conditions at which the food is likely to be held during distribution and storage (FAO/WHO Codex Alimentarius Commission, 1983). Canning of food for preservation requires the use of a hermetically sealed container, which is impermeable to liquids, gases and micro-organisms, and the use of a heat process sufficient to inactivate micro-organisms capable of proliferating under normal non-refrigerated conditions of storage and distribution. Any canned food that meets these two requirements is considered 'commercially sterile'. Commercial sterility is different from 'absolute sterility'. The latter means total absence of viable micro-organisms, whereas viable micro-organisms can be recovered from commercially sterile canned fish.

Containers for thermal processing/ Packaging Materials

- Tin plate
- Tin-free steel (TFS)
- Aluminium alloys
- Retort pouch
- Glass containers

Metal containers are normally divided into two groups:

- 2-piece cans (Cylindrical, square, flat rectangular, oval or round)

- 3-piece cans with soldered or welded body. They are generally cylindrical with two lids attached to the cylindrical body by double seaming.

Historically, heat processing started in glass containers. Over the years, different containers like metal, rigid plastic containers and flexible retortable pouches have been developed for thermal processing. The most common material used for manufacturing containers for fish products are tin plate, aluminium and lacquered steel plate (TFS). Enamel coatings are used to protect tin plate, aluminium alloys and TFS. Flexible packaging as an alternative to metal cans has become more common during the last years and glass jars are sometimes used for speciality packs. Nowadays, retort pouch processing is very popular.

Retort pouches

The most common retort pouch is 3-layered laminate. The 3 layers are joined with adhesive lamination. These three layers are; Polyester layer which helps in providing strength and abrasion resistance, Aluminium foil for providing barrier against moisture, gases and light and Polypropylene/ polyethylene for heat sealing properties.

Thermally Processed Fishery Products

Fishery products, being categorized as low-acid foods require heat processing severity with respect to *C. botulinum*. These products have to be processed in such a way that all the points in the container should achieve a minimum lethality of 2.52 minutes, when processed at 121.1 ^{o}C (250 ^{o}F), which corresponds to 12 decimal reduction of *C. botulinum*. In practice, fish products are processed beyond this lethality for safety reasons. The selection of prime-quality fish is important for heat processing. Thermal processing of various ready-to-eat fish products has been studied and reported by ICAR-CIFT, Kochi. Mohan et al. (2015) studied the effect of filling medium on cooking time and quality of canned yellowfin tuna (*Thunnus albacares*).

Steps in fish canning

The important steps in canning process are:

1. Raw material selection and preparation
2. Blanching/ Precooking
3. Filling into containers
4. Addition of fill (brine/ oil/ gravy)
5. Exhausting
6. Seaming/ sealing
7. Retorting (heat processing)
8. Cooling
9. Drying
10. Labelling and storage

Hazards in fish canning

- Survival of pathogens during heat processing
- Presence of heat-stable toxins (biotoxins, histamine) in the raw material

- Recontamination of product after heat processing (faulty containers, poor sealing, contaminated cooling-water, faulty container handling).

Retort pouch processing

As in canning, retort pouch food is sterilized after packing, but the sterilization procedure differs. The pouches are processed in an over-pressure retort. The time and temperature will be standardized depending on the product. Besides, cost reduction, retort pouch packages have unique advantages like boil-in-bag facility, ease of opening, reduced weight and don't require refrigeration for storage. The energy saving is more in processing in flexible pouches compared to cans. Processed food products can be kept for long periods at ambient temperatures. Bindu et al. (2007) reported that ready-to-eat black clam (*Villorita cyprinoides*) product in indigenous retort pouches remained in good condition even after storage for one year at ambient temperature (28 ± 2 °C).

Non-thermal Processing Techniques

The demand from consumers for safe and nutritious food products has promoted the rapid development of non- thermal processing technologies. Non-thermal food processing simply refers to methods where the food materials receive microbiological inactivation without the direct application of heat. They are relatively young technologies, which use mechanisms other than conventional heating to reduce or eliminate microorganisms.

1. High-pressure processing

High-Pressure Processing is also known as high hydrostatic pressure (HHP) or ultra-high pressure (UHL) processing. It is a non-thermal, cold pasteurization technique, which generally consists of subjecting food, previously sealed in flexible and water-resistant packaging, to a high level of hydrostatic pressure (pressure transmitted by water) up to 600 MPa / 87,000 psi for a few seconds to a few minutes (1 – 20 min). HHP utilizes a very common medium, i.e., water, to apply the pressure on the product to be treated. HHP transmits isostatic pressure (100–1000 MPa) instantly to product at low temperature and might have comparable preservation effect as thermal processing through inactivating undesirable microorganisms and enzymes. An HPP unit consists of a pressure compartment in which food is kept and water is introduced into the chamber. Food is then pressurized using this water. HPP compromises cellular functions such as DNA replication, transcription, translation already at lower pressures (≤100 MPa) which impairs bacterial growth. At higher pressures, microorganisms start suffering lethal injuries due to loss of cell membrane integrity and protein functionality. The most sensitive to pressures are moulds, yeast and parasites.

Studies at ICAR-CIFT, Kochi

Ginson et. al. (2015) studied the effect of high-pressure treatment (250 MPa for 6 min at 25 °C) on microbiological quality of Indian white prawn (*Fenneropenaeus indicus*) during chilled storage. All microbes were reduced significantly after high pressure treatment and there was significant difference in microbial quality of control and high pressure treated samples in the entire duration of chilled storage. Kunnath et. al. (2020) reported that synergistic effect of high pressure and microbial transglutaminase (MTGase) could enhance

the textural and functional properties of fish gels, when compared with conventional cooking. MTGase enzyme along with pressure treatment enhanced the conformational stability and produce stronger networks through the formation of non sulfide bonds between proteins and setting reinforced these networks. Devatkal et. al. (2015) employed high-pressure processing (300 MPa for 5 min) as a non-thermal post-processing intervention to improve the shelf life and quality of cooked refrigerated chicken nuggets. Kundukulangara Pulissery et. al. (2021) compared the textural and nutritional profile of high pressure and minimally processed pineapple. On the basis of microbial quality and sensory assessment, high pressure treatment at 300 MPa for 10 min was found to be suitable for preserving the quality of pineapple up to 16^{th} day in refrigeration condition. Ginson et. al. (2020) investigated the piezotolerance and diversity indices of microflora of Indian white prawn (Fenneropenaeus indicus) after high pressure (HP)-treatment. *Arthrobacter spp., Listeria grayi* and *Corynebacterium spp*. were the most piezo tolerant bacteria in HP-treated samples.

2. Pulsed electric field (PEF) processing

PEF is an efficient non-thermal food processing technique using short, high voltage pulses. It is used for inactivation of spoilage and pathogenic microorganisms in various food products. Electric pulses are applied for destroying harmful bacteria in food. Microbial inactivation is achieved by dielectric breakdown of the bacterial membranes. Food material is placed between electrodes. The field intensity is typically 20–80 kV cm^{-1}) and the exposure time is a few milliseconds or nanoseconds. It enhances the shelf life of the food without quality loss. The PEF mechanism is called ***electroporation***. Very short electric pulses of high voltage are applied to the food. Small pores are formed in the cell membrane of the food by the electric pulses without damaging the cell compounds, such as vitamins. Pulsed electric field is generally used for liquid food or semi-solid food that can flow easily.

3. Irradiation/Radiation processing

Irradiation refers to the process by which an object is exposed to radiation (A deliberate exposure to radiation). There are two forms of radiation: Ionizing radiation (IR) and non-ionizing radiation (NIR). IR includes high-energy electron beam, X-rays and γ-rays. IR leads to the production of charged particles or ions in material it comes in contacts with. Irradiation is a process of applying low levels of ionizing radiation to food material to sterilize or extend its shelf life. Radiation inactivates food spoilage organisms, including bacteria, moulds, and yeasts. It is effective in lengthening the shelf-life of fresh fruits and vegetables by controlling the normal biological changes associated with ripening, maturation, sprouting, and finally aging. Radiation also destroys disease-causing organisms, including parasitic worms and insect pests, that damage food in storage. Irradiation is harmful or noxious to humans. However, the dose for seafood pre-treatment is low, therefore making it safe for consumption. Food irradiated under approved conditions does not become radioactive.

Studies at ICAR-CIFT, Kochi

Annamalai et. al. (2020) assessed the effect of electron beam irradiation ((0, 2.5, 5.0, 7.5 and 10 kGy) on the biochemical, microbiological and sensory quality of vacuum packed headless

Litopenaeus vannamei during chilled storage (2 °C). There is a significant ($p<0.05$) reduction in *Brochothrix thermosphacta* and *lactobacillus* count in the irradiated sample. Based on the microbial and sensory analysis control had a shelf life up to 12th day. However, electron beam irradiated sample had an extended shelf life of 15-23 days with respect to dose level.

4. Ultraviolet (UV) Radiation

UV radiation is a form of energy considered to be non-ionizing radiation having in general germicidal properties at wavelengths in the range of 200–280 nm (usually termed UV-C). UV irradiation has demonstrated to be effective not only in reducing microbial load but also inactivating enzymes activity in plant products. When food is exposed to UV-C, with 200–280 nm, these short wavelengths are absorbed by the microbial cell nucleic acids. These absorbed photons cause the breakage of the bond and interlinking between thymine and pyrimidine of different strands and the formation of dimers of pyrimidine. These dimers (Photo products) prevent DNA transcription and translation, thus leading to the malfunctioning of the genetic material, which causes microbial cell death. In principle, the UV radiation operates by destroying the genetic constituent of the pathogen to prevent division, multiplication and subsequently hinder its propagation. Usually, different kinds of food products require different doses of UV radiation (termed as UV-inactivation dose measured in mJ/cm^2) to inactivate different kinds of pathogens.

5. Pulsed Light (PL) Preservation

Pulsed light (PL) is an alternative technique to continuous ultraviolet treatment for solid and liquid foods. PL consists of successive repetition of high-power pulses of light/short time high-peak pulses of broad-spectrum white light. Comparatively, PL has a thousand times strength greater than the normal UV light which is quite continuous. Pulsed xenon UV uses the full spectrum of ultraviolet light to disperse germ-killing energy. The light spectrum includes wavelengths from 180 to 1100 nm with a considerable amount of light in the short-wave UV spectrum. Similar to other non-thermal food processing technologies, PL also has potential in the inactivation or elimination of microbes in food. Specific examples of foods processed by PL include fish, vegetables, fruits, and meat. PL can be used alongside other novel technologies as a hurdle in the inactivation of microbes on the surfaces of foods.

Studies at ICAR-CIFT, Kochi

Ananthanarayanan et. al. (2019) studied the effect of pulsed light (PL) treatment on the shelf-life extension of yellowfin tuna (*Thunnus albacares*) steaks stored at 2±1 ^{o}C. Tuna steaks of 1 cm thickness weighing 80 g packed in 300-gauge cast polypropylene pouches were subjected to PL treatment using Xenon pulse light machine RC-847. Shelf-life studies were carried out in terms of reduction of aerobic flora as inferred from the total plate count (TPC) and the psychrophilic count. An overall extension of 13 days of shelf life was achieved for PL treated samples.

6. Ultrasound (US) processing

US is a compressional wave with a frequency of over 20 kHz. Sound wave bearing certain frequency that is more than the normal human hearing frequency. The frequency of US used

in the food industry for microbial inactivation ranges from 20 kHz to 10 MHz. The bactericidal action of US is mainly due to the cavitation process, in which microbubbles are produced and collapsed within a liquid medium. During the cavitation process, the temperature can increase to as high as 5500 °C and the pressure can increase up to 100 MPa, resulting in localized microbial sterilization. The bactericidal mechanisms of ultrasound include breakage of cell walls, disruption and thinning of cell membranes and free radical activity due to the collapse of cavitation bubbles.

7. Cold Plasma (CP) Technology

Ionization of gas molecules gives rise to plasma. Cold plasma is a non-thermal treatment that works in the temperature range 25–65 ^{o}C. Cold plasma has high antimicrobial activity and efficient enzyme inactivation capacity. The composition of the plasma reactive species largely depends on the composition of gas which is ionized. The gases commonly used for the generation of plasma include argon, helium, oxygen, nitrogen and air. The gases are subjected to any of the types of energy like thermal, electrical, magnetic field, etc., to generate plasma containing positive ions, negative ions, and reactive species like ozone and singlet oxygen.

8. Ozone treatment

Ozone is extensively employed as an effective antibacterial against many bacteria in food. Due to its high oxidizing potential and the ability to attack cellular components, ozone has broad-spectrum of disinfection. Ozone treatment is a chemical method of food decontamination that involves exposing contaminated foodstuffs (fruits, vegetables, beverages, spices, herbs, meat, fish, and so on) to ozone in aqueous and/or gaseous phases. Ozone alters the permeability of cells by damaging the microbial cell membranes. Ozone is also known to damage the structure of proteins, leading to the malfunctioning of microbial enzymes, which affects the metabolic activity and finally results in microbial cell death. Chemical composition, pH, additives, temperature, initial bacteria population, and ozone contact time with food and food surface type are factors determining the efficiency of ozone treatment on microbial reduction in seafoods

Conclusion

Fish require immediate processing and packaging to retain its quality. In addition to the existing fish preservation methods, many advanced processing techniques have been developed over the years to meet the consumer demand of fresh, safe and minimally processed fish. With non-thermal treatments, consumers get high quality, healthy, and safe food products. But there are two sides of the coin: with advantages come some disadvantages as well. If food is exposed for a longer period or treated at a higher intensity, these non-thermal technologies may lead to some undesirable changes in food, such as oxidation of lipids and loss of colour and flavour. But these technologies have many advantages compared to thermal processing. After overcoming the limitations properly in a planned manner, non-thermal technologies will have a broader scope for development and commercialization in food processing industries.

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