

# **DEVELOPMENT OF CANNED AMBULTHIAL**

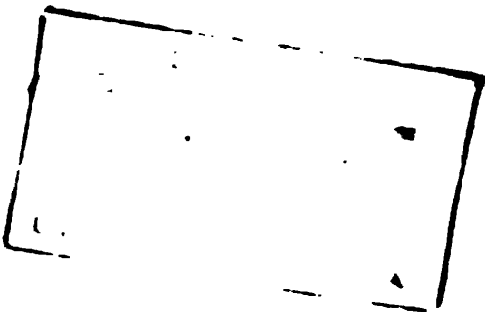
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**Thesis submitted in partial fulfillment of the requirements for the Degree of Bachelor of science in Food science and technology of the Faculty of Applied Sciences Sabaragamuwa University of Sri Lanka, Buttala, Sri Lanka**

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## DECLARATION

The work described in this thesis was carried out by me at the Faculty of Applied Sciences under the supervision of Mr Ranaweera and Mr A Sandanayaka. A report on this has not been submitted to any other University for another degree.

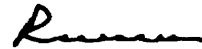


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***AFFECTIONATELY DEDICATED***  
***TO MY EVERLOVING***  
***PARENTS, BROTHER, SISTERS***  
***AND FRIENDS***

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## **Abstract**

Fish is naturally available food resources in Sri Lanka. Large fish production is obtained annually. Higher amount of this become waste because fish is highly perishable food. Therefore developments of fish preservation methods are important in Sri Lanka.

Fish canning is a world wide established preservation method. There is higher consumption of canned fish in the world. But fish canning technology is not established in Sri Lanka because of main two reasons.

One of them is Sri Lankan fish harvest contain higher amount of histamine content due to difficulties to transport fish to factory in chill condition with maximum safety. The other one is higher initial microbiological contain and loss of sensory properties of the raw material due to bad handling and unsafe conditions.

Ambul thial is a traditional fish curry that prepared from tuna fish. This curry can be thermally processed in hermetically sealed cans as a ready to serve product. This product is safer because the curry has great preservative action to inhibit the microorganisms.

The canned ambul thial, examination for the commercial sterility and pH was determined on the investigation of shelf life. Histamine content of the product was determined to ensure the product safe. The consumer acceptability was determined by sensory evaluation.

The final sample had negative for any microorganisms. The histamine level is low compare with Sri Lanka standards in the range of acceptance. Result of the sensory evaluation shows the product has consumer acceptability.

Since the samples were free from microorganisms, histamine hazard and it is sensorily accepted, there is a great potential of grabbing the any market.

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# Chapter 1

## Introduction

Fish is a naturally available food resource in Sri Lanka, which can be obtained from the sea and inland water streams. Marine fish production is higher than inland fish and it was about 260,100 metric tonnes for last recent year (Statistical analysis, 1999). Fish is highly perishable food. Higher amount of this large fish production becomes waste due to bad handling and lack of transport facilities. To overcome this problem we have to reduce the post harvest losses and increase the preservation methods.

Ambul thial is one of the traditional food preservation methods that can be used as a solution for this problem. This curry is prepared specially using Goraka, salt, pepper and other spices and stored in clay pots. Shelf life of ambul thial in ambient temperature is about three days. The preservation ability of this curry has mainly been obtained from the low pH value of the curry and the preservation action of the added ingredients.

Goraka and salt have preservation actions that lower the initial microbial content (Weerasinghe, 1988) and the pH value of ambul thial curry is less than pH 4.6, which has a great effect on inhibiting growth and toxin production of *Clostridium botulinum*. (Amarasinghe and Jayaweera, 1994).

Canning is a technology that has been developed today as a food preservation method. This can be applied for a wide content of food like vegetables, meat, fruits and fish. In the process of canned ambul thial, edible fish is cooked as ambul thial curry and packed in hermetically sealed metal containers and then processed by heat treatment to preserve it. The product can be kept in ambient temperature for a much longer time. Handling and transport of canned ambul thial will be much easier than handling raw fish and the product can be used as a ready-to-eat food without further processing.

Pre-cooked products have become popular in Sri Lanka. Although this product has potential to be marketed as a ready-to-eat product.

Fish plays a significant role in human nutrition in Sri Lanka. Fish provides a good balance of proteins, lipids, vitamins and minerals. Protein (17% to 20%) provided by fish are highly digestible and are rich in essential amino acids. Fish contain limited amounts of carbohydrates (less than 1%) and high amounts of polyunsaturated fatty acids such as Omega-3 fatty acids. Fish liver lipids are rich in vitamins A and D. Therefore, fish is an essential food for regularity.

This study was undertaken to develop fully sterilized canned fish product has long shelf life with out biological and chemical hazards and with minimum loss of nutrients and sensory properties

### **Objectives**

1. Preservation of tuna fish ambul thial using canning technology with out adding any chemical preservatives
2. Developing ambul thial as a ready to eat food product for the busy communication.
3. Developing a fish canned product in Sri Lanka as a substitute of imported can fish.

## Chapter 2

### Literature review

#### 2.1 Process of fish canning

The contents of cans are an ideal growth media for microorganisms. They will support of harmful anaerobes over aerobic organisms in the hermetically sealed canned products. There for canning is a technology if some mistake cause to death of consumer if can content will be contaminated it is toxic before noticeably spoilt. Therefore followings requirement in canning process are essential.

1. Free processing hygiene of factory environment and condition of the row material
2. Container seal integrity
3. Complete thermal destruction of microorganisms
4. Post processing hygiene of the product.

##### 2.1.1 Pre processing hygiene of factory

Factory should be located in areas free from dust objectionable odors and other contaminants. There should be adequate facilities for the disposal of effluent locates where such distance as to avoid possibility of contamination. Factory environment should be free from insects, birds, and pest to avoid contamination. Vehicles, which transport fish, are potential source of contamination. Therefore vehicles and roadways is not conveyed in to the process area.

The factory structure should be planned with considering essential requirement in the production area such as Insect screen on all opening windows (removable for cleaning), Plastic strip barriers on all doorways to use during processing, enough lightening and ventilation (air conditioning system)

The production area should be separated to avoid contamination the product from microorganisms, foreign bodies. Such as should be separated manufacturing area for filling, seaming, sterilization, area for cooled and dry freshly process cans and operation area for the cartoning and palletizing. Although there should be separated stocks for unused empty cans, cartons wrapping materials and final finish goods.

There is a main risk to contaminate the product from water. There for portable water stored in clean tank without contamination should be used. The following tests should be done periodically. Those are the test for color, taste, odor, turbidity pH, hardness, free residual chlorine, suspended solid, total aerobic plate count, total coliform and nitrate iron. The personal hygiene of the workers also is a main reason for product contamination. Therefore the separate person should provide to area, which have high accumulation probability such as free processing and post processing section (cutting room and packing room). Protective over clothing, dust masks, hair net should be provided to the workers. Workers must be wash their hand thoroughly using soap fingernails must be kept short and clean. Wearing of watches jewelry should be prohibited personal items must be left in lockers at the out side the production area. The consumption of food and drink must be prohibited in the production area. Specially injured person with cuts wound their hands and legs and infectious person should not be work.

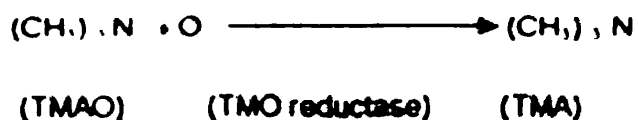
#### 2.1.1.1 Condition of raw material

Good quality fish must be used as the main raw material to obtain a good product. Spoiled and diseased condition fish should be discarded only clean and sound fish are used. There are many quality parameters to specified freshness of the fish. Those can be divided as physical chemical and biological parameters.

Physical parameters are fish should be firm body texture with out slim, brown red appearance gills should be characteristic odor, eyes should be clearly thrust out and belly cavity and internal organs should be smooth, bright color no evidence of odor or burn.

As Chemical parameters trimethylamine (TMA) content and total volatile base (TVB) and histamine content can be used.

Trimethylamine oxide (TMAO) occurs in marine fish as a part of the osmoregulatory system is used as electron acceptor by non-fermentative bacteria such as *Sewanella putrefaciens* and produce TMA. The reduction of TMAO to TMA is used as an indicator of fish spoilage.



Normally fresh fish is contained TMA less than 15 mg/100g fish. Stale fish contain TMA more than 30mg/100g fish. TMA is a volatile compound. Also there is rapid increasing of

volatile compound during the fish spoilage. The level of volatile base (TVB) is also used as an indicator of fish spoilage.

#### **2.1.1.2.1 Histamine**

Histamine content is also an indicator of fish spoilage. Scombrotoxic fish such as tuna contain large amounts of free histidine in their muscles. Bacteria contaminated during fish spoilage decarboxylate free histidine to form histamine. Histamine is a heat-resistant compound. Therefore, it can be an indicator in canned fish if the raw material was contaminated. Bad quality fish contain high levels of histamine content. The product when prepared using tuna species should not contain more than 100 mg/kg fish according to SLS 1106:1995.

#### **2.1.1.2.2 Determination of histamine content**

Histamine content can be determined using a fluorometric method. (AOAC method 977.13, 1997). In this method, the sample is extracted with 75% methanol. The extract is passed through an ion exchange column. O-Phthalaldehyde solution is added to elute to form fluorescent histamine derivatives. Fluorescent intensity of derivatives is measured using a fluorometer and histamine is quantified using external standards.

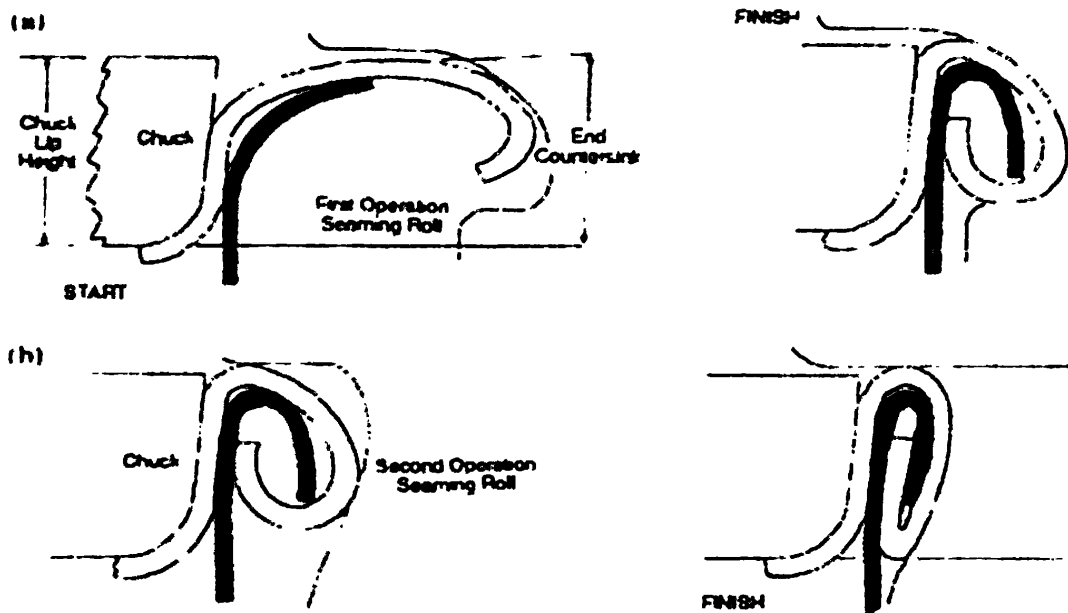
#### **2.4.2 Container seal integrity**

Containers should be hermetically sealed to avoid contamination, especially during cooling of the cans. Double seaming technology is used. The hermetic seal formed between the can body and the can end is reheated to a double seam. There are three steps as follows:

- 1 Body hooks butting (primary seal formation)
- 2 Actual overlap (secondary seal formation)
- 3 Tightness rating (ensuring the seam is held under sufficient compression)



**Figure: 2.1 Double seaming technology**



### 2.3.1 Heat sterilization of the product

The hermetically sealed cans are sterilized to sufficiently high temperature and sufficiently long time to destroy microbial and enzyme activity. As a result of that canned food have long shelf life. But over heat sterilization will be cause loss of sensory quality and nutritional value of the food. Therefore determination of the optimum temperature and time for the sterilization is necessary. In order to determine the process temperature the pH value of the content is important. The food which pH higher than the 4.6 are required full sterilization process that temperature based on 121 °C. The foods which pH lower than the 4.6 have high acid content do not want fully sterilization process. The reason for that the foods have high pH value allows to grow spore forming anaerobic pathogenic bacteria such as *Clostridium botulinum*. But foods have low pH value does not allow harmful spore forming bacteria.

In order to determine the process time for given food it is necessary to have information on heat resistance of microorganisms or enzymes and the rate of heat penetration in food container within the retort. Information about heat resistance microorganisms is described from D and D<sub>c</sub> values of specific microorganisms.

The time taken to reduce spores to 10% from the 100% at the specific temperature is described as D value. If that temperature is 121 °C D value is called D<sub>c</sub> value. Thermal death

time (TDT) is calculated from  $D_0$  values of specified microorganisms. TDT is the shortest time, which required to killing all microorganisms at a specified temperature with a minimum lose of nutrient

**Table: 2 .1 some microorganisms important in canning and their  $D_0$  value**

Organism	$D_0$ (minutes)
Spores of <i>bacillus Stearo thermophilus</i>	4-5
Spores of <i>Clostridium Thermosaccarolyticum</i>	3-4
Spores of <i>clostridium nigrificans</i>	2-3
Spores of <i>clostridium botulinum</i> types A and B	0.1-0.25

(Source: G.M Hall, 1997)

Rate of heat penetration of the food is the other factor to determine the process time Saturated steam is the best media for heat sterilization. Heat penetration to the center is faster in different condition Those are the heat penetration is faster in small containers than in large containers Also there is a fast heat penetration in liquid food than solid food Therefore solid foods within sauce have fast heat penetration. Agitation of can using rotating retort also gives fast heat penetration. Metal containers are good on heat penetration than plastic or glass containers Pressure of the steam will increase the temperature in the retort The correct retort operation technique must be used to get maximum efficiency of steam

F value is requiring minimum thermal process value to sterilize the product considering these entre requirements

## 2.4 Post process operation

Stanlized cans are rapidly cooled to avoid over cooking Also slow cooling of the cans will be caused to germinating growth of remain spores of thermophilic bacteria such as *Clostridium Botulinum* The water used to cool the process containers must be chlorinated because while the containers are hot due to the pressure difference of the head space of the cans slight possibility that a drop of cooling water could be pulled in to the can through a seam There is a high risk of contamination the can content Therefore water used for cooling process should be tested at least weekly for total aerobic plate count (TPC) and for coliform monthly It should be in limit 100 organisms per ml at 20-22 °C for 5 days for TPC and for coliform count no organisms per 100ml 20-22 °C for 5 days according to SLS B73 1989 But high level of free chlorine cause to accelerate external corrosion of the containers Therefore it should be in limit (10 p p m )

## **2.5 Spoilage of canned fish**

Spoilage caused by chemical, biological and physical hazards or all of them. Because of the several failures of the process, the spoilage can appear. Spoiled cans are identified externally by blowing of the cans. But some time can content may be spoiled without external changes of the can appearance.

### **2.5.1 External changes of the cans during spoilage**

Normally the ends of a can food are termed Flat, which means that no evidence of swelling. If the pressure develops inside, the can goes through a series of distortions. A Flipper ends are flat but with insufficient vacuum to hold the ends in place, thus a sharp blow will cause them to become convex but both ends may be pressed to their normal position. A Springer has both ends of the can bulged, but one or both ends will stay concave if pushed in, an opposite flat end will pop out. Some to designate slight pressers in the cans not caused by gas production use the terms flipper and springer but by such things as a poor exhaust, over filling, dishing of the can, changes in temperature. But the can may have some outward characteristics at the start of gas production from either a microbial or chemical cause or both. A soft swell both ends of can are not firmly bulged. The gas pressure is low enough to permit the ends to be dented by pressure of fingers. A Hard swell has such high gas pressure in the both ends of can are firmly bulged. The final step is bursting the can.

### **2.5.2 Chemical changes**

The most important kind of chemical spoilage of canned foods is the hydrogen swell, resulting from the pressure of the hydrogen gas, realized by the action of the acid of the food on the iron of the can. Hydrogen swell are favored by increasing acidities of foods, increasing temperature of storage, imperfection of the tinning and lacquering of the interior of the can, a poor exhaust and presence of soluble sulfur and phosphorus compounds. Other defects caused by interaction between the steel based of the can and the contain food include discoloration of the inside of the can and food, production of off-flavors, cloudiness of liquors or syrups, corrosion of the metal and loss of nutrition value. Chemical spoilage distinguish from microbial spoilage since the rate of blowing ends of the can is very slow and start after long time of storage.

### 2.5.3 Biological spoilage

Biological spoilage of canned foods by microorganisms may result from either or both two results such as survival of Microorganisms because of inadequate heat process and post process contamination from cooling water through the container leakage. If the presence of organisms low heat resistance and especially more than one kind of such organisms is evidence post process contamination by leakage.

The organisms that can be caused to spoilage are mesophilic bacteria, thermophilic bacteria and their spores. The thermophilic bacteria spores are more heat resistant than mesophilic bacteria spores. The three common types of spoilage by thermophiles are flat sour spoilage, TA spoilage.

#### 2.5.3.1 Flat sour spoilage

Flat sour spoilage derives its name from the fact that the ends of the can remain flat during souring. This type of spoilage cannot be detected by examining appearance of the can but must be detected by cultural method. The various species of bacillus that are able to form acid without gas in food may be mesophilus, facultative thermophilus and obligate thermophilus. The spores of mesophilus are the least heat resistant and are usually killed by the heat processing and hence are fairly concerned with flat sour spoilage of low acid foods. Surviving obligate thermophilus, such as *Bacillus stearothermophilus*, would not cause spoilage unless the food were held hot for a while, as in slow cooling or storage in the tropics, but facultative thermophilus could grow at ordinary temperatures. The source of the flat sour bacteria is usually comes from sugar starch or soil. The most spores present, the lower the minimal pH for germination about 5.0 and for growth about 4.2 to 4.3. The organism, which is homo-fermentative under almost anaerobic conditions and hetero-fermentative under aerobic conditions, can grow in low concentrations of oxygen.

#### 2.5.3.2 TA spoilage

The bacterium causing this type of spoilage has been nicknamed TA which is short for "thermophilic anaerobic not producing hydrogen sulfide", or for the species *Clostridium thermosaccharolyticum*. This is a sugar splitting obligatory thermophilic spore forming anaerobe that forms acid and gas in low and medium acid foods. The gas, a mixture of carbon dioxide and hydrogen, swells the can if it is held long enough at a high temperature and may eventually cause bursting. The spoiled food usually has a sour odor. Since the organisms do not form colonies readily in agar, it is detected usually by the inoculation by the media liver broth.

### **2.5.3.3 Sulfide spoilage**

This spoilage called Sulfide or "Sulfur stinker", caused by *Clostridium nigrificans*. The spores of this bacterium have considerably less heat resistance than those of flat sour and TA. The organism is an obligate thermophile and therefore also requires poor cooling of the heat processed foods or hot storage its development. It is detected by means of black ferrous sulfide (FeS) colonies it forms in an iron sulfite agar at 55 °C. Hydrogen sulfide, formed in the canned is evident by odor when the can is opened.

### **2.5.4 Physical spoilage**

Over filling, seam defects and bad handling also reasons for the can spoilage. Over filling is caused to immediate swelling of the can during sterilized in the retort, seam defects cause to microbial spoilage of the cans and bad handling cause to external and internal corrosion of cans.

## **2.6 Quality changes during thermal processing**

Following Quality changes are occurred during thermal processing because of over heating.

### **2.6.1 Cook out**

The heat denaturation of protein causes water loss from 9 to 28%. This water loss cause to curdled appearance in the contents called as 'cooked out'. Pre processing such minimizes this as curing, smoking and cooking.

### **2.6.2 Vitamin loss**

There are slight losses of b-group vitamins. Such as thiamin, riboflavin, nicotinic acids, folic acids and cyanocobalamine in canned product than fresh fish.

### **2.6.3 Flavor changes**

Some flavor changes occur in fish when it espouses to high temperature. But if the product gravy will be contain spices, salt and pepper that will be masked by them.

### **2.6.4 Textural changes**

Excessive protein denaturation at the high temperature cause to textural changes and reduction of water holding capacity.

### **2.6.5 Color changes**

Color changes can be indicated because of poor quality of raw material and heat changes. Due to the iron content of the raw material and free sulfur of the tissue react together during heat processing precipitating black iron sulfide on the container. Those reactions are called as browning. Those reactions are called as browning.

### **2.6.4 Adhesion formation**

Tendency of fish pieces to adhere to the side of the can are made more probable if, raw, previously frozen fish are canned and less probable if pre-sterilization treatment including brining, pre-cooking and inclusion of tartaric acids.

## Chapter 3

### Materials and methods

#### 3.1 Preparation of ambul thial curry

Following materials were used in preparation of ambul thial curry.

##### 3.1.1 Material

- ◆ Salt 125 g
- ◆ Pepper 60g
- ◆ Goraka 80 g
- ◆ Rampe 3g
- ◆ Ginger 15 g
- ◆ Garlic 30g
- ◆ Cloves 3g
- ◆ Curry leaves 15g
- ◆ Cinnamon 3g
- ◆ Curry powder 25g
- ◆ Tuna fish cube 5kg
- ◆ Stainless steel steam jacketed kettle

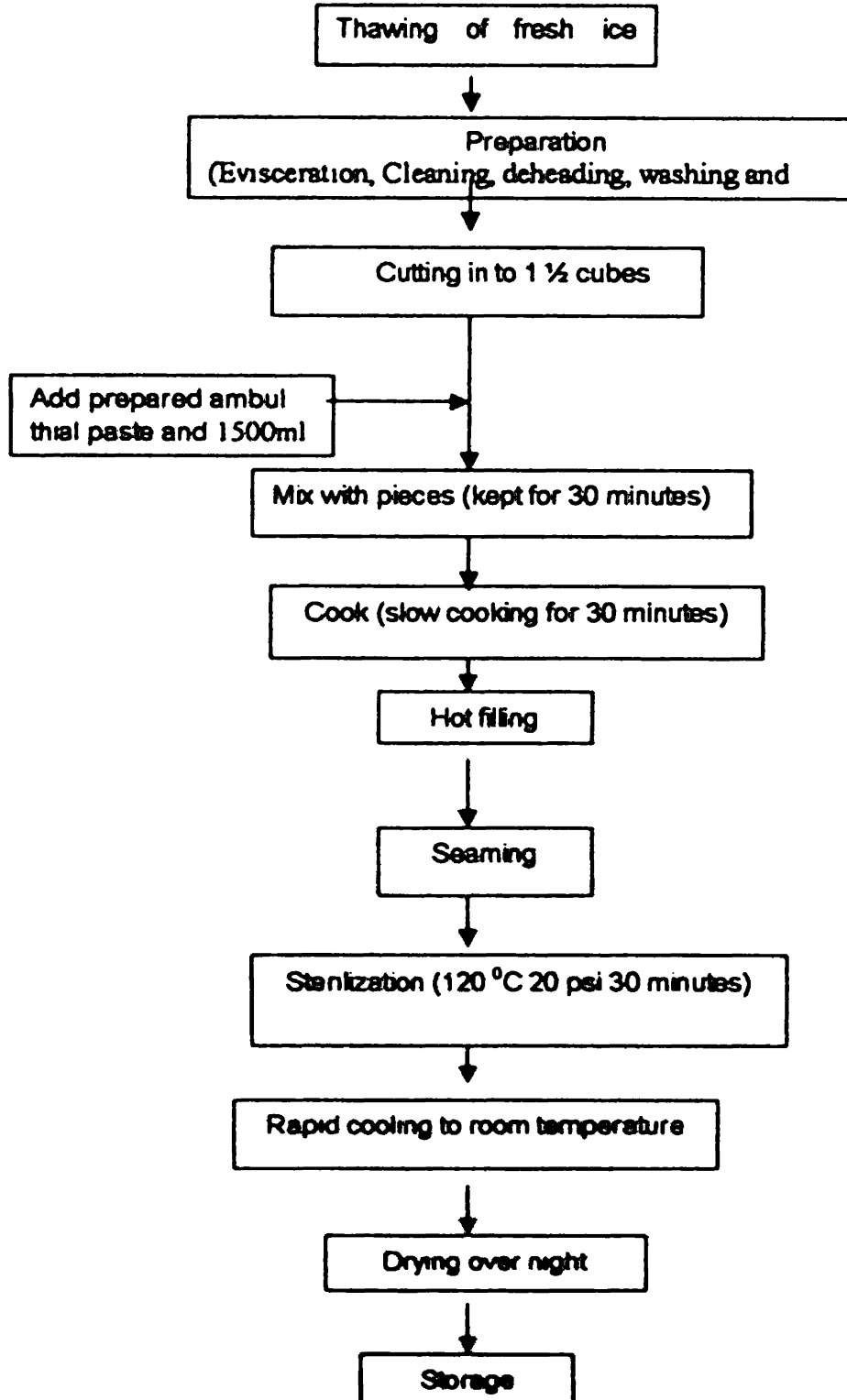
##### 3.1.2 Method

The salt, pepper, Goraka, and other spices were ground to a fine paste and prepared as a paste adding salt water. Iced fish was thawed to room temperature using potable water. Head and fins were removed and fish were eviscerated and cut to the cubes.

A 35 % was removed as the waste from whole fish. The fish cubes were mixed with prepared ambul thial paste and kept for 30 minutes. After that 3L of water was added mixed well and cooked for 30 minutes.

### 3.2 Preparation of canned ambul thial

Figure: 3.1 Flow chart for the production of canned ambul thial





### **3.2.1 Material**

The following materials were used in Preparation of canned ambul thial

- ◆ Prepared ambul thial curry
- ◆ Internal sulfur resistant food grade can
- ◆ A Seamer
- ◆ A Batch retort
- ◆ Pepper serviette
- ◆ A Stainless steels forceps
- ◆ A permanent marker
- ◆ Heat resistant gloves

### **3.2.2 Method**

The cans were sterilized 120 °C for 20 minutes. Previously prepared hot cooked ambul thial was poured in to prepared cans as 350-g fish and 50 g gravy as remain the head space as 1.5 cm in each can. Then those were hermetically sealed and sterilized in the retort 120 °C for 30 minutes. That cans were cooled within 1 1/2 hours in cooled water, and wiped using pepper serviettes and storage.

### **3.3 Laboratory analysis**

#### **3.3.1 Examination of commercial sterility**

The Tests were done for prepared three Samples of canned ambul thial as following

Sample -The sample was sterilized at 100°C for 5 minutes

Sample-The sample was sterilized at 120°C for 30 minutes

Sample-The sample was sterilized at 120°C for 30 minutes

Sample 1 was tested to study observation of the culture media when the microorganisms growth and standardized the method. Prepared cans of this sample were kept in refrigerator until use for the test

Sample-1 and sample-2, were tested to present of viable microorganism and microbial spores in an anaerobic media and aerobic media (Commercial sterility test). The cans were incubated at 35°C 10 days before the testing

### **3.3.1.1 Preparation of glass wears and equipment**

#### **3.3.1.1.1 Materials**

The following materials were used in the preparation of glassware and equipment.

- ◆ Detergent
- ◆ Autoclave
- ◆ Incubator
- ◆ Glass wares (Petry dishes, Test tubes, 1-ml pipette)

#### **3.3.1.1.2 Method**

The glassware was cleaned using detergent and dry in an incubator. The glassware was autoclaved at 120°C for 30 minutes just before use for the test.

### **3.3.1.2 Preparation of liver broth**

#### **3.3.1.2.1 Materials**

The following materials were used for the preparation of liver broth.

- ◆ Cheesecloth
- ◆ Beef liver
- ◆ 0.5 M NaOH solution
- ◆ Peptone
- ◆ Na<sub>2</sub>HPO<sub>4</sub>
- ◆ Distilled water
- ◆ Stainless steel Knife
- ◆ Blender
- ◆ 1L, 500 ml conical flasks
- ◆ Electronic balance
- ◆ Measuring cylinder
- ◆ Glass rod
- ◆ pH meter
- ◆ Gas Cooker
- ◆ Stainless steel pan (2)
- ◆ Water Bath
- ◆ Deep Freeze

### **3.3.1.2.2 Method**

A 250g of beef liver with 500ml of water was boiled in an hour. That boiled liver was sliced in to small cubes and chopped in grinder. The ground medium was adjusted to pH 8.5 by adding 0.5M NaOH solution. Then was boiled in 10 minutes. That hot media was filtered using cheesecloth.

The residue was poured in to 500ml conical flask plugged with cotton wool. A 5g Of peptone and 0.5 g of  $\text{Na}_2\text{HPO}_4$  were added to the filtered broth. Then diluted the broth to 500ml by adding distilled water mixed using a glass rod. That media was adjust to pH -7 adding few drops of 0.5 NaOH M solution. The prepared broth was poured in to a 1L conical flask plugged with cotton wool. Both samples, which prepared previously, were sterilized at 121 °C for 20 minutes in an autoclave. Immediately after autoclave both samples were exhausted in 20 minutes in a water bath. Prepared broth and ground beef liver were packed and sealed. The samples are freeze for further use.

### **3.3.1.3 Preparation of nutrient agar**

#### **3.3.1.3.1 Materials**

Following materials were used for the preparation of nutrient agar

- ◆ Nutrient agar
- ◆ Spatula
- ◆ Distill water
- ◆ 250 ml conical flask
- ◆ Electronic balance
- ◆ Measuring cylinder
- ◆ Glass rod
- ◆ Water bath
- ◆ Autoclave

#### **3.3.1.3.2 Method**

A 7g of nutrient agar was measured in to conical flask. Agar was suspended by adding 250-ml of distil water while stirring with a glass rod. Then it was kept in a boiling water bath for complete dissolve and conical flask was plugged with cotton wool. Sterilized at 120 °C for 20 minutes. Thus prepared culture media were used for the test.

### **3.3.1.4 Preparation of samples**

#### **3.3.1.4.1 Materials**

- ◆ Detergent
- ◆ Isopropyl alcohol
- ◆ Cotton wool
- ◆ Sprit lamp

#### **3.3.1.4.1 Method**

The cans and opener were washed with detergent and tap water. Then dried with clean paper serviette and moistened with isopropyl alcohol. The cans' ends and cutter blade were flamed on blue portion of the flame just before open the can.

#### **3.3.1.5 Inoculation**

When inoculation four samples were inoculated with fish pieces using sterilized forceps. Other four samples were inoculated with gravy using sterilized glass pipette. One sample was used as control one with out inoculation of each test.

### **3.3.1.6 Test for anaerobic viable bacteria**

#### **3.3.1.6.1 Materials**

The following materials were used for the test.

- Sterilized test tubes
- Sterilized 1-ml of pipette
- Forceps
- Paraffin
- pH meter
- Autoclave
- Incubator
- Cotton wool
- Aluminum foil
- Liver broth and ground liver media
- Nutrient agar
- Spatula
- 10ml pipette with rubber cap
- Sprit lamp
- Can opener

### **3.3.1.6.2 Method**

About 2g of previously boiled ground beef liver and 10ml of prepared liver broth were added to a test tube. The test tube was plugged with cotton wool, sterilized at 120°C for 20 minutes and cooled to 35°C. The sample was inoculated following the method previously described. Then prepared nutrient agar media and sterilized paraffin 2ml were poured into test tube and plugged with cotton wool and wrapped with an aluminum foil. The ten samples were prepared as this. One of them was used to measure the pH value and the other samples were incubated at 35°C for 72 hours.

### **3.3.1.7 Test for anaerobic bacteria spores**

The 3.3.6.1 method was followed and prepared nine test tubes were inoculated at 55°C for 72 hours.

### **3.3.1.8 Test for the aerobic microorganisms**

#### **3.3.1.8.1 Materials**

Following materials were used for the test of aerobic microorganisms.

- ◆ Nutrient agar
- ◆ Sterilized 1ml pipette with rubber cap
- ◆ Forceps
- ◆ Para film
- ◆ Sterilized petry dishes
- ◆ Incubator

#### **3.3.1.8.2 Method**

Prepared nutrient agar was poured in to petry dish, was inoculated wrapped with para film and placed in incubator at 35 °C for 72 hours

### **3.3.1.9 Test of aerobic spores**

The 3.3.1.8 method was used. Finally prepared petry dishes were incubated at 55°C for 72 hours

### **3.3.2 Determination pH of value**

#### **3.3.3.1 Materials**

The following materials were used in determination of pH

- ◆ pH Meter
- ◆ A 100ml beaker
- ◆ pH- 7, pH-4 buffer Solution.
- ◆ Electronic Balance
- ◆ Blender

#### **3.3.3.2 Method**

After using for microbiological test remaining portion of the can was blended. A 20g of that was weighed in to 100ml of measuring cylinder and added distilled water up to the mark and mixed with glass rod. A pH was measured with reference to buffer solution of pH-7 and pH-4

### **3.4 Sensory evaluation**

#### **3.4.1 Material**

The following materials were used for the test

- ◆ A can opener
- ◆ White curry dishes
- ◆ Stainless steels spoons
- ◆ Score sheets glass of water

#### **3.4.2 Method**

The samples were arranged with sufficient spaces between the assessors. The cans were started the just before starting the test. Fish pieces and gravy were poured in to curry dishes as sufficient one can for the five curry dishes. The assessors were scored the samples in score sheets according to the given guide line which had given before starting the test.

### **3.4.3 Statistical analysis**

The basic purpose of this evaluation is ensuring this product is marketable considering consumer acceptability base on four parameters. Those were appearance, odor, flavor and texture. Priority has given for the odor and flavor when analyses the data.

The total weighted scores (S) and the average weighted score (S) for each assessor was calculated.

## Chapter 4

### Result and Discussion

#### 4.1 Result of the Commercial sterility test

##### 4.1.1 Sample-1 The sample was sterilized at 100 °C for 5 minutes

Table: 4.1 Results of the sample 1.

Test	Treatment	Presence of micro organisms	pH value of the media
1	Anaerobic 35 °C for 72 hours	Positive	4.16
2	Anaerobic 55 °C for 72 hours	Positive	4.22
3	Aerobic 35 °C for 72 hours	Positive	—
4	Aerobic 55 °C for 72 hours	Positive	—

According to results of the sample -1 microorganisms were observed in all test treatments. The anaerobic culture media was changed to cloudy appearance with splitting of agar, black and white spots and sour odor.

Those observations showed the presence of mesophilic anaerobes and thermophilic anaerobes. The pH value was reduced from 6.48 to 4.22 and 4.16. It indicates the presence of acid producing bacteria

White color colonies were observed in the aerobic bacteria culture media.

The microorganism can be subculture and can be identified by microscopic examination

The source for spoilage can be found due to type of microorganism presence



#### 4.1.2 Sample-2 The sample was sterilized at 120 °C for 30 minutes

**Table: 4.2 Result of the Sample-2.**

Test	Treatment	Presence of micro organisms	pH value of the media
1	Anaerobic 35 °C for 72 hours	Negative	4.16
2	Anaerobic 55 °C for 72 hours	Positive	4.22
3	Aerobic 35 °C for 72 hours	Positive	—
4	Aerobic 55 °C for 72 hours	Positive	—

The sample-2 shows presence of aerobic and anaerobic spores. It must be due to inadequate heat treatment or post process leakage of the can.

The main reasons for presence of microorganisms may be the higher microbial population of the raw material, inadequate heat penetration and wrong calibration values of the retort and defects of the seamer.

Presence of viable bacteria of sample-2 may be due to laboratory contamination. Because the sample cans were kept 2 months period without seeing a major spoilage characteristics such as swelling of the cans' ends, spoilage characteristics of the can content and variation of the pH value (Figure 4 1)

#### 4.1.3 Sample-3 The sample was sterilized at 120 °C for 30 minutes.

**Table: 4.3 Results of the sample 3**

Test	Treatment	Presence of micro organisms	PH value of the media
1	Anaerobic 35 °C for 72 hours	Negative	4.16
2	Anaerobic 55 °C for 72 hours	Negative	4.22
3	Aerobic 35 °C for 72 hours	Negative	—
4	Aerobic 55 °C for 72 hours	Negative	—

The sample-3 was prepared after correction of the defect in the process. Therefore sample-3 was free from the biological hazards

#### 4.2 Histamine test

**Table: 4.4 Result of the Histamine test**

Criteria	Content (p.p.m)
Sample-1	42.5
SLS Recommendation level	100

Determination of histamine content is very complicated and expensive method. Histamine content has determined in this study to ensure safe of the product. This test was done by external laboratory.

There is a main risk of histamine poisoning of the product because Yellowfin tuna was used as the main raw material in this study. According to the result, histamine content of the sample is less than the recommend SLS Level. Hence the quality of the ambu that cans can be assured in terms of histamine content. The result is shown the method has followed to get low histamine content of the product has successful.

### 4.3 pH value of the product

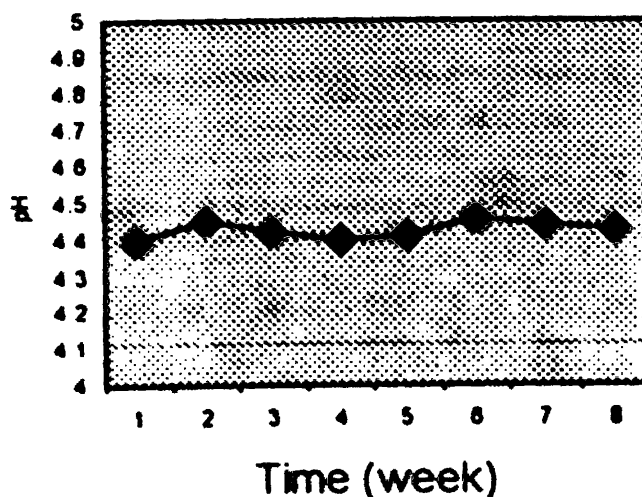
Table 4.5: pH value of prepared samples

Sample	PH
1	4.44
2	4.26
3	4.23
Mean	4.23

The mean pH value of the product was 4.23. It is less than the minimum growth of *Clostridium botulinum* and its toxin production.

Acidity of the can content increases hydrogen swelling due to internal corrosion. Internal sulfur resistant (ISR) cans have been used to solve this problem. Also the defective cans must be rejected because acid of content reacts with iron of damage places of cans and produce hydrogen. Ends of the cans swell due pressure of hydrogen but swelling is indicated after some time of can produce. The marketed product can be return after long time. Therefore selection of correct can type, rejection of damage cans, proper handling practices are essential in canning.

Figure: 4.1 Variation of pH with time



The cans haven't any external defects and internal spoilage characteristics of the content may be spoiled. Any deference of the pH value indicates start of the spoilage especially biological spoilage due to thermophilic flat sour bacteria.

The variation of pH was evaluated for sample-2. There is no much variation of pH of the product. But according to result of commercial sterilization sample had microbial spores. The presence of microbial spores had not effect on can spoilage at the ambient temperature.

#### 4.4 Sensory evaluation

**Table 4.6 Results of sensory evaluation**

Criteria	Average weighed scores
Non-defective (marketable)	> 12
Defective (non marketable)	< 12
Average weighed score of the sample	15.67

The result of sensory evaluation shows the product is marketable

According to the comments of the assessors the flavour, colour and the odour of the product is much better. To obtain this quality the natural ingredient added to the product was effective. Those are Garcenia, pepper, curry leaves, curry powder and onions.

The tartaric acid of Garcenia has a great effect to avoid curdling problem. Ambul thial is pre-cooked product. It has great effect to get good canned product with curdling problem of content.

The texture of the product is not much better. The pieces of fish were dry appearance. The heat de-naturation of protein causes water losses and textural defects. The correct cooking time and temperature must be defined to avoid the problem.

Gravy of canned ambul thial curry was prepared as much higher than normal curry because liquid content of can increases heat penetration.

Considering all those texture of ambul thial curry must be developed due to consumer preferences.

## **CHAPTER 5**

### **CONCLUSION**

The F value of the process (120 °C for 30 minutes) is correct. According to the result of the commercial sterility test the product has fully sterilized.

The histamine level of the product is too low compared with Sri Lanka standards and in the range of acceptance. It indicates the fish used in this study had not been high initial histamine level.

According to the result of the commercial sterility test Tuna used in this study as a main raw material had been low initial microbiological load.

The prepared ambient cans have consumer acceptability according to the results of the sensory evaluation.

Considering all the facts, the production method currently in use is the most suitable method to produce ambient cans in terms of quality and safety.

### **Recommendation**

Shelf life of the product should be practically evaluated.

Further studies should be carried out to reduce the histamine level of tuna fish during the commercial handling of the raw material.

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## Appendix

Table: 7 .1 The statistical analysis of the sensory evaluation

Raw score for each parameter of 20 assessors				weighed score for each parameter				Total weight score for each assessor
Appearance (A)	Texture (T)	Odor (O)	Flavor (F)	Ax0.8	Tx0.8	Ox1.2	Fx1.2	
3	4	4	4	2.4	3.2	4.8	4.8	15.2
4	4	4	4	3.2	3.2	4.8	4.8	16
3	4	4	3	2.4	3.2	4.8	3.6	14
4	4	5	5	3.2	3.2	6	6	18.4
5	5	5	4	4	4	6	4.8	18.8
4	3	4	5	3.2	2.4	4.8	6	16.4
4	4	3	4	3.2	3.2	3.6	4.8	14.8
2	3	2	4	1.6	2.4	2.4	4.8	11.2
3	5	4	5	2.4	4	4.8	6	17.2
4	5	5	5	3.2	4	6	6	19.2
5	4	3	4	4	3.2	3.6	4.8	15.6
3	5	4	4	2.4	4	4.8	4.8	16
4	4	5	3	3.2	3.2	6	3.6	16
4	4	4	4	3.2	3.2	4.8	4.8	16
4	5	5	5	3.2	4	6	6	19.2
4	4	4	4	3.2	3.2	4.8	4.8	16
4	4	5	4	3.2	3.2	6	4.8	17.2
4	3	4	4	3.2	2.4	4.8	4.8	15.2
5	5	5	4	4	4	6	4.8	18.8
5	4	5	4	4	3.2	6	4.8	18
The average weighed score ( $\bar{X}$ ) $\sum_{i=1}^{20}$								16.46



Name .....  
Date .....

**Evaluation of sensory quality characteristics of canned  
Ambul thial**

**Scale for evaluation**

The best sample	05
The much better sample	04
The better sample	03
The bad sample	02
The very bad sample	01

The samples are to be sensorial evaluated for following properties.

PARAMETRE	CODE . .
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1.appearance

2 Texture

3.Odour

4.Flavour

Other comments

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