STUDY ON THE PRACTICE OF FISH HANDLING AND PRESERVATION TECHNIQUES OF *Crrhinus mrigala* FOR ENHANCING FISH FOOD SAFETY IN SELECTED AREAS OF BARA, PARSA AND KATHMANDU DISTRICTS OF NEPAL

A Dissertation

Submitted for the Partial fulfillment of the requirement for Master's Degree of Science in Zoology (Fishery)

By

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To

Central Department of Zoology

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LETTER OF RECOMMENDATION

This is to recommend that the thesis entitled **"STUDY ON THE PRACTICE OF FISH HANDLING AND PRESERVATION TECHNIQUES OF *Cirrhinus mrigala* FOR ENHANCING FISH FOOD SAFETY IN SELECTED AREAS OF BARA, PARSA AND KATHMANDU DISTRICTS OF NEPAL"** has been carried out by Ms Silpa Bhandari for the partial fulfillment of the Masters Degree of science in Zoology with special paper Fishery. This is her original work and has been carried out under my supervision. To the best of my knowledge, this thesis work has not been submitted for any other degree in any institutions.

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LETTER OF APPROVAL

On the recommendation of supervisor "Prof. Dr. Surya Ratna Gubhaju" this thesis is submitted by Ms. Silpa Bhandari entitled **"STUDY ON THE PRACTICE OF FISH HANDLING AND PRESERVATION TECHNIQUES OF *Cirrhinus mrigala* FOR ENHANCING FISH FOOD SAFETY IN SELECTED AREAS OF BARA, PARSA AND KATHMANDU DISTRICTS OF NEPAL"** is approved for the examination and submitted to the Tribhuvan University in partial fulfillment of the requirements for Master's Degree of Science in Zoology with special paper Fishery.

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CERTIFICATE OF ACCEPTANCE

This thesis work submitted by Ms. Silpa Bhandari entitled **"STUDY ON THE PRACTICE OF FISH HANDLING AND PRESERVATION TECHNIQUES OF *Cirrhinus mrigala* FOR ENHANCING FISH FOOD SAFETY IN SELECTED AREAS OF BARA, PARSA AND KATHMANDU DISTRICTS OF NEPAL"** has been accepted as partial fulfillment for the requirements of Master's degree of Science in Zoology with special paper Fishery.

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I hereby declare that the work presented in this thesis has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the authors or institutions.

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ABSTRACT

The study was done at Bodhban 9 Parsauna VDC of Bara, Jhounkuti and Birjung of Parsa and different fish market at Kathmandu. In this research work the study of fish handling method at all levels of supply chain from catching to marketing was conducted to identify the major factor affecting the fish quality. The samples from different levels of chain were analyzed at "Fisheries research unit Godabari". Sensory analysis by QIM method was applied to assess the quality of fish available in different fish markets at Kathmandu. QIM score of fish samples from Kalimati was observed to be the least i.e. 2 while of bicycle seller the highest i.e.12. Microbial analysis result shows the presence of salmonella species in most of the samples. Similarly, the total microbial load in all the samples except taken from landing site of Bodhban pond was more than $5x10^5$cfu/gm, which indicates the poor sanitation condition in handling and processing of fish. Average moisture content, total ash and acid insoluble ash were in acceptable range for human digestibility. Peroxide value in most fish samples were more than 10meq/kg. High peroxide value indicates that fat was oxidized to become rancid. High peroxide value was an indicator of storage problem or some technical problem. The overall results suggested that there was quality deterioration in fishes in markets which was mostly due to bacterial growth.

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ABBREVIATIONS

ADB	Asian Development Bank
AGDP	Agriculture Gross Domestic Production
AOAC	Association of Official Analytical Chemists
ATP	Adenosine Triphosphate
BGA	Brilliant Green Agar
CFU	Colony forming unit
CIFT	Central Institute of Fisheries Technology
CUT	Citrate Utilization Test
DOFD	Directorate of Fisheries Development
EFSA	European Food Safety Authority
FAO	Fisheries and Aquaculture Organization
FDD	Fisheries Development Division
FGD	Focus Group Discussion
GDP	Gross Domestic Production
H_2s	Hydrogen Sulfide
ICMSF	International Commission for Microbiological Specification for Food
IDRC	International Development Research center
IJFS	Internet Journal of Food Safety
KG	Kilogram
MOAC	Ministry of Agriculture and Cooperative
Mt	Metric ton
P^H	Percentage of Hydrogen
PV	Peroxide Value
PCA	Plate Count Agar
QIM	Quality Index Method
SIM	Sulphur reduction, Indole production and Motility Test
SSA	Salmonella Shigella Agar
SSO	Specific Spoilage Organism
TMA	Trimethyl Amine
TMAO	Trimethyl Amine Oxide
TPCA	Total plate count Agar

TSI	Triple Sugar Iron Test
TVB-N	Total Volatile Basic Nitrogen
TVC	Total viable count
UNDP	United Nations Development Program
VDC	Village Development Committee
XLD	Xylose Lysine Deoxycholate Agar

1. INTRODUCTION

1.1 Background

Nepal is a landlocked country which lies between India and China having unique and varied ecological features. Nepal is situated between the longitudes 80°00' to 30°15'E and latitudes 26°30' to 30°15'N geographically. Being landlocked, the country is deprived of any oceanic resources and occupy the total area of 1,47,181 sq. km; approximately 5% of which is known to be occupied by different freshwater aquatic habitat.

1.2 Water Resources of Nepal

Nepal is divided into three physiographic regions, from south to north: the Terai plain, the mid hills and the Himalayas. Mountains and hills make up 83 percent of the area of Nepal while the Terai occupies only 17 percent. The country may be divided into three climatic zones according to altitude: subtropical in the Terai, temperate in the hills, and alpine in the mountains. Nepal is endowed with many forms of water resources scattered throughout the country. Total water surface area in the country is estimated about 820,077ha, made up of rivers (48.20%) ,lakes (0.61%),irrigated paddy fields (48.50%) , reservoirs (0.18%), ponds (0.89%), swamps (1.60%). Water surface area of Nepal covers 0.1% of global freshwater (DOFD,2010/11).

Table 1: Estimated water surface area in Nepal (DOFD, 2010/11)

Resource	Estimated area (ha)	Coverage (%)	Potential for fisheries (area in ha)
Rivers	395, 000	48.20	-
Lakes	5, 000	0.61	-
Reservoirs	1, 500	0.18	78, 000
Village ponds	7, 277	0.89	14, 000
Marginal swamps, irrigated fields	13, 300	1.60	-
Irrigated paddy fields	398, 000	48.50	-

Total	**820, 077**		

1.3 Fishery resources

The aquatic ecosystems of Nepal offer excellent habitats to at least 217 indigenous and 15 exotic fish species of high economic, environmental and academic value (Shrestha 2008). They are distributed from Terai, through the hills to the Himalayan Mountains up to the altitude of about 4000 m. They inhabit rivers and lakes of mid hills and mountains, with water temperature of 10°-20°C.

Fish is the major source of protein .All communities in the country accept fish as delicious food and considered auspicious among many communities. In our country Nepal the production of fish is from different aquaculture practices and capture fisheries (Table 2).

Table 2: Summary of fish production in Nepal,2009/010

particulars	Ponds(no.)	Total area (ha)	Production(Mt)	Yield(Kg/ha)
A. Production from aquaculture practices 28,230				
Pond fish culture	24,418	6,900	24,837	3,600
Other areas (ghol)	-	2,000	2,600	1,300
paddy cum fish culture	-	100	45	450
Case fish culture(m^3)	-	80,000	480	6
Enclosure fish culture	-	100	140	1,400
Trout fish in raceway(m^2)	-	5,000	100	20
Fish production in public sector	-		28	-
Production from capture fisheries 21,500				
Rivers	-	395,000	7,110	18
Lakes	-	5,000	850	170
Reservoirs	-	1,500	385	257
Marginal/swamps/ghol	-	11,100	5,990	540
Irrigated paddy fields	-	398,000	7,165	18
Total fish production			**49,730**	

Source: Statistical Information on Nepalese Agriculture, MOAC(2009)

Total fish production in country was 49,730 metric tons during 2009/10, which has increased to 52,970 metric tons in 2010/2011 (Nepal, MOAC 2010).

1.3.1 Capture fishery in Nepal

The tremendous altitudinal difference produces great biological variations, including a great variety of fish in Nepal's 6000 rivers and streams, lakes and swamps. Koshi, Gandaki and Karnali are the principal river systems fed by hundreds of small

rivers and streams originating in the Mahabharat and Siwalik mountain ranges. Water bodies cover about 5% of the total land area of the country and are ideal habitats for indigenous fish fauna which supports capture fishery in the country. Traditionally, the capture fishery is carried out by fisher communities belonging to 23 ethnic groups (Majhi, Chepang, Bote, Tharu, Badi, Raji etc) as the main profession. Most of them were living scattered along the rivers and lakes and were not organized or supported. Therefore, the river basins play a vital role in the socio-economic status of the people of Nepal. The waters of Nepal provide a variety of habitats for indigenous fish, water for irrigation and for hydropower generation. Capture fisheries has been an important occupation in many parts of Nepal. In spite its significance and the institutional efforts the contribution of fisheries to employment, national income and exports has been far from satisfactory. Government fisheries institutions have been able to make estimates of fish catches in some fresh waters and have studied the people involved in it. Capture fisheries in Nepal is widely scattered and not organized. Scanty records are available on capture fisheries. As Nepal is a landlocked country, capture fishery has an important role. The communities used traditional fishing gear for subsistence fishery, generating marginal economic benefits. Destructive devices such as dynamite, fish poisons, electrofishing, etc., were common, having a negative impact on fish by destroying broodstock, spawning and nursery grounds.

Data published by MOAC (2009) given in table 2 showed the production from capture fishery had reached to 21,500M ton/yr. In contrast with aquaculture, capture fishery contributed only 43% of total fish production during 2009/2010.

1.3.2 Aquaculture in Nepal

Aquaculture has a relatively short history in Nepal. It was initiated in the mid 1940s as a small scale in ponds with indigenous India major carp seed brought from India. Further development took place in 1950s with the introduction of the exotic species of common carp (*Cyprinus carpio*). It's breeding success in the 1960s followed mono culture practices and gained considerable popularity in private sector. More significant progress was seen in the 1970s with the introduction of three exotic Chinese carp species like silver carp (*Hypophthalmichthys molistrix*), big head carp (*Aristichthys nobilis*) and grass carp (C*tenopharyngodon idella*). Their breeding success in capitivity had been a major breakthrough in the development of aquaculture in Nepal. Similarly, the induced breeding

of three commercially valuable indigenous major carps: rohu *(Labeo rohita)*, mrigal (*Cirrhibnus mrigala*) and catla (*Catla catla*) were successfully established aquaculture in the country. This success was followed the polyculture system of fish production in ponds with several species of fish living in different feeding habits. The actual development of this practice was seen from the beginning of 1980s with the execution of the Aquaculture Development Project supported by Asian Development Bank (ADB) and United Nations Development Program (UNDP). Over the year, pond aquaculture has developed as the most viable and popular aquaculture production system. The major part of pond fish production takes place in the southern part of the country in Terai region where about 94% of the fish ponds are located (FAO 2004).

Table 3: Records of different exotic carp fishes introduced in Nepal.

S.N.	Species	Scientific Name	Year, Origin	Remarks
1	Common carp	***Cyprinus carpio***	1956, India 1960, Israel 1987, Israel	Scale carp Mirror carp Nasice carp
2	Silver carp	***Hypothalmychthys Molitrix***	1968,1969 Japan Israel	
3	Bighead carp	***Aristicthys nobilis***	1971, Hungary	
4	Grass carp	***Ctenopharyngodon idella***	1968, Japan	

During 1970s the cage fish culture in lake and reservoir was initiated with the support of FAO/ UNDP and later the international Development Research Center (IDRC), Canada. In cages, silver carp (*Hypophthalmichthys molitrix*) and big head carp (*Aristichthys nobilis*) were used as major species. Sometimes *Labeo* were also stocked as they help to keep the case clean by feeding the detritus attached to the cage mesh.

Rice-fish farming technology was known to have started in 1960s in Nepal in hills and valleys with the application of improved management techniques and careful planning. The culture of high value cold water species i.e. Rainbow trout (*Oncorhynchus mykiss*), had been an ongoing activity from some year.

Table 4. Area, production, productivity of pond fish culture in Nepal 2000/01-2011/2012, MOAC(2011/2012)

Year	Water surface area (ha)	Fish production(Mt)	Productivity(Kg/ha)
2000/01	5,945	15,320	2,577
2001/02	5,954	15,516	2,606
2002/03	5,987	16,000	2,672
2003/04	6,093	18,060	2,964
2004/05	6,220	20,213	3,250
2005/06	6,337	22,545	3,558
2006/07	6,500	23,750	3,654
2007/08	6,735	24,295	3,607
2008/09	6,700	23,780	3,549
2009/010	6,900	24,869	3,604
2010/011	-	26,941	3,702
2011/012	10,718	29,999	3,779

The table 4 shows sharp increase in pond fish production, which was 29,999Mtons in 2011/2012 from about 10,718 ha of water surface area.

1.3.3 Socio-economic Aspect

Socioeconomic aspect of aquaculture and fisheries are one of the least focused topics in Nepal. The fisheries and aquaculture subsector plays a relatively limited role in the overall Nepalese economy, it contributes only 2.7% of agriculture GDP. Its contribution in national GDP is 0.97 (1.0) %, and per capita fish consumption is 2.07 kg (DOFD 2010). It was estimated that during 2003/2004 approximately 136000 families were engaged in aquaculture, fisheries and associated activities, with about 504000 individuals actively involved in the sector. It has been estimated that capture fisheries production in 2003/2004 employed about 425000 people and benefited over 741000 individuals in the country (FAO 2004). It is estimated that about 65.0 percent female are involved in capture fisheries. About 3 percent of total population is benefited from this sector (FAO, 2004). In 2003/2004, this sector also employed an estimated number of 9000 families, with about 21000 individuals actively involved in associated activities

such as seasonal workers (e.g., fish harvesting processing, earthwork,) and support services (e.g. marketing, storage transportation, research).

1.4 Fish Handling and Preservation

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people as well as provide foreign exchange earning to many countries(Al-Jufaili and Opara, 2006). In Nepal major preservation methods of fish are freezing, drying, smoking,chilling etc.

Fish is highly susceptible to deterioration without any preservative or processing measure (Clucas and Sctcliffe, 1987) and (Okonta and Ekelemu, 2005). Emokpae (1979) reported that immediately after the fish dies, a number of physiological and microbial deterioration set in and thereby degrading the quality of fish.

Fresh fish handling procedure consists all the operations aimed at maintaining food safety and quality characteristics from the time fish is caught until it is consumed. The quality of the freshly caught fish and its usefulness for further utilization in processing is affected by the fish capture method. Unsuitable fishing method does not only cause mechanical damage to the fish, but also creates stress and the condition which accelerate the fish deterioration after death. Microorganism contamination of fresh fish is a major cause of spoilage (Clucas, 1981).It is common knowledge that if fish are kept clean and at low temperature, growth of bacteria, consequently spoilage is kept at minimum. There are numerous requirements, which include adequate supply of clean water. The water should preferably be chlorinated. Chlorinated clean water will remove maximum bacteria invading fish surfaces.

1.4.1 Fish Market

The concept of organized fish marketing was developed in 1981/ 1982 with the start of the Aquaculture Development project. The fish marketing system seemed to have evolved and self regulating with increasing production and demand. The consumer in Nepal prefers fresh and healthy fish. Demand of processed fish and fishery products is gradually increasing in market. Fish marketing infrastructures have been developed in most cities in Nepal along with agriculture marketing networks but are not well managed. In Nepal, fishes are sold as live fish, iced fish from India and Nepal, dried/smoked fish, and canned

fish (department stores). The system of labeling/ certification of fish and its product has not yet been initiated.

1.5 Objectives

The main objectives of this research work are:

i. To study fish handling and processing methods followed in study area.
ii. To identify the major risk and hazard factors in fresh fish marketing.
iii. To analyze the quality of fish available in the fish market of Kathmandu.

1.6 Justification

The present research work was conducted because very little work had been conducted so far for fresh fish handling and processing for the enhancing fish quality and food safety. In Nepal, still people are following the traditional methods of fish handling, processing and preservation. The present work will pinpoint demerits of unconventional method of fish handling, processing and preservation. The present work will provide the information about the quality of fish available in the fish markets of Kathmandu.

1.7 Limitation

The present study was carried with limited time and financial resources. The microbiological and physiochemical analysis of fish could not be carried out immediately for the samples collected from study area Bara and Parsa.

2. LITERATURE REVIEW

Fishes are recognized as highly perishable, relatively short shelf life (C.E. Regenstein and M.J. Regenstein 1991). Therefore fish requires proper handling and preservation to increase its shelf life and retain its quality and nutritional attributes. Quality is defined as the aesthetic appearance, freshness or degree of spoilage undergone. Immediately after fish caught, it loses its natural resistance to be attacked by microorganism and also starts to undergo both physical and chemical changes that in return bring change in appearance, texture, smell and taste.

2.1 Forms of fish spoilage

"Spoilage" can be defined as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption (Hayes 1985). Fish undergoing spoilage has one or more of the following signs: slime formation, discoloration, changes in texture, off- odours or off- flavours and gas production. The development of these spoilage indicators in fish and fish products is due to the combination of microbiological, chemical, enzymatic and physical phenomena (Huis in't vel 1996).

2.1.1 Microbiological spoilage

When the fish dies, its entire body resistance mechanisms breakdown, giving way to microorganisms or the enzymes they secrete to invade or diffuse into the flesh where they invade with the complex mixture of natural substances present. During storage a characteristic flora develops, but only a part of this flora, known as the specific spoilage organism (SSO), contribute to spoilage. The SSO counts reach a minimal spoilage level where the fish is sensorially rejected (Dalgaard 1993). Bacteria present in the fish are normally associated with those found in their natural environment and influenced by the season and the harvesting conditions. The proportion of the initial population can easily be changed after the harvesting process depending on the ability of those bacteria to adapt to the new conditions (ICMSF 1998). Spoilage bacteria are predominant on newly caught fish, but some pathogenic bacteria could also be present in the skin, gills or guts. According to Vanderzant and Spilttstoesser (1992) and Huss (1995), the type and number

of pathogenic bacteria found in fish can be divided in two group (i) indigenous pathogenic bacteria, which are commonly found in the aquatic environment they are present on the live fish and their presence in the final product is predictable (e.g. *Clostridium botulinum*, *Listeria mincytogenes, Aeromonas hydrophila and vibrio* sp.) and (ii) non indigenous pathogenic bacteria, which are normally associated with human or warm- blooded animals and their faeces, and not naturally present in fish and sea food products (e.g. *Salmonella, Escherchia coli* and *Staphylococcus aurous*).

Pseudomonas and *Altermonas putriefaciens* are probably and major bacteria species that cause fundamental spoilage of usually iced fish. These can use the non- protein nitrogen compounds present in the fish such as trimethyl amine oxide (TMAO) that result is several volatile odoriferous compounds such as trimethyl amine TMA (C.E. Regenstein and M.J. Regenstein 1991). These volatile compounds are responsible for the off odours and flavours characteristic of spoiled fish.

Microbiological quality evaluation of fish aims to quantify the hygienic quality of fish, including temperature abuse and the possible presence of pathogenic microorganisms in the fish. Total aerobic bacteria, also called total plate count (TPC); specific spoilage organisms (SSO) and various pathogenic bacteria are examined using appropriate agar media. Quality levels are based on the plate counts for acceptance or rejection of fishery products for human consumption with representative sample units not less than five, plate counts below 5×10^5 are considered of good quality, between 5×10^5 and 10^7 marginally accepted quality and plate counts at or above 10^7 are considered unacceptable in quality (ICMSF 1998).

2.1.2 Autolytic spoilage

The autolysis process relates to enzyme activities in fish (autolysis means self digestion). When the fish dies adenosine triphosphate (ATP), which is the energy rich organic compound present in its muscles, will mostly be synthesized from glycogen, but also from creatine- phosphate (for finfish) and from argentine phosphate (for cephalopods) under anaerobic conditions. The glycolysis (glycogen reduction process) still occurs continuously to create the end product of lactic acid. Because the end product of this process is lactic acid, the pH of the muscle will decrease. The ATP concentration gradually decreases and when it goes below 1 µmol/g in the muscle tissues the enzyme ATP-ase is activated. This leads to the stiffening of the muscle known as Rigor Mortis.

The ATP is gradually degraded during time to some degraded products e.g. adenosine diphosphate, adenosine monophosphate, inosin monophosphate, inosin and hypoxanthine. Hypoxanthine is considered to cause the off flavour in spoiled. When the fish raw material is handled carelessly, the autolytic enzymes lead to the production of some spoilage substances. These substances create a very good environment for microorganisms. Cathepsin, chymotrypsin, trypsin. calpain, collagenase and TMAO – dimethylase are all autolytic enzymes. Therefore, in order to maintain fish quality, enzyme activities should be prevented. Using low temperature is the most frequently used measure to limit enzyme activities (Huss 1994).

2.1.3 Chemical oxidation

Fat oxidation usually occurs after autolysis and bacterial spoilage. The lipid concentration in fish can contribute to the spoilage process in fish. The fat compounds in fishes, are mainly unsaturated fatty acids that are easily oxidized from the oxygen of atmosphere. High temperature or exposure to light can increase the oxidation rate. For fatty fish preserved in ice, spoilage due to rancidity is mainly caused by oxidation. This produces bad and unpleasant odour as well as a rancid taste (Hobbs 1982). Lipids are oxidized to peroxides, aldehydes, ketoses and lower aliphatic acids. The hydroperoxides are tasteless but can cause brown and yellow discoloration of the fish tissue. The production of hydroperoxides and ketoses produce rancid off- flavours.All the chemical by-products eventually reach a level where the fish is rejected(Figure 1). High temperature is partly responsible for the speed of the oxidation processes. In addition, direct sunlight, wind, heat, light(especially UV-light) and several organic and inorganic substances may also accelerate oxidative processes.

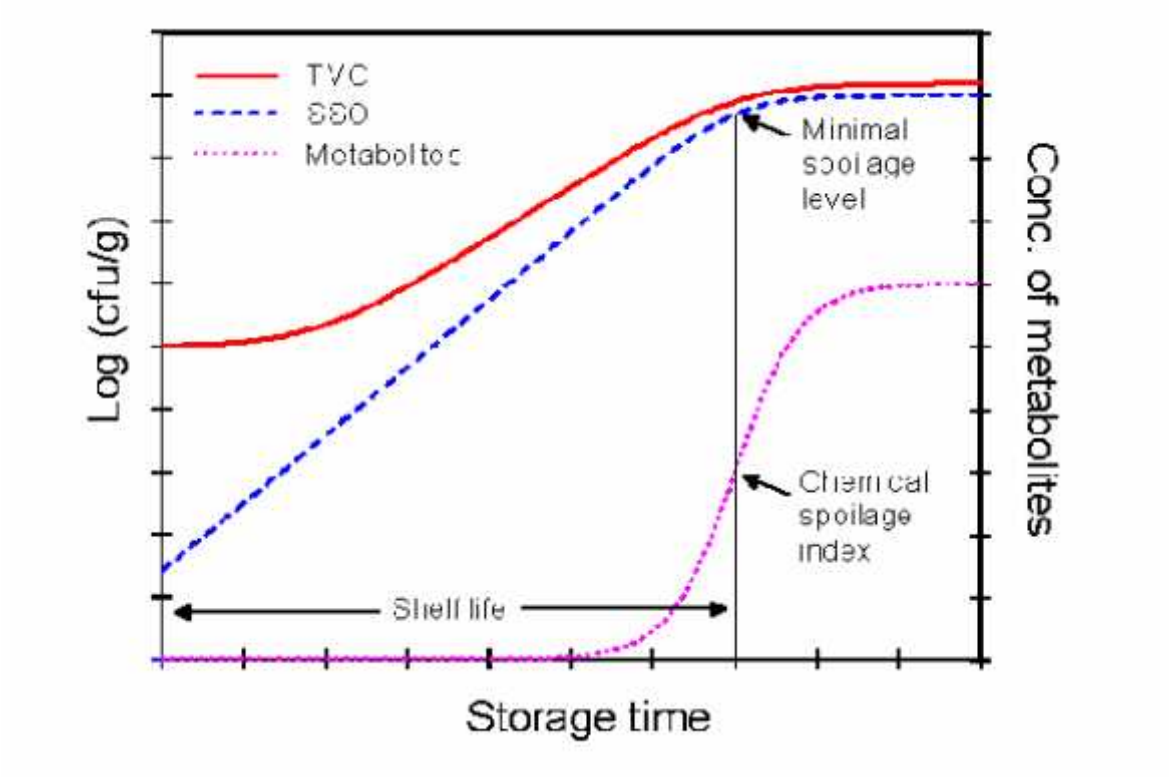

Figure 1: General pattern of microbial spoilage changes in total viable count (TVC), specific spoilage organisms (SSO), and chemical spoilage indices during chilled storage of a fish product (adapted from Dalgaard 1993).

2.2 Analysis methods for quality evaluation

The methods of assessing freshness can be divided to two groups: sensory methods and non-sensory methods. Non-sensory methods include microbiological, chemical and physical analysis. Sensory assessment is a direct measure but the non-sensory methods are indirect measurements. The disadvantage of the sensory method is that it is subjective depending on the person who evaluates and people have to be trained for sensory evaluation of fish. The non-sensory methods are biological, chemical, physical. Their disadvantage is complexity because they require laboratory equipment (Jonsdottir 1992). The sensory and non sensory methods should be used in combination (Howgate 1992).

2.2.1 Sensory method

Sensory evaluation is a systematic assessment of the odour, flavor, appearance and texture of food. The Quality Index Method (QIM) is a sea food freshness quality control system that was developed by European Fisheries Research Institutes. It is considered to

be a rapid and reliable method for assessing freshness (Martinsdottir et al. 2001). QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 3 defect points (with 0 being highest and 3 the lowest) with a total score of 0-20 for whole fish(Larsen et al.1992) as shown in appendix 1. QIM score is given in appendix 1 and table 9. QIM is the practical rating system where the defect points are recorded. The sum or scores for all the characteristics is the overall sensory score. QIM gives scores of zero for very fresh fish, while increasingly larger totals result as the fish deteriorates (Huss 1995). When the score is 18 or more the fish is considered spoilage.

2.2.2 Microbiological method

There are a lot of microbiological methods to determine fish bacteria e.g. plate count, direct microscopic count, ATP measuring. But the plate count is traditional and common method with some different media like plate count agar or iron agar. Some spoilage bacteria can produce H_2S (e.g. *Sewanella putrefaciens*) and reduce TMAO. The iron agar medium can be used in order to isolate spoilage bacteria that produce H_2S and form black colonies on the agar media. Black and white colonies are observed and counted respectively. The black ones are referred to as spoilage bacteria, while the totals (black + white) are referred to as the total count. The pour plate method is often used with plate count agar, which is a common method to determine the total content of bacteria in seafood. The iron agar method can sometimes detect higher bacteria amounts than plate count agar (Gram 1992).

2.2.3 Chemical method

Chemical methods, to measure freshness quality, have been considered to be objective and superior methods to methods involving sensory evaluation. During post mortem storage microbiological spoilage causes the formation of volatile bases, which can be determined to measure indirectly the freshness quality of such seafood. There are a few substances that are usually determined to evaluate fish raw material freshness, e.g. total volatile basic nitrogen (TVB-N), trimethylamine (TMA), ammonia, biogenic amines, ethanol and indol. The TVB-N remains constant for the first days of storage or increases slowly but it rises fast later in the spoilage process. Therefore TVB-N is very good indicator of spoilage in the fish (Oehlenschlager 1992). For some types of ground fish

species like Atlantic cod (*Gadus morhua*), European hake (*Merluccius merluccies*) and haddock (*Meranogrammus aeglefinus*), the TVB-N determination is not as good to detect the early stages of deterioration in freshness quality like the TMA measurement, but it can be used for measuring later stages of deterioration (Botta 1995).

2.3 Causative factors of spoilage

Spoilage is caused by the action of enzymes, bacteria and chemicals present in the fish. In addition, the following factors contribute to spoilage of fish.

- Temperature
- Rough handling
- Unhygienic practices
- Method of capture
- Mode of storage
- Initial bacterial load

2.3.1 Temperature

It is well known that high temperatures increase the rate of fish spoilage and low temperatures slow it down. Therefore, if the temperature of fresh fish is low, then quality is lost slowly. The faster a lower temperature is attained during fish chilling, the more effectively the spoilage activity is inhibited. Generally, the rate at which fish loses quality when stored in ice (0^0 C) is used as the baseline when comparisons are made regarding self-life at different storage temperatures. The relationship between the shelf-life of fish at 0^0C and at t^0C is known as the relative rate of spoilage at t^0C (RRS) and is defined by the equation (Spencer and Baines 1964):

$$\text{Relative rate of spoilage at } t^0\text{c} = \frac{\text{Keeping time at } 0^0\text{C}}{\text{Keeping time at } t^0\text{C}}$$

$$\text{Shelf life} = \frac{\text{Final – initial level of a quality indicator}}{\text{Rate of spoilage at the actual storage conditions}}$$

2.3.2 Rough handling

Rough handling will result in a faster spoilage rate. This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. Physical mishandling

in the net, such as very large catches, fishermen stepping on fish or throwing boxes, containers and other items on top of the fish, may cause bruises and rupture of blood vessels.

2.3.3 Unhygienic practices

Apart from the microorganisms that fishes have at the time of capture, more is added via unhygienic practices and contaminated equipment such as storage facilities. This was demonstrated by studies that compared the quality and storage life of completely aseptically treated fish (aseptic handling), washed fish, iced in clean plastic boxes, with clean ice (clean handling) and with unwashed fish, iced in old, dirty wooden boxes (normal handling). A considerable difference was found in the bacterial contamination of the three batches, the later heavily contaminated with a reduction in storage life compared with the other samples (Huss *et al*. 1974).

The design of a fish hold is of great importance as far as hygiene in the hold is concerned. Hold design should enable the purge (drip loss) to be collected easily. The amount of purge was suggested to be higher at 5-7^{0}C; at which temperature there is greater spoilage since the purge is a very good medium for bacterial growth (Hermansen 1983).

2.3.4 Methods of capture

The fishing gear and method employed determines the time taken between capture and death. Fish caught in gillnets struggle much to escape, and in doing so, they are bruised by the net which increases exposure to microbial entry and subsequent deterioration. Fish caught by hook and line methods, on the other hand, die relatively quickly and therefore bruises and stresses are likely to be minimal. Physical mishandling in the net due to long trawling nets and very large catches accelerates spoilage. The large catches in the net are compacted against each other resulting in the fish getting bruised and crushed (especially small sized fish) by the heavy trawl net.

2.3.5 Mode of storage

In bulk-storage, the weight of the pile may crush the fish at the bottom, leading to a loss of weight (yield) as well as other physical damage. It has been reported that when haddock is kept in a short, deep pile of about 3 ft, the bottom fish lose 15 % of their

weight compared to a normal weight loss of 3-8 %, which is entirely due to biochemical changes that causes a loss of water holding capacity leading to drip (C.E. Regenstein and M.J. Regenstein 1991). Crushing of the fish by ice or other fish can seriously affect the quality of fish by releasing enzymes from the gut into the fish muscle there by accelerating autolytic processes. When the fish is in rigormortis(a complicated series of chemical changes that result in stiffening of the fish muscles shortly after death), rough handling can cause gaping(Huss 1995).

2.3.6 Initial bacterial load

The microflora on tropical fish often carries a slightly higher load of Gram positives and enteric bacteria but otherwise is similar to the flora on temperate –water fish (Liston 1980). Basically, bacteria populations on temperate fish are predominatly psychrotrophic reflecting water temperature of about 1^0Cwhile fish from the tropics have largely mesophilic bacteria(Gram and Huss 1996).

2.4 Fish preservation

The processing and preservation of fresh fish were of most importance since fish is highly susceptible to deterioration immediately after harvest and to prevent economic losses (Okonta and Ekelemu 2005).If fish is not sold fresh, preservation methods should be applied to extend self life. These include chilling, freezing, drying, smoking, etc.

2.4.1 Chilling

Chilling may be defined as cooling of fish to low temperatures without necessarily hardening fish. Chilling does not prevent spoilage. However, the colder the fish the better and the lower are the incidences of microbial or enzymatic spoilage. Bacteria or enzyme action are not completely stopped but they may be temporarily halted by chilling. To chill fish, the fish has to be surrounded by colder medium, which could be solid such as ice or liquids such as refrigerated water (Ita 1972).

2.4.2 Super cooling

This is not a common method. Super chilling implies reducing the temperature of fish uniformly below 0^0C. At this temperature half the water in the fish freezes, bacteria action

is greatly reduced and self-life is extended. Fish are initially chilled using ice before storage in refrigerator holds at temperatures below freezing of ice. The temperature in the hold is maintained by means of cold or circulating refrigerated brine. This method is known to extend shelf life of fish by up to 14 days (Ita 1972).

2.4.3 Freezing

Freezing is distinct from chilling of fish. Freezing can keep products in near perfect condition for very prolonged periods. Freezing is essential for export purposes. Freezing becomes extremely effective, if it is combined with cold storage (Anthonio 1970).

Freezing is the process by which the water in the fish muscles is crystallized into ice. Pure water freezes at 0^0C. Fish contains about 80% water, salts and minerals. As would be expected therefore, fish can be frozen at temperatures lower than 0^0C. As the water freezes out, the concentration of salts and chemicals increase thereby lowering the freezing temperature. At about -5^0C, up to 20% of water in fish is still unfrozen. The freezing stage in fish has been divided into three. The first stage includes the period when the temperature falls rapidly to about -1 ^{0}C. At -1^0C, the temperature remains fairly constant and up to 75% of the water freezes. This is the thermal arrest stage of which there is no change in the temperature. In the third stage, the temperature begins to drop and most of the remaining water freezes (Bolaji 2005).

In the process of freezing, heat is transferred from the fish to be frozen to some surrounding of adjacent material. It is necessary that a sufficiently cold surrounding must be supplied to effect this change (Davies 2006).

2.4.4 Drying

Drying is defined as the removal of water by evaporation. When applied to fish, drying is the removal of water by any method as a means of fish preservation to prolong the shelf life. In the areas where sun drying is used traditionally, the effects of wind and weather conditions are important. Basically, the drying effect of the sun depends on the emission of heat from the sun. This is transferred to the fish and; it is accompanied by, heat transfer within the fish. During drying, the fish shrinks and undergoes irreversible changes. Water is removed from the surface in the following sequence. Firstly, water on the surface of fish evaporates. Water migrates to the surface of the fish from within fish tissues and evaporates. The air surrounding the fish then experiences a drop in temperature. This is

accompanied by cooling of the surface of the fish. The energy required to drive the moisture from the surface of the fish can be obtained from a variety of sources including wood smoke, sun drying, solar drier electricity and mechanical drier (Davies *et al*.2008).

The surrounding air conditions remain constant. The rate of drying will also remain constant. This stage of the process of drying is referred to as the "constant rate drying". As the removal of moisture from the fish continues, the drying effect continues. Eventually, the concentration of the moisture at the fish surface falls consequently, the movement of moisture to the surface also drops and the drying rates slows down. This stage is referred to as the "falling rate drying" (Emokpae, 1979).

Both rates drying are under the influence of numerous factors. Notably is the relative humidity of the air. If the air is fully saturated with water vapor, drying will not take place. The relative humidity must be less than 100% for drying to occur. It is obvious that the lower the relative humidity, the faster the drying rate. Increased air speed results in faster drying rates (Eyo 1997).

2.4.5 Smoking

Smoking is a popular traditional method of fish preservation in most developing countries. Smoking combines the effect of the destruction of bacteria by compounds in the smoke, such as phenols and the cooking of the fish, since, high temperatures will be generated. Smoked fish products have long shelf life, which has been attributed to the drying and cooking effects. When wood and sawdust are burnt, smoke is produced as a result of incomplete combustion. The smoke produced depends on the amount of air available and the quality of wood or sawdust. Soft woods produce a lot of smoke, which may lead to blacking of the final products. Wood smoke is a mixture of complex chemical product gases, vapor and volatile substances. The volatile substances are absorbed on the wet surfaces of fish during the smoking and produce the characteristic aroma (FAO/UN 1970a).

3. METHODOLOGY

3.1 Study area

Study is done at Bodhban 9, Parsauna VDC of Bara district, Jhounkuti and Birjung of Parsa district, and different fish markets at Kathmandu. Bodhban is considered as 'Fish Village'. According to Statistical Information on Nepalese Agriculture 2011/2012, total fish production in Bara was about 4,488,000 kg and in Parsa was about 915,000kg. Total number of fish pond in Bara is 2,276 out of which 800 pond lies in Bodhban. There are about 184 fish farmers in Bodhban. Total number of fish pond in Parsa district is 1,275.

3.2 Research design

It is based on observation cum experimental methods. Fish handling process from the time of catch to the fish market were observed. Similarly quality assessment for freshness of fish was done by Quality Index method (QIM). Microbiological examination for detection of *Salmonella* species was done by different biochemical tests such as Citrate Utilization Test (CUT), Sulphur reduction, Indole production and Motility test (SIM), and Triple Sugar Iron (TSI) tests. Total load of microorganism were counted by pour plate method in plate count agar (PCA).Physiochemical characters (moisture percentage, average ash percentage, acid insoluble ash percentage, peroxide value) were determined by standard methods of Proximate analysis.

3.3 Source of data

The study was based on primary as well as secondary data collection. The primary data has been collected by visiting the study site Bodhban 9, Parsouna in Bara, ,Jhounkuti and Birjung of Parsa from 27th January 2012 to 29th January 2012. Different fish market of Kathmandu were visited twice from January 2012 to July, 2012. Focus group discussion (FGD) was done to study the fish handling and processing method and to identify the major risk and hazard factors for end product quality. The set of questions asked during Focus group discussion (FGD) are given as in Appendix 4. The secondary data had been collected from different sources like related publications, websites, government and nongovernment institutions.

3.4 Sample collection

Total 48 Naini (*Cirrhinus mrigala)* of about 75 to 100 gm were collected as experimental samples from different ponds and places of study area. Four fishes as samples from each places were collected. Samples were collected from ponds, from the net inside the water, from landing site of Bodhban and Jhounkuti and from different fish market of Kathmandu. The collected samples were labeled including the place of collection, date and were brought to the ``Fisheries research Division Godavari'' for analysis in the icebox. The samples were then preserved in deep fridge at about 0^0C for further analysis. The temperature recorded was 5-15^0C during sample collection at Bara, Parsa and different fish market at Kathmandu.

3.5 Method for Assessing Freshness

The method for assessing freshness can be divided into two groups: Sensory and Non sensory methods. Sensory method or assessment is a direct measure which is subjective depending upon the person who evaluates. Similarly, non sensory methods include Microbiological and Physiochemical analysis which are indirect measurement.

3.5.1 Sensory analysis by Quality index method (QIM)

Fish samples brought from different fish market of Kathmandu were brought to sensory room for analysis. Where the samples were evaluated .Appearance of skin, slime formation, firmness of flesh, colour, form of eyes, smell and mucus formation of gills was evaluated according to the Quality Index method (QIM) where quality attributes were rated on 0-3 demerit point score (with 0 being the highest and 3 the lowest) with total score of 0-20 for whole fish (Larsen et al. 1992) as shown in Appendix 1.The score from each fish samples were added and the sum of the individual fish averaged to give the overall sensory score(quality score) of the fish sample.

3.5.2 Microbiological analysis

3.5.2.1 Processing of the sample

i. Grinding of fish samples: Twenty grams of fish sample including all parts was aseptically transferred into a sterile mortar and grinded by sterile pistle and added to

180 ml of normal sline(0.85% Nacl). This fish homogenate was now itself at 10-1 dilution.

ii. Serial dilution of homogenate: The fish homogenate was mixed well by shaking. 1ml of fish homogenate was pipetted out into a tube containing 9ml of normal sline and carefully mixed and labeled as 10-2 dilution. Similarly the dilution was carried out up to 10-9 dilution and labeled as 10-4,10-5,10-6 and so on to10-9dilutions respectively

iii. Enumeration of aerobic mesophilic bacteria(Total plate count):

One ml of homogenate and dilutions(aliquots) of the homogenate were pipetted out and kept into each of sterile approximately marked duplicate plates. Sterilized total plate count agar (TPCA), cooled to 450 C and was poured into each petridish within 15 minutes of the time of original dilution. The sample dilution and agar medium were mixed thoroughly and uniformly and allowed to solidify.

iv. Incubation of the culture: The prepared dishes containing TPCA was incubated at 370C for 48 hours in an inverted position.

v. Counting of the colonies and calculation: The dishes containing 30-300 colonies after 48 hours incubation were counted. When the dishes examined contained no colonies, the result was expressed as zero bacteria per gm/ml. When the dishes (dilution 1 in 10) contain less than 30 colonies and no other plates of the sample contain colonies then it was counted and the result was expressed as the (number of colonies) CFU of bacteria per gm/ml.

3.5.2.2 Isolation of Salmonella species

Five ml of the enriched sample of fish homogenate was transferred to Selenite-F broth, and incubated at 370C for 24 hours for enrichment of Salmonella spp. After 24 hours, a loopful of enriched culture from Selenite-F broth was streaked on Brilliant Green Agar (BGA) and Xylose-Lysine-Deoxycholate (XLD) Agar and Salmonella –Shigella Agar and incubated at 370C for 24 hours.

3.5.3 Identification of Organisms

After obtaining the pure culture the organisms were identified by using standared microbiological techniques as described in BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY which involves morphological appearance of the colonies, staining reactions, biochemical properties (Mackie and Mc Cartney,1989 ; Bailey and scotts, 1990; Monica Cheesbrough, 1984).

3.5.3.1 Biochemical tests used for identification of isolated bacteria

Following biochemical tests were carried out to identify the isolated organisms.

Citrate Utilization Test (CUT).

Sulphur Reduction, Indole production and Motility (SIM) test.

Triple Sugar Iron (TSI) Test.

3.5.4 Proximate analysis

3.5.4.1 Moisture content

Moisture content in fish sample is determined by hot air oven method.The fish sample is weighed and heated in an insulated oven to constant weight. The difference in weight is the water that has evaporated (Appendix 3)

3.5.4.2 Total ash

The total ash was determined from the fish sample by dry ashing method (Appendix 3).Total ash is determined by incinerating all the organic matter of the food sample at 550^0c.

3.5.4.3 Acid insoluble ash

The acid insoluble ash is ignited residue obtained after treatment of total ash in 10% Hcl and subsequent filtration. Acid insoluble ash mainly consists of Silica, compounds that are resistant to dissolution in 10% Hcl (Appendix 3)

3.5.4.4 Peroxide value

To find the peroxide value first fat is extracted by solvent extraction method. Crude fat is solid samples which is determined by using soxhlet apparatus by recycling hot solvent, usually petroleum ether. The apparatus consists extraction tube (into which sample in a timble is kept immersed in solvent for fat extraction), the receiving flask (which receives through a siphon system and extracted fat from the extraction tube and vaporizes the solvent selectively for recycling) and the condenser (which condences the the vaporized solvent onto the sample placed in the extraction tube).The recycling is done for a certain number of times until the extraction is complete and the fat is recovered by evaporating away the solvent. (Appendix 3)

Peroxides are formed due to oxidation of fats. Peroxide value is expressed as milliequivalent of peroxides formed per 1000 gram of material. Peroxide value was determined by dissolving the sample in an acetic acid chloroform mixture and an aqueous solution of potassium iodide was added in it. The iodide gets oxidized to iodine due to oxidizing peroxides. The liberated iodine gives the measure of peroxide contents and was found from titration with standard $Na_2S_2O_3$ solution.(Appendix 3)

3.6 Statistical tools

Arithmetic mean is used as statistical tools during the study.

3.7 Photography

The photograph during research was taken by Sony shot 5.1 digital camera. The photography of fish handling and processing were done.

1. Crushing ice for Fish Preservation

2. Foam boxes used for fish packing

3. Jute Sack used for packing fish

4. Fish catching

5. Researcher collecting sample

6. Jeep used for transportation

7. Carring fish to jeep from landingsite

8. Researcher during FGD.

9. Drying of net

10.suplimentary fish feed

11. FGD during dissertation

12. Fish dressing in fish shop at old Baneshore

4. RESULTS

4.1 Fish handling steps in Bara and Parsa

The complex chain through which the catch flows from pond to the fish market at different places is summarized in Figure 2. Fish from pond of different village in Bara and Parsa are brought to fish collection site at Birjung by jeep, tanga, local bus etc. From here the fishes are distributed to the wholesaler of different places by night bus. The time taken form catch to reach to wholesaler of different places may reach about or more than 24 hours. Then the fish is distributed to the retailer.

4.1.1 Catching method

For catching the fishes in the pond, usually the cast net and drag nets are used. The hauling of the net takes place at morning usually from 7am to 10 am. It takes about 45 minutes to 1 hour to catch the fish and to bring at the landing site. It depends on size of pond.

4.1.2 Landing

Fishes are landed on the dike or side of pond where the fishes are shorted out of nets, graded, weighed and sold to the supplier. Fishes are packed with crushed ice in plastic crate or polystyrene box or directly in the jeep or tanga and brought to collection site at Birjung. Fish get packed within 45 minutes at landing site.

4.1.3 Reicing

At collecting site in Birjung, reicing and packing of fishes is done in plastic crate or thermocool or styrobox. On the same day these packed fishes are supplied to the wholesalers of different places of Nepal by night buses. The wholesalers of Kathmandu receive the fishes early in the morning at about 4.30 to 6 am. From whole saler the fishes are distributed to the retailers of different fish markets on the same day.

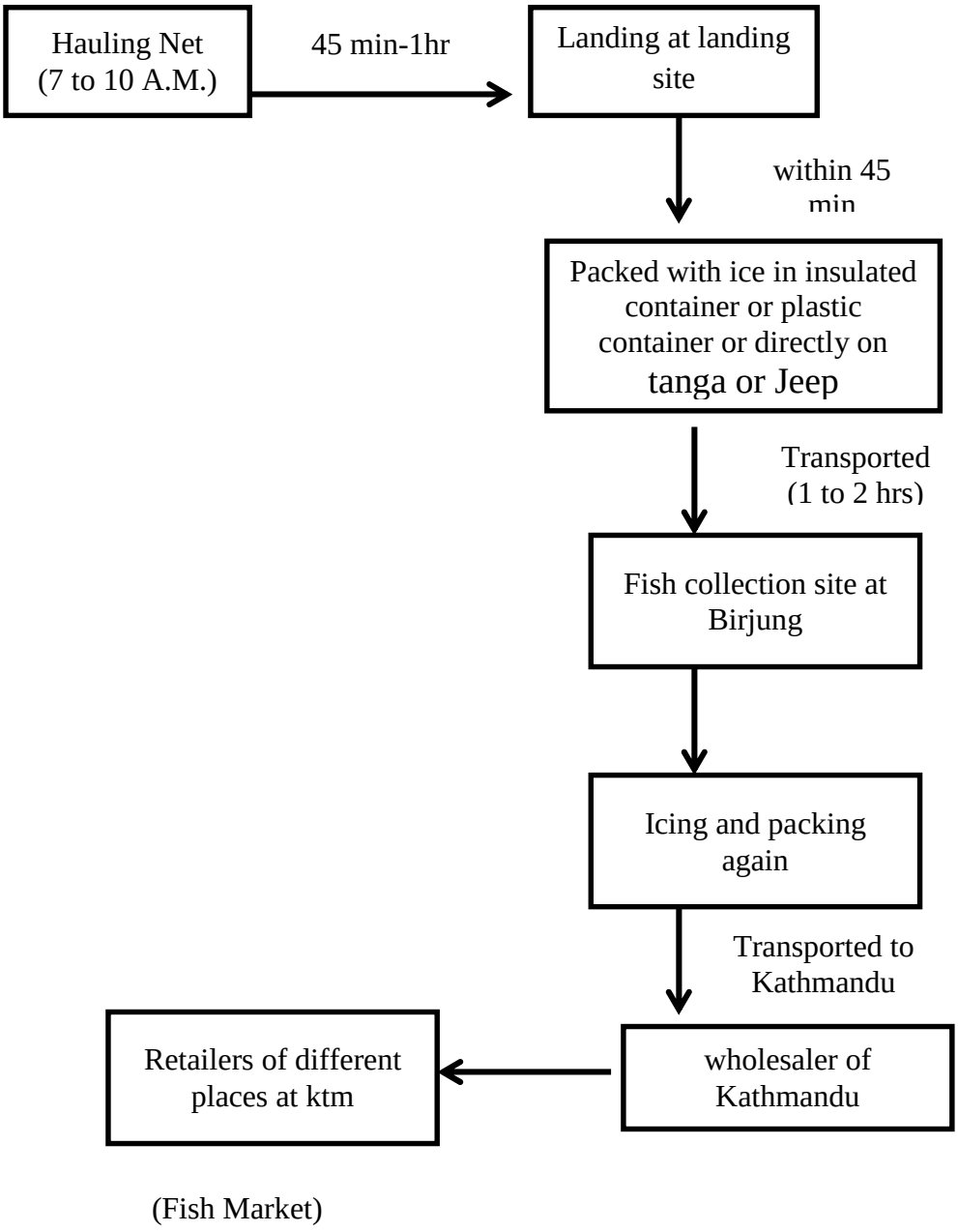

Figure. 2. Flow diagram of fish catch from pond to fish market

In sum, there are risk for a cumulative reduction in the original quality of fish at every step, which are accelerated by the practices employed in handling the fish and need to be corrected or avoided (Table 5).

4.1.4 Survey at each handling steps (from landing site to fish market)

It was noticed that the nets was washed only in pond water without using any type of disinfectant like Chlorinated water or Teepol quaternary ammonium compounds or any detergent and was dried in sun.

The dike and surrounding area of the pond used as landing site was found contaminated with human faces as well as animal dung. Jute bags, plastic crate as well as styrobox used for packing the fish was not clean, even the floor of Jeep used to carry the fish was not cleaned, which can be observed in photos taken during research work. Similarly the fish market at Kalimati shows the worse sanitary condition. In most of the fish shop it was also found that same water was used repeatedly during fish dressing. Fish shops were found close and humid supporting the growth of microorganisms. During selling hours the fishes were kept on table without ice on direct exposure of sunlight.

Table 5: Summary of risks at each handling steps.

Handling Step	Potential danger (Hazard)	Source of danger
On the pond to landing site	Growth of bacteria	Exposure to sun and wind
	Contamination	Landing site
	Bruises	Rough handling
At landing site	Growth of bacteria	Delayed icing
	Contamination	Contaminated landing site
	Growth of bacteria	Poor insulation of container
During transportation	Growth of bacteria	Delayed re-icing
		Improper icing
	Fish crushing	Mechanical load
At wholesaler Market (Kalimati)	Growth of bacteria	Delayed off load
	Contamination	Unhygenic environment
At fish market (Retailar)	Growth of bacteria	Exposure to sun & wind
	Contamination	Unhygenic environment

4.2 Sensory Assessment

The sensory analysis by quality Index Method (QIM) showed that the Quality Index score of fishes available in different fish market of Kathmandu.The Quality Index score of fish taken from Kalimati was 2, from Khichapokhari was 5, from old Baneshwor was 5,from Lagankhel was 5 and from bicycle seller was 12. The least score 2 was found in fish samples from Kalimati and highest score 12 was recorded in fish samples from the bicycle seller (Table 6).

Table 6: Sensory assessment of fish samples from different fish markets

Sensory Score of Fish from diff. fish market							
Quality Parameter	**Character**	**Observation**	**Kalimati**	**Khichapokhari**	**Lagankhel**	**Old Baneshwor**	**From Bicycle seller**
	Skin	Bright and shining	0	0	0	0	0
		Bright	**1**	**1**	**1**	**1**	1
		Dull	2	2	2	2	**2**
	Blood spot on gill cover	None	**0**	**0**	**0**	**0**	0
		Small (10-30%)	1	1	1	1	**1**
		Big (30-50%)	2	2	2	2	2
		Y. Big (above 50%)	3	3	3	3	3
	Stiffness	Stiff	**0**	0	0	0	0
		Elastic	1	**1**	**1**	**1**	1
		Firm	2	2	2	2	**2**
		Soft	3	3	3	3	3
	Belly	Firm	**0**	**0**	**0**	**0**	0
		Soft	1	1	1	1	**1**
		Belly brust	2	2	2	2	2
	Smell	Fresh	0	0	0	0	0
		Neutral	**1**	**1**	**1**	**1**	**1**
		Musty/Sour	2	2	2	2	2
		Stale meat/rancid	3	3	3	3	3
Eyes	Clarity	Clear	**0**	0	0	0	0
		Cloudy	1	**1**	**1**	**1**	**1**
	Shape	Normal	**0**	**0**	**0**	**0**	0
		Plain	1	1	1	1	1
		Sunken	2	2	2	2	**2**
Gills	Colour	Red	**0**	**0**	**0**	**0**	0
		Foded	1	1	1	1	**1**
	Smell	Fresh	**0**	0	0	0	0
		Neutral	1	**1**	**1**	**1**	**1**
		Sweaty/slightly rancid	2	2	2	2	2
		Sour stink/stale rancid	3	3	3	3	3
Sum of score			**2**	**5**	**5**	**5**	**12**

4.3 Microbiological Analysis

The fish sample was taken from different sampling site i.e. from net and landing site of Bodhban and pond 1st and 2nd of Jhaunkuti, and from fish market at Kathmandu. The samples were studied for Total microbial load (cfu/gm) and detection of Salmonella species.

4.3.1 Total microbial load

From the study, it was found that the fish sample taken from net inside the water at Bodhban pond showed Total plate counts of 57.2 x 10^6 cfu/gm, samples from landing site at Bodhban contained 38.4 x10^6 cfu/gm, from net of pond 1st of Jhounkuti contained 96.4 x10^6 cfu/gm, landing site of pond 1st of Jhounkuti contained 93.4 x10^6 cfu/gm, from net of pond 2nd of Jhounkuti 59.6 x10^6 cfu/gm, landing site of pond 2nd of Jhounkuti 101.2 x 10^6 cfu/gm,and fish market of Kathmandu 68.4 x 10^6 cfu/gm.

4.3.2 Isolation of Salmonella species

From the different biochemical test during study it was found that the Salmonella sp. was not isolated from the samples taken from net inside the water of Bodhban pond, net inside the water of pond 1st of Jhunkuti and landing site of pond 1st of Jhounkuti.As the samples from these sites gives negative result. Similarly the samples taken from landing site of Bodhban pond, net inside the water and landing site of pond 2nd of Jhounkuti, and fish market at Kathmandu gives the positive result indicating the presence of Salmonella spp.

Table 7: Microbial analysis for detection of *Salmonella* species and total microbial load

Samples		Salmonella SPS	Total microbial count, cells/gm
Place	**From collected**		
Bodhban (Bara)	Net	-ve	57.2×10^6
	Landing site	+ve	38.4×10^6
Jhounkuti (Pond 1) (Parsa)	Net	-ve	96.4×10^6
	Landing site	-ve	93.4×10^6
Jhounkuti (Pond 2) (Parsa)	Net	+ve	59.6×10^6
	Landing site	+ve	101.2×10^6
Kathmandu	Fish Market	+ve	68.4×10^6

4.4 Proximate Analysis

4.4.1 Moisture percent

From the study, it was found that the moisture percentage in fish samples taken from net inside the pond of Bodhban was 71.11%, landing site of Bodhban pond was 75.81%, sample taken from net inside water of pond 1st of Jhounkuti showed 74.635%, landing site of pond 1st of Jhounkuti 75.415%,sample taken from net inside water of pond 2nd of Jhounkuti 72.405%,landing site of pond 2nd of Jhounkuti 72.625% and from market of Kathmandu 72.29%.

4.4.2 Total ash percent

From the study, it was found that the total ash percentage in fish samples taken from net inside the pond of Bodhban was 2.957%, landing site of Bodhban pond was 2.572%, sample taken from net inside water of pond 1st of Jhounkuti showed 4.598%, landing site of pond 1st of Jhounkuti 4.469%, sample taken from net inside water of pond 2nd of Jhounkuti 2.581%, landing site of pond 2nd of Jhounkuti 2.650% and from market of Kathmandu 3.822%.

4.4.3 Acid insoluble ash percent

From the study, it was found that the acid insoluble ash percentage in fish samples taken from net inside the pond of Bodhban was 0.41%, landing site of Bodhban pond was 0.26%, sample taken from net inside water of pond 1st of Jhounkuti was 1%, landing site of pond 1st of Jhounkuti 0.82%,sample taken from net inside water of pond 2nd of Jhounkuti 0.14%,landing site of pond 2nd of Jhounkuti 0.28% and from market of Kathmandu 0.73%.

4.4.4 Peroxide value (meq/kg)

From the study, it was found that the peroxide value in fish samples taken from net inside the pond of Bodhban was 7.52 meq/kg, landing site of Bodhban pond was 1.56 meq/kg, sample taken from net inside water of pond 1st of Jhounkuti was 25.09 meq/kg, landing site of pond 1st of Jhounkuti 12.74meq/kg,sample taken from net inside water of pond 2nd

of Jhounkuti 21meq/kg,landing site of pond 2nd of Jhounkuti 11.94meq/kg, and from market of Kathmandu 11.91 meq/kg.

Table 8: Proximate analysis of fish samples from different places

Sample		Average Moisture %	Average ash %	Average acid insoluble ash	Peroxide value mg/kg
Place	**Taken from**				
Bodhban	Net	71.115	2.957	0.41	7.52
	Landing site	75.81	2.572	0.26	1.56
Jhounkuti (Pond-1)	Net	74.635	4.598	1.00	25.09
	Landing site	75.415	4.469	0.82	12.74
Jhounkuti (Pond -2)	Net	72.405	2.581	0.14	21.00
	Landing site	72.625	2.650	0.28	11.94
Kathmandu	Market	72.29	3.822	0.73	11.91

5. DISCUSSION

Handling of raw material roughly be divided into two categories the artistinal type and industrial type. In Nepal the handling process is mainly artistinal and uses mainly human force.

After landing, the raw fish material still has to go through many steps before entering the fish market. This may take 2 to 3 days and the temperature can easily fluctuate during the process. During study it was observed that the net used for catching the fishes are not washed by any type of disinfectant before and after catch. Farmer used to wash it with pond water. The fishes were landed on the landing site (side or dike of pond) on the plastic sheet or jute bag. In most places, the dike and sides of ponds were covered by the human and animal faeces indicating the poor hygienic condition of that place. Fishes are recognized as highly perishable, relatively short shelf life (C.E. Regenstein and M.J. Regenstein 1991). Therefore fish requires proper handling and preservation to increase its shelf life and retain its quality and nutritional attributes.

During transportation from landingsite the fishes were packed in the plastic crate or polystyrene crate or directly on the floor of Jeep or Tanga without cleaning them.

Quality is defined as the aesthetic appearance, freshness or degree of spoilage undergone. Immediately after fish caught, it loses its natural resistance to be attacked by microorganism and also starts to undergo both physical and chemical changes that in return bring change in appearance, taste, smell and texture. “Spoilage” can be defined as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption (Hayes 1985). Fish undergoing spoilage has one or more of the following signs: slime formation, discoloration, changes in texture, off- odours or off-flavours and gas production. The development of these spoilage indicators in fish and fish products is due to the combination of microbiological, chemical, enzymatic and physical phenomena (Huis in’t vel 1996).

Spoilage of product can be detected by sensory analysis. QIM is based on the significant sensory parameters for raw fish when using many parameters and a score system from 0 to 3 defect points. It is considered to be a rapid and reliable method for assessing

freshness (Larsen et al. 2001). The sensory analysis result showed that the sensory score of fish available in the Kalimati fish market had score of 2 point, that was, least score of all, but fishes carried in bicycle were not kept in ice, so it had highest score of 12 point. QIM gives score of zero for very fresh fish, while increasingly larger totals results as the fish deteriorates (Huss 1995). If QIM score is 18 or more the fish is considered spoilage (Larsen et al. 1992).

In the study, the microbial analysis result shows the presence of salmonella species in samples taken from most of the places. Total microbial load of all samples ranges between $38.4x10^6$ to $101.2x10^6$ cfu/gm. The total microbial count was lowest in the samples taken from landing site of Bodhban pond while the highest in the samples taken from landing site of pond 2 of Jhaunkuti. The total microbial load in all samples were more than $5x10^5$ cfu/gm except the samples taken from landing site of Bodhban pond. The study showed that the fishes were loaded with microorganism. The presence of bacteria like Salmonella species harmful to man generally indicates the poor sanitation during handling and processing. The contamination is almost of human or animal origin. The total microbial load is the indicative of possible contamination and very poor hygienic condition. The high value of microbial analysis was unexpected. Plate count below $5x10^5$ cfu/gm are considered as good quality, between $5x10^5$ and $5x10^7$ cfu/gm marginally accepted quality and plate count above $5x10^7$ cfu/gm are considered unacceptable in quality(ICMSF, 1986). Hence the samples taken from landing site of Bodhban are of good quality while rests all are marginally accepted. In the study conducted by Prasai (2000) shows that the total plate count of samples taken from different fish market of Kathmandu ranges between $2x10^6$ to $7x10^5$ cfu/gm, which is lesser than the total plate count value of present study.

The study on the proximate analysis shows that the moisture content by all samples ranges between 71.115 to 75.81%. The range of values for the moisture in the edible portion of common fish species lies between 65-90 %(CIFT). According to Sailender et al. 2013 the moisture content by *Cirrhinus mrigala* under different treatments ranges between 77.1 to79.35%. The moisture content by different major carp ranges between 71.91 to 79.80%(Ahmed et al. 2012). This was slightly higher than the value obtained from present study. The moisture is the main constituent of muscles **o**f the fish,which

plays an important role in their metabolism. The water content of fish is varied within the limited range in various species.

The present study shows that the total ash content by the samples ranges between2.572 to 4.598%. The total ash content in *Cirrhinus mrigala* under different treatment ranges between 1.31 to 1.5% (Sailendra et al. 2013). Similarly, the total ash present in different major carp fish ranges between 1.08 to 1.66%.In cirrhinus mrigala was 1.66% (Ahmed et al. 2012). These values were lesser than the values obtained from present study. Proximate analysis in some selected fresh water fishes in Nigeria total ash content ranges between 4.00 to 9.00% (IJFS 2007). Nutrient content of fresh water fish in Srilanka (previous analysis) shows that the total ash content by Tilapia was 6.92%. The ash content represent the total mineral content in the food.

The present study shows that the acid insoluble ash percent ranges between 0.14 to 1.00. All the samples was below 1.5, it meant all samples were in acceptable range according to Srilanka standerd, as the permissible limit for acid insoluble ash is 1.5% (Srilanka Standard 643:2007). This value can be considered as indicator for consumer digestibility.

In this study peroxide value which is a primary indicator of oxidation of fat (rancidity) ranges between 1.56 to 25.09 meq/kg. Connell (1995) reported that when peroxide value is above 10-20, fish develop rancid taste and smell. According to Chakrabarty (2003) Peroxide values of fresh oil are less than 10 milliequivalents per kg, when the peroxide value is between 30 and 40 milliequivalent per kg rancid taste is notisible.

Thus, the peroxide values obtained from this study indicated the beginning of spoilage in most of the samples. The peroxide value of samples taken from net and landing site of Bodhban were below 10meq/kg i.e. permissible for human consumption. Fish contain high amount of unsaturated fatty acid which are liable to oxidize with atmospheric oxygen and form peroxy radicals. This is the induction process and can be induced by high temperature, prooxidants (fe^{++},Cu^{+}etc.), oxidative enzymes, sunlight etc.

High peroxide content, in present study was an indicator of storage problem or some technical problem during present proximate analysis. It also indicated that fat was oxidized to become highly rancid when stored at normal condition.

6. CONCLUSION AND RECOMMENDATION

6.1 Conclusion

The raw fishes had to pass through different stages before reaching the fish market as shown in figure 1. At every step, there is risk of original quality deterioration of fish, which are accelerated by practices employed during handling the fish and needed to be corrected.

The presence of bacteria like *Salmonella* species harmful to the man generally indicates poor sanitation in handling and processing. The contamination is almost of human or animal origin. The total microbial load is the indicative of possible contamination and very poor hygienic condition. In present study the high value of microbial load was unexpected. This might be due to delayed microbial analysis done after few days of sample collection.

High peroxide content, in present study was an indicator of storage problem or some technical problem during present proximate analysis. It also indicated that fat was oxidized to become highly rancid when stored at normal condition.

Average ash percent, insoluble ash percent and moisture content in all fish sample were in acceptable range for human digestibility. The high moisture content is responsible the growth of the microorganism. Present study showed the contamination of *Salmonella* pathogenic bacteria due to the sanitation and hygiene problem associated at every stage of handling, transportation and storage.

6.2 Recommendation

These recommendations drawn from the present study was intended to supply high quality fishes by fisherman, suppliers, wholesaler or retailer in the market:

- After the nets are hauled, fish should be immediately removed from the nets. Prolonged time in nets leaves marks on the skin and hence affects its physical quality attributes.
- Fish should not be thrown around as this practice can damage the fish.
- Fish catch should not be kept in dirty plastic sheet, or jute bag on the landing site.

- Landing site must be free from contamination like human and animal faeces.
- Fish should be properly iced. Ice should be used to chill the fish as soon as possible after landing to preserve their fresh flavor by slowing down the rate of chemical and bacterial spoilage.
- An ice to fish ratio of 1:1 and 1:2 is recommended for tropical and temperate areas respectively, though the amount eventually would depend on experience. Begin with a layer of ice at the bottom, followed by a layer of fish that alternates with a layer of ice until the hold is appropriately filled, ending with a layer of ice on top. All spaces between the fish must be filled with ice.
- Ice used for chilling must not be dirty but safe.
- Cleanliness is an important part of good handling. Fish container and surface of vehicles where fish are directly kept during transportation should be cleaned with suitable disinfectant like chlorinated water, Teepol quaternary ammonium compounds etc or with suitable detergent to avoid contaminating ice and fish
- fishes kept in the market for sale should avoid direct exposure to the air and sun as it increases the chances of contamination by insects, flies or air borne pathogenic bacteria
- Most fish shops in the market lack sanitary and hygiene practices. Clean hygienic water should be used to wash the equipment, fish surface, surrounding etc
- Microbial pathogen contamination found as major problem in present study as a serious warning signal for human consumption. Therefore important majors needed to be taken to train local people in hygienic practices.

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APPENDIX 1
ANALYSIS METHOD

1.Sensory method

1.1 Material

- Samples: whole fish and fish fillets
- Nylon sheet: 1 unit
- Table for evaluation: 1 unit
- Sticky paper for coding: 1 unit
- Evaluation form: 3 forms

1.2 Method

2 fishes were taken from the box and put into the PE bag the sample coded. (Then the 2 samples are taken to the sensory room, put on the table and coded). Three people from the fellow group carried out the sensory evaluation on whole fish.

Appearance of skin, firmness of flesh, slime formation, colour and form of eyes and finally colour, smell and mucus formation of gills was evaluated according to the Quality Index Method (QIM).

1.3. Sensory form

Table 9: Quality assessment scheme used to identify the quality index demerit score (Larsen *et. al;* 1992)

Quality parameter	Character	Score (ice/seawater)
General appearance	Skin	0 Bright, shining 1 Bright 2 Dull
	Bloodspot on gill cover	0 None 1 Small, 10-30% 2 Big, 30-50% 3 Very big, 50-100%
	Stiffness	0 Stiff, in rigor mortis 1 Elastic 2 Firm 3 Soft
	Belly	0 Firm 1 Soft 2 Belly burst
	Smell	0 Fresh 1 Neutral 2 Musty/Sour 3 Stale meat/Rancid
Eyes	Clarity	1 Clear 1 Cloudy
	Shape	0 Normal 1 Plain 2 Sunken
Gills	Colour	0 Characteristics, red 1 faded, discolored
	Smell	0Fresh, seaweed/metallic 1 Neutral 2 Sweaty/slightly rancid 3 Sour stink/Stale, rancid
Sum of scores		(min. 0 and max. 20)

APPENDIX 2

Microbiological Method

2.1 Preparation of culture media

Different types of culture media were used. Composition and preparation of different types of culture media are given below.

I.Total plate count agar(TPCA)

The total plate count agar(TPCA) is used for the enumeration of bacteria in food and water.

Composition:

Ingredients	Gms./litre
Tryptone	5.0
Yeast	2.5
Dextrose	1.0
Agar	15.0
Final pH(at 25^0C)	7.4 ± 0.2

Direction for preparation:

Suspend 23.5 grams of TPCA in 100ml distilled water. Boil to dissolve the medium completely. Sterilize at 15 lbs pressure (at 121^0C) for 15 minutes.

II.Salmonella-Singella Agar

SS agar is selective medium used for isolation of Salmonella and Shigella, So it is essentially a modification of deoxycholate citrate agar, described by Leifson. A bile salt mixture replaces deoxycholate for inhibition of coliform organisms and gram-positive bacteria. It can also differentiate lactose fermenting from non-lactose fermenting.

Composition:

Ingredients	Gms./litre
Fish extrate	5.0
Peptone	5.0

Lactose	10.0
Bile salts	8.5
Sodium thiosulphate	8.5
Sodium chloride	10.0
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	15.0
Final pH (at 25^0c)	7.4 $\pm$0.2

Direction for preparation:

Suspend 63 grams in 1000ml distilled water. Heat to boil with frequent agitation to dissolve the agar completely. Do not autoclave. Cool to about 500C and pour into petridishes.

III.Brilliant Green Agar:

Brilliant Green Agar is highly selective media for the isolation of *Salmonella* other than *Salmonella typhi* and *Singhella* species.

Composition:

Ingridients	Gms./litre
Proteose peptone	10.0
Lactose	10.0
Yeast extract	3.0
Sodium chloride	5.0
Sucrose	10.0
Phenol red	0.08
Brillient green	0.0125
Agar	20.0
Final pH (at 25^0C)	69-+0.2

Direction for preparation:

Suspend 58 grams of Brilliant green agar in a litre of distilled water.It was dissolved completely and sterilized at 15 lbs (121^0C) for 15 minutes.It was then cooled to 50^0C and poured aseptically into sterile petriplates and allowed to solidify.

IV.Nutrient broth

Composition:

Ingredients	Gms./litre
Peptone	5.0
Sodium chloride	5.0
Fish extract	1.5
Yeast extract	1.5
Agar	15.0
Final pH(at 250C)	7.4±0.2

Detection for Preparation:

Suspend 28 grams of powder in 1000ml distilled water and then boil to dissolve completely.Then sterilize the medium by autoclaving at 1210C (15 lbs pressure) for 15 minutes.

V.Selenite F enrichment broth:

Selenite F enrichment broth is used as enrichment medium for members of salmonella groups and few species of Shigella groups, when isolating these organisms from foods, dairy products etc.

Georgala and Boothroyd found that this medium is more selective for the isolation of Salmonella from foods when incubated at 43^0C instead of 37^0C. Proteus and paracolon bacteria are inhibited at 43^0C in this medium.

Composition:

Ingredients	Gms./litre
Part A	
Tryptone	5.0
Lactose	4.0
Sodium phosphate	10.0
Part B	
Sodium acid selenite	4.0
Final pH (at 250C)	7.4±0.2

Direction for preparation:

Suspend 19 gram of part A and 4gram of part B in 1000 ml distilled water. Warm to dissolve and mix well. Dispense and sterilize in a boiling water bath or in free flowing steam for 10 minutes. Do not autoclave, excessive heat is detrimental.

2.2 Preparation of biochemical test media and reagents

I. Indole test:

Indole test is done to determine the ability of an organism to split indole from tryptophan molecule with the help of various intracellular enzymes collectively known as "Tryptophanase". Indole is then tested by a colorimetric reaction with P-dimethylaminobenzaldehyde.

Medium

Peptone (containing tryptophan)	20gm
Sodium chloride	5gm
Distilled water	1000ml
pH	7.4

Dispense and sterilize by autoclaving at 121^0C for 15 minutes.

Kovac's Reagent

Amyl or Isoamyl alcohol	150ml
p-dimethylaminobenzaldehyde	10gm
Conc. Hydrochloric acid	50ml

Dissolve the aldehyde in the alcohol and slowly add the acid. Prepare in small quantities and store in the refrigerator, shake gently before use.

Procedure:

Inoculate the medium and incubate for 48 hours at 370C. After incubation add about 0.5ml Kovac's reagent and shake gently. A red colour in the alcohol layer (a red ring) indicates a positive reaction.

II. Citrate Utilization Test:

Citrate Utilization test is done for the ability of certain organism to utilize citrate as the sole source of carbon and energy source for the growth. The ability to use citrate can be used to differentiate among the members of the Enterobacteriaceae.

Medium	Gms./lit
Magnecium sulphate	0.2
Sodium Citrate	5.0
Ammonium dihydrogen phosphate	1.0
Potassium dihydrogen Phosphate	1.0
Sodium Chloride	5.0
Agar	20.0
Bromothymol blue (0.2%)	40ml

Dispense, autoclave at 121^0C for 15 minutes and allow setting up slopes.

Procedure:

Since the reaction required oxygen, the organism was inoculated to the surface of the agar slant of the medium. A very light inoculums was picked up with straight wire (to prevent false positive reaction) and streaked over slant, and incubated for 24 hours at 37^0C with loose cap.

Growth of organism on the slant with reversion of the color indicator from green to blue was evidence of positive test, indicating that the organism was able to grow and produce acetate and other alkaline carbohydrates and products.

Note: There may be rare citrate positive organism that can utilize the substance without producing enough alkaline reaction to change the pH indicator.Luxuriant growth on the slant without a blue color may indicate the positive test, but the test should be repeated with minimal inoculum.

III.Triple Sugar Iron Agar(TSI) Slant

Medium	Gms./litre
Peptone	10.0
Tryptone	10.0
Yeast extract	3.0
Fish extract	3.0

Lactose	10.0
Saccharose	10.0
Dextrose	1.0
Ferrous sulphate	0.2
Sodium Chloride (NaCl)	5.0
Sodium thiosulphate	0.3
Phenol red	0.024
Agar	12.0
pH(at 25^0C)	7.4±0.2

Procedure:

65 gram was dissolved in 1000 ml distilled water and boiled to dissolve the medium completely, Which was then sterilized by autoclaving at 15 lbs pressure (121^0C) for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of long.

2.3 Biochemical tests used for identification of bacteria:

After obtaining pure culture, colony was passed into nutrient broth and incubated at 370C for 4 hours, then inoculated into different biochemical media for different biochemical tests as given below.

For each set of biochemical test, both positive and negative control strains were inoculated and one uninoculated media was also incubated and examined along with the test.

The following tests were done:

I. Indole Production test (Mc Cartney, 1989):

This test demonstrates the ability of certain bacteria to decompose the Amino acid Tryptophan to Indole that accumulates in the Medium. Test tubes with SIM medium sulphide-indole-motility were stabbed with fresh culture from Nutrient Broth and then incubated at 37^0C for 48 hours. After incubation 2-3 drops of Kovac' s reagent was added and the appearance of red color in the alcohol layer indicates a positive reaction.

II. Motility test (Benson, 1989):

This test is to determine either the organism is motile or non motile. The tubes with the SIM medium were stabbed with fresh culture of the test organism and incubated at 37^0C for 24 to48 hours, and then the tubes were observed. Positive test shows that the organisms migrate from stab line and diffuse throughout the medium causing turbidity.

III. Citrate Utilization test (Mc Cartney, 1989):

This is the test for the ability of an organism to utilize citrate as the sole carbon and energy source for the growth and an ammonium salt as the sole source of nitrogen. Simmon's citrate medium slants were prepared and the organisms was inoculated and incubated at 370C for 48 hours. After inocubation, the change in color of the indicator from green to blue indicate positive results.

2.4 Enumeration of microorganisms

After 24 hours incubation, all the colonies on the dishes containing 30-300 colonies were counted.

Calculation:

The following standard rules were followed during the calculation of colonies.

(a) When the dishes examined contained no colonies, the result was expressed as zero bacteria per gm/ml or mold/yeast per gm/ml.

(b) When the dishes (dilution 1 in 10) contain less than 30 colonies and no other plates of the sample contain colonies then it was counted and the result was expressed as the (number of colonies) CFU of bacteria or Yeast/mold per gm/ml.

(c) When the colonies were more than 30, in more than one plate, the colonies in the plates were counted and the average was counted, retaining only two significant digits and multiplied by the inverse of the corresponding dilutions to obtain the number of bacteria per gm/ml or mold/yeast per gm/ml.

APPENDIX 3

PROXIMATE ANALYSIS

3.1 Moisture content by hot air oven method

I. Material

-Grinded fish sample (5gram) - petri dish or aluminium can

-Weighing arrangement (electrical balance) -Desiccator

-Hot air oven

II. Method

5 gram of grinded fish sample is taken in clean and dried aluminium can and is weighed. The sample is than placed in hot air oven set at1000c.The difference in the weight of plate is noted every hour until two consecutive weights differ only by +-5mg.Before each weighing, the can or dish is cooled in desiccator.

3.2 Calculation

$$\text{Moisture content},\% = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

3.2 Determination of total ash by dry ashing method:

I. Material

-Food sample -Silica crucible

-Muffle furnace -Electronic balance

-Desiccator

II. Method

Silica crucible is washed with water, HNO_3 and distilled water and is dried in hot air oven at temperatures above 150c for half an hour than it is cooled in a desiccator and is weighed. 3 gram of grinded fish sample is taken in the crucible and is chared over a low Bunsen flame to volatilize as much of organic matter until no more smoke is given out by the material as possible. Than the crucible is transferred to the temperature controlled

muffle furnace with the help of long tongs. Furnace is kept at 3000c until the carbon has ceases to glow and then temperature is rose to 5000c. Ashing is continued for 3-4 hours. The furnace is turned off and crucible is allowed to cool around 2000c to see whether the traces of black residue is left or not, which is the indicator of incomplete ashing. If carbon is present about 1-2ml of conc HNO_3 is added and is evaporated and ashing is done again for an hour. The furnace is turned and crucible is cooled slowly in desiccators and is weighed.

III. Calculation

Total ash=Ash(g) x100/Sample(g)

Total ash %=Ash(g)x100x100/Sample(g)xDry matter%

3.3 Determination of acid insoluble ash

I. Material

-Extracted ash sample
-10% Hcl solution
-Muffle furnace
-Whatman filterpaper
-Heating arrangement
-Filtration assembly

II. Method

The ash prepared by dry ash is taken in crucible and 25ml of 10% Hcl solution is added in it and is covered with watch glass and is boiled gently over a low flame for about 5 minutes. It is than filtered quantitatively through ash-less filterpaper. The filter paper with residue is kept in the crucible. The contents is ignited in the Muffle furnace, and is cooled and weighed.

3.4 Calculation

Acid insoluble ash (% wet basis) =Acid insoluble ash (g) x100/Sample (g)

3.5 Extraction of fat by solvent extraction method:

I. Material

-Petrolium ether sample
-Soxhlet apparatus
-Desiccator
-Heating arrangement
-Sample (5 gram of grinded fish sample)
-Acetone
-Timble
-Balance

II. Method

1. About 250 gram of fish sample is grinded into fine particles in Mortar and Pestle. The thimble is stuff with 3 gram of ground sample and remaining space is packed with fat free cotton wad. The timble is than kept slowly in upright position in the reflux (fat extraction tube) and the reflux is fitted in the receiver flask. The solvent (Petroleum ether) is poured slowly onto the sample until the solvent starts siphoning to the receiver. The condenser is connected on the top of the reflux tube and the tap water is opened to run it. Heat is on and temperature is adjusted. The solvent vaporizes, condenses to the extraction tube, and siphons down after the volume of collected solvent reaches a critical level. During the residence of the solvent in the reflux (extraction tube) the fat from the sample gets slowly extracted. The extracted fat siphon down to the receiver along with the solvent. Because of continuous boiling, the solvent soon begins to vaporise and the fat free vapour begins to condense once again into the reflux. It is done for about 6 hours. More solvent is added to compensate the loss of solvent that might occurs during the prolonged boiling. After the extraction is completed, the last siphoning is allowed for emptying the reflux. Immediately the heat is turn off and the apparatus is removed from heating arrangement. The timble is taken out and apparatus is reset and is heated to allow one more siphoning. The solvent is collected in the reflux without allowing the solvent to siphon down, so most of the solvent is removed from the extracted fat. At last the apparatus is separated and the solvent from the extracted fat is evaporated in hot air oven as completely as possible, keeping the temperature of the heater below 1000c. After this about 5ml of

acetone is added and is evaporated and the receiver is cooled in desiccators and weighed it. This extracted fat is used for determining the peroxide value.

3.6 Determination of peroxide value by Iodometric titration method

I. Material

-Extracted oil
-Acetic acid-chloroform solvent
-Saturated potassium iodide
-0.01N and 0.1N sodium-thiosulphate
-Measuring cylinder: 25ml cap
-Iodine flasks:250ml cap
-Burette:25-50ml cap
-Pipette 25ml cap
-0.05% starch indicator
-Weighing arrangement

II. Method

The extracted fat is taken in the Iodine flask and 25ml of solvent is added in it .The air is displaced with co_2. 1ml of KI solution is added and is allowed to stand for 1 min. About 35ml of distilled water and a few drops of starch indicator is added .Blue colour appears on addition of starch indicates presence of free iodine. Liberated iodine is titrated with 0.01N or 0.1N sod.thiosulphate until the blue colour just vanishes. The blank determination is carried out simultaneously.

Peroxide value is calculated by using following equation:

$$PV(meq/kg)=\frac{N\ (V_S-v_{B)x1}}{W\ .O\ \ St\qquad (g)}$$

Where,

N=normality of sod-thiosulphate, V_s=sod-thiosulphate consumed by sample(ml),and V_B=sod-thiosulphate consumed by blank(ml).

APPENDEX-4

Questionnaires for fresh fish harvesting to marketing.

Name: Sex:

Address:

1. How many ponds do you have?

1. What is the method applied to harvest fish from fish pond?
2. How many fish species are cultured and what are they?
3. What types of gears are used to harvest fish from pond?
4. Are the gear washed before and after fish harvesting?
5. Are the gears disinfected with any type of disinfectant, such as potassium permagnet?
6. How the gears are stores after fish harvesting?
7. Where the fish is landed after harvesting?
8. Is there any provision of ice to preserve the fish after harvesting at landing sites?
9. How long the harvested fish are kept in the landing side until transport to market or preservation?
10. How far is the market from the pond distant (in hour).
11. What type of container is used to carry harvested fish from ponds to the nearby market or storage?
12. What type of transportation is used to transport fish to short distance market?
13. How the fish are processed for marketing?
14. How the fish is preserved during transportation to short distance?
15. How the fish are packed for long did\stance market, from Gurvu Shah's fish collection site to Kathmandu, Pokhara, Malekhu etc?
16. What transportation is used to transport fish to long distance market (from Gurvu Shah's fish collection site to Kathmandu, Pokhara, Malekhu etc)?
17. How the fish are preserved after arrival to fish market at Kathmandu before processing?
18. How the fresh fish are preserved in the market for marketing at Kathmandu?
19. How long the fish are kept without ice for marketing in a shop (in hours)?