

Study on the Micro-flora and Chemical Constituents of Preserved Fishes Available in Kathmandu Market



**A Dissertation Submitted to the
Institute of Science and Technology
Central Department of Zoology
Tribhuvan University
For the partial Fulfillment of Master of Science in Zoology**

**BY
PRAFULLA JOSHI**

**Central Department of Zoology
Tribhuvan University, Kirtipur
Kathmandu**

2007

LETTER OF RECOMMENDATION

It is my pleasure to mention here that Ms. Prafulla Joshi has carried out the dissertation entitled "**Study on the Micro-flora and Chemical Constituents of Preserved Fishes Available in Kathmandu Market**" under my supervision and guidance. It is the original work of the candidate and incorporates the discovery of new facts and fresh approach towards their interpretations. Therefore, I strongly recommend the dissertation for the partial fulfillment of the Master of Science in Zoology.

Prof. Dr. Jiwan Shrestha

(Supervisor)

Central Department of Zoology

T.U., Kirtipur

Kathmandu, Nepal

Dr. Kayo Devi Yami

(Co-supervisor)

Senior Scientist

NAST, Khumaltar

Lalitpur, Nepal

Date: 2064-01-04

April 17, 2007

LETTER OF APPROVAL

This letter is to certify that the dissertation presented by Ms. Prafulla Joshi entitled **"Study on the Micro-flora and Chemical Constituents of Preserved Fishes Available in Kathmandu Market"** has been accepted for the partial fulfillment of the Master of Science in Zoology.

Evaluation Committee:

Prof. Dr. Jiwan Shrestha

(Suervisor)

Central Department of Zoology

Tribhuvan University

Kirtipur

Kathmandu, Nepal

Dr. Kayo Devi Yami

(Co-Supervisor)

Senior Scientist

NAST, Khumaltar

Lalitpur, Nepal

Prof. Dr. Tej Kumar Shrestha

(Head)

Central Department of Zoology

Tribhuvan University

Kirtipur

Kathmandu, Nepal

External Examiner

Date: 2064-01-04

April 17, 2007

ACKNOWLEDGEMENTS

I would like to extend my sincere thanks to my supervisor, Prof. Dr. Jiwan Shrestha, Central Department of Zoology, T.U., Kirtipur, for her kind guidance throughout the research work.

I am very much grateful to my co-supervisor Dr. Kayo Devi Yami, Senior Scientist, NAST (National Academy for Science and Technology) for not only providing me with all the necessary laboratory facilities such as chemicals and equipments required for the study of micro-flora of fish but also helping and guiding me throughout the laboratory work.

I would also like to thank Prof. Dr. Tej Kumar Shrestha, Head, Central Department of Zoology, T.U., Kirtipur for his valuable suggestions.

I am very much indebted to the Dr. Amriteshwari Rajbhandari, Former Director, Central Food Research Laboratory, Babarmahal for providing necessary laboratory facilities for the bio-chemical analysis of fish.

Thanks are also due to Mrs. Tista Sharma, NAST, for helping me to identify different species of bacteria isolated during the microbial analysis. My sincere thanks go to Mr. Pramesh Lakhey (Former Research Assistance, NAST) and Ms. Trishna Manandhar (Research Assistance, NAST) and my friends of Micro-biology Department, Tribhuvan University: Bina, Anup, Olivia, Kamil, Dinesh, Srijana, and Junu for their help. I am also very much obliged to the staffs of NAST especially Mr. Pramod Shrestha for his timely cooperation.

I would also like to thank the staffs of Central Department of Zoology, Tribhuvan University for their help.

I would like to express my sincere thanks to Mr. Sandesh Malla for supporting me through out the research period and also helping me with the photographs. I am very

much thankful to my elder brother Mr. Sagar Krishna Joshi for printing all the photographs that are included in this work and my younger brother Mr. Shirish Joshi for his help during the preparation of this thesis.

Last but not the least; I would like to thank my parents who gave me unconditional support, help and encouragement throughout the study period and preparation of this present dissertation.

Prafulla Joshi
Examination Roll no: 539
Registration no: 5-1-256-46-97
Batch: 2060\61



ABSTRACT

Preserved fish are quite popular among the people of Kathmandu. There are many shops in Kathmandu and Lalitpur where preserved fish are sold. These fish are available in four different forms, viz, ice-preserved or chilled, salted, sun-dried and smoked. Chilled fishes include Rohu (*Labeo* spp.), Carps, Cat fishes etc. Salted fish include *Harpadon nehereus*, sun-dried fish include *Puntius* spp., *Oxygaster* spp. etc. and smoked fish include Naini (*Cirrhinus mrigala*), *Schizothorax* spp., *Garra gotyla*, *Tor* spp. etc. Most of these fish are supplied from different parts of India such as Andhra Pradesh, Bombay while some are brought from different parts Nepal as well. The salted and sun-dried varieties sell in cheaper price than smoked ones. The hygienic condition of fish markets is quite poor which make these fishes susceptible to micro-flora contamination. The micro-flora contamination also depends upon the process followed during the preservation. The bacterial test revealed that in smoked fish (*Cirrhinus mrigala*) the bacteria isolated are *Pseudomonas*, *Micrococcus* and *Staphylococcus*. In salted fish (*Harpadon nehereus*) colonies of Enterobacteriaceae spp. such as *Salmonella* spp. were isolated. Similarly, in sun-dried fish (*Oxygaster* spp.) also species of *Salmonella* and *Escherichia coli* were isolated. Besides bacteria the preserved fish were also subjected for fungi culture. Two different types of fungal colonies were isolated. In sun-dried fish colonies of *Trichoderma* spp. and *Aspergillus fumigatus* were isolated. In case of salted fish colonies of *Trichoderma* spp. are isolated. The chemical analysis of fish revealed that the protein content of *Cirrhinus mrigala* (smoked fish) is 70.78 percent whereas that of *Harpadon nehereus* (salted fish) is 62.15 percent. Similarly, the protein content of *Oxygaster* spp. (sun-dried fish) was found to be 64.93 percent. The fat content of fish flesh is the factor that determines the quality of fish and hence its price. The fat content of smoked fish, *Cirrhinus mrigala* was highest among the three. It was about 7.23 percent whereas that of *Harpadon nehereus* (salted) and *Oxygaster* spp. (sun-dried) were 6.03 and 4.21 per cent respectively.

CONTENTS

Chapter I

INTRODUCTION	1- 5
A. General Background	1
B. Fish Spoilage	3
C. Fish Micro-flora	3
D. Fish Preservation	4
Justification	4
Limitation	5

Chapter II

OBJECTIVES	6
-------------------	----------

Chapter III

LITERATURE REVIEW	7-11
--------------------------	-------------

Chapter IV

MATERIALS AND METHODS	12-30
A. Study Area	12
B. Market Survey	12
C. Sample Collection	12
D. Microbial Analysis	13
I. Bacterial Culture	13
i. Media preparation	13
ii. Serial Dilution	14
a. Materials required for Serial Dilution	14
b. Procedure in Serial Dilution	15
iii. Pour plate and Streak Plate	16
a. Materials required	16

b. Procedure Involved	16
iv. Colony Count	17
v. Sub Culture	17
II. Identification of Isolated Bacteria	18
i. Gram stain	18
a. Differentiation of Gram-positive and gram-negative cell wall	18
b. Procedures involved in Gram-staining	18
ii. Biochemical tests	19-22
a. Catalase Test	20
b. Oxidase Test	20
c. Methyl Red and Voges-Proskauer (MR-VP) Test	21
d. Indole Test	21
e. OF Test (Oxidative\Fermentation)	21
f. Urease Test	22
g. Citrate Utilization Test	22
h. SIM (Sulphide-Indole_Motility) Test	22
i. Triple Sugar Iron(TSI) Test	22
III. Fungal Culture	25-27
i. Media Preparation	25
ii. Serial Dilution	26
iii. Pour Plate	26
iv. Colony Count	26
v. Sub-culture	26
vi. Preparation of Slides	26
a. Materials required	27
b. Procedure involved	27
E. Chemical Analysis	27-31
I. Determination of Protein	27
a. Materials required	28

b.	Procedure involved	28
	II. Determination of Fat	29
a.	Materials required	29
b.	Procedure involved	29

Chapter V

OBSERVSTIONS AND RESULTS 31-34

A. Fish market survey 31

I. Hygienic condition of fish market 31

II. Preserved fishes available in the markets of Kathmandu 32

a. Chilled fishes 33

b. Smoked fishes 33

c. Sun-dried fishes 33

d. Salted fishes 34

B. Microbial Analysis 35

I. Isolation of Bacteria 35

i. Identification of Gram negative bacteria 37

ii. Identification of Gram positive bacteria 39

II. Isolation of Fungi 30

C. Chemical Analysis 40

Chapter VI

DISCUSSION 44-51

Chapter VII

CONCLUSION 52-53

Chapter VIII

RECOMMENDATION

54

REFERENCES

55-60

APPENDICES

61

Appendix 1

62

Appendix 2

64

Appendix 3

73

LIST OF TABLES

Table-1	Gram Satin Procedure	19
Table-2	TSl reaction*	23
Table-3	List of different types of preserved fishes found in Kathmandu and their sources	34-35
Table-4	Bacteria isolated from the head and body regions of sun-dried fish. (<i>Oxygaster</i> spp)	36
Table-5	Bacteria isolated from the head and body regions of salted fish (<i>Harpadon nehereus</i>)	36
Table-6	Bacteria isolated from the head and body regions of Smoked fish (<i>Cirrhinus mrigala</i>)	36-37
Table-7	Result of biochemical tests performed to identify Gram-ve bacteria isolated from sun-dried fish (<i>Oxygaster</i> spp.)	39
Table-8	Result of biochemical tests performed on Gram-ve bacteria isolated from salted fish (<i>Harpadon nehereus</i>)	40
Table-9	Result of biochemical tests performed on Gram-ve bacteria isolated from smoked fish (<i>Cirrhinus mrigala</i>).	41
Table-10	Gram positive bacteria isolated from the head and body regions of smoked fish (<i>Cirrhinus mrigala</i>)	42
Table-11	Fungi isolated from the head and body regions of salted fish (<i>Harpadon nehereus</i>) and Sun-dried fish (<i>Oxygaster</i> spp.)	42
Table-12	Protein Content in Smoked, Salted and Sun-dried fish	43
Table-13	Fat Content in Smoked, Salted and Sun-dried fish	43

Photo Plates 1-4

- Fig. 1** Different type of preserved fish on display at Mangal Bazar, Lalitpur.
- Fig. 2** Woman selling different types of smoked-fish at Asan Bazar, Kathmandu.
- Fig. 3** View of different types of chilled fish ready for sell at Lagankhel, Lalitpur.
- Fig. 4** A boy chops fish for his customer at Lagankhel, Lalitpur.

Snaps taken during survey work



Fig. 1 Different types of preserved fish on display at Mangal Bazar, Lalitpur.



Fig. 2 Woman selling different types of smoked-fish at Asan Bazar, Kathmandu.



Fig. 3 View of different types of chilled fish ready for sell at Lagankhel, Lalitpur.



Fig. 4 A boy chops fish for his customer at Lagankhel, Lalitpur.

Photo Plates 5-8

- Fig. 5** Live marine crabs ready for sell in basket at Ranamukteshore
- Fig. 6** Shopkeeper being interrogated at Asan
- Fig. 7** Congested fish market at Lagankhel
- Fig. 8** Preserved fish sold along with various other items at Asan



Fig. 5 Basket with live marine crabs for sell at Ranamukteshore.



Fig. 6 Shopkeeper being interrogated at Asan.



Fig. 7 Congested fish market of Lagankhel.



Fig. 8 Preserved fishes sold along with various other items.

Photo Plates 9-11

- Fig. 9** Sun-dried fish (*Oxygaster* spp.)
Fig. 10 Salted fish (*Harpadon nehereus*)
Fig. 11 Smoked fish (*Cirrhinus mrigala*)

Selected fish species for microbial analysis



Fig. 9 Sun-dried fish (*Oxygaster* spp.)



Fig.10 Salted fish (*Harpadon nehereus*)



Fig. 11 Smoked fish (*Cirrhinus mrigala*)

Photo Plates 12-15

Fig. 12 Pour plate showing mixed culture

Fig. 13 Pour plate showing mixed culture

Fig. 14 Streak plate

Fig 15 Streak plate

Pour plate and streak plate culture of bacteria

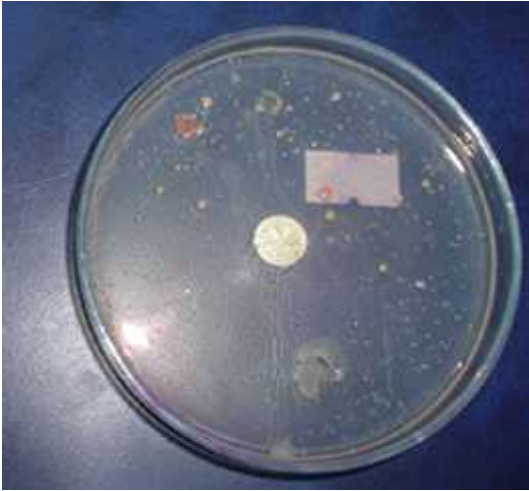


Fig. 12 Pour plate showing mixed mixed culture



Fig. 13 Pour plate showing mixed culture

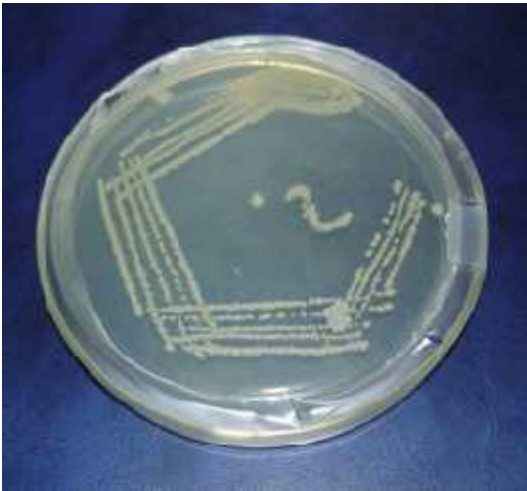


Fig.14 Streak plate



Fig.15 Streak plate

Photo Plates 16-18

- Fig. 16** Biochemical test results of *Salmonella* spp.
- Fig. 17** Biochemical test results of *Escherichia coli*
- Fig. 18** Biochemical test results of *Enterobacter*

Biochemical test results



Fig. 16 Biochemical Test Results of *Salmonella* spp.



Fig. 17 Biochemical Test Results of *Escherichia coli*

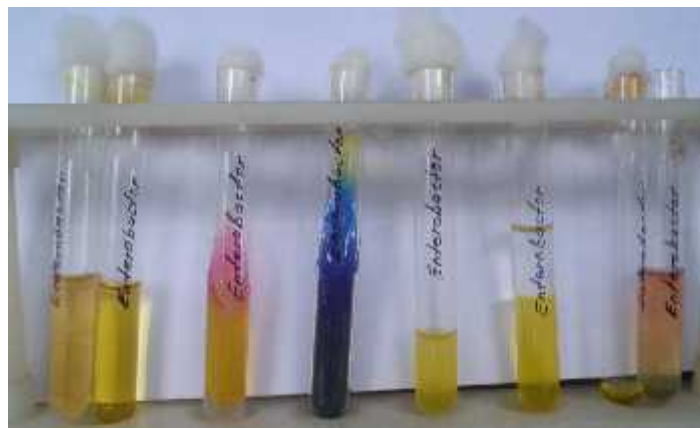


Fig. 18 Biochemical Test Results of *Enterobacter*

Photo Plates 19-22

- Fig. 19** *Aspergillus fumigatus*
- Fig. 20** *Trichoderma* spp.
- Fig. 21** Hyphae of *Aspergillus* as seen under microscope
- Fig. 22** Spores of *Trichoderma* as seen under microscope.

Culture plates and microscopic studies of fungi

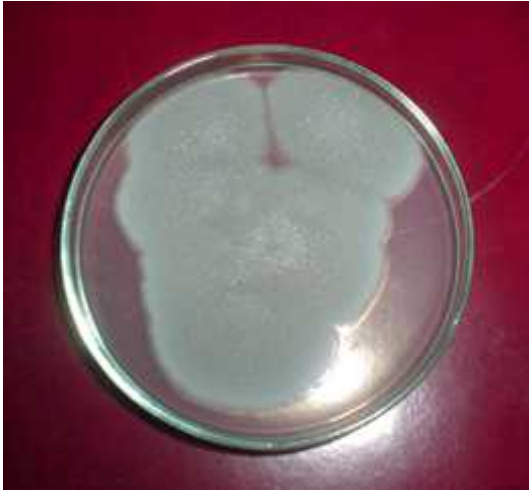


Fig. 19 *Aspergillus fumigatus*



Fig. 20 *Trichoderma* spp.



Fig. 21 Hyphae of *Aspergillus fumigatus* as seen under microscope.

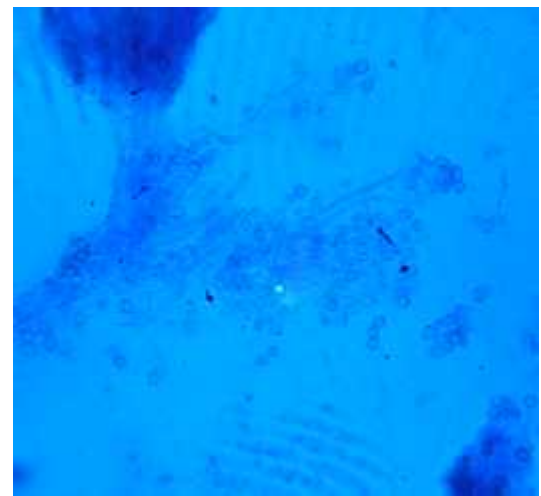


Fig. 22 Spores of *Trichoderma* as seen under microscope

ACRONYMS

FAO	Food and Agricultural Organization.	
IUCN	World Conservation Union	
UNESCO	United Nation Educational Scientific and Cultural Organization	
NAST	Nepal Academy for Science and Technology	