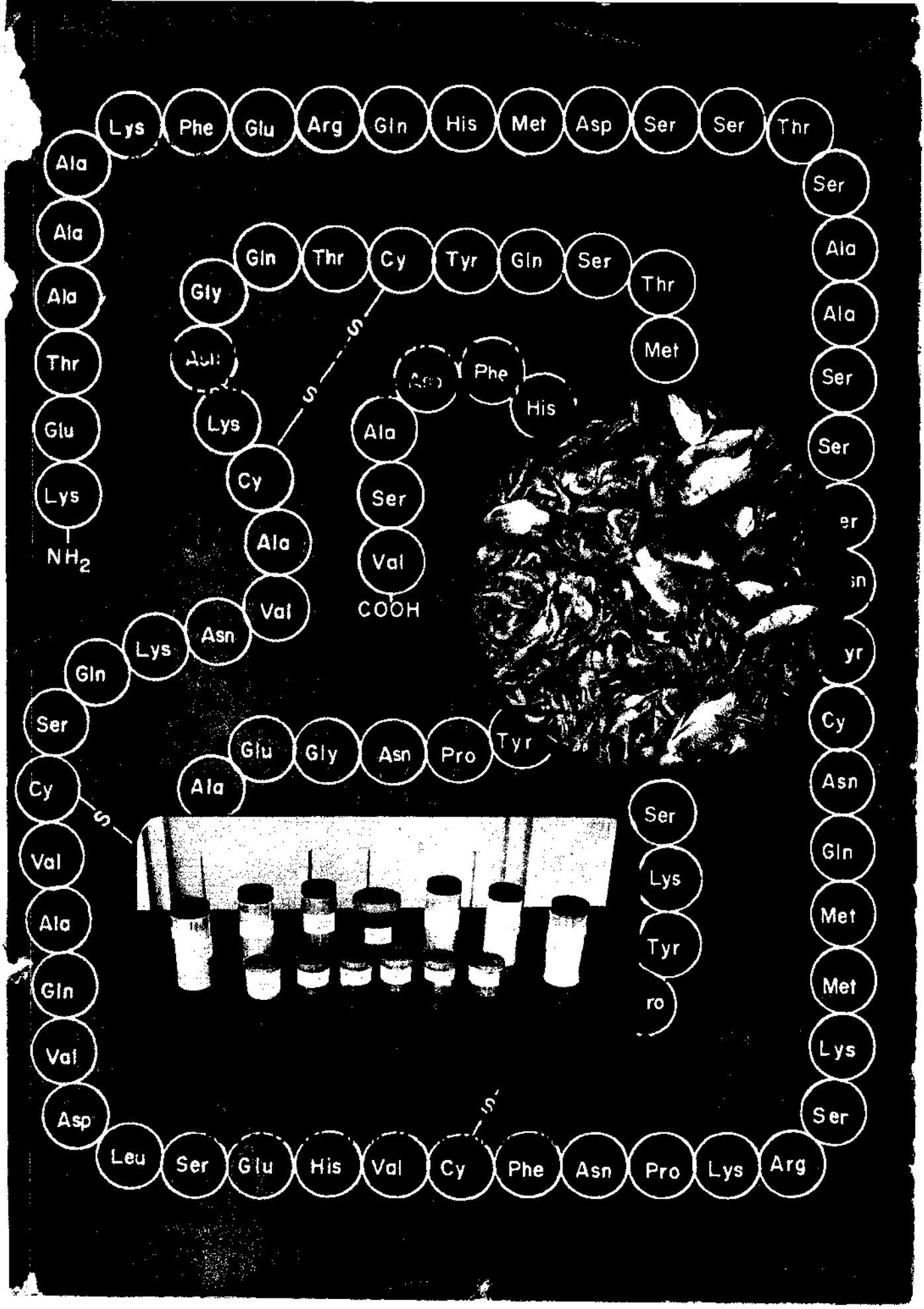
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Ala Ala Ala Thr Glu Lys NH2
Ser Ala Ala Ser Ser er in yr Cy Asn Gln Met Met Lys Ser
Gln Thr Cy Tyr Gln Ser Thr Met
Gly
Lys Cy Ala Val Asn Lys Gln Ser Cy Val Ala Gln Val Asp Leu Ser Glu His Val Cy Phe Asn Pro Lys Arg
Ala Ser Val COOH
Phe His
Glu Gly Asn Pro Tyr
Ala
Ser Lys Tyr ro

CMFRI SPECIAL PUBLICATION

Number 8

MANUAL OF RESEARCH METHODS FOR FISH AND SHELLFISH NUTRITION

Issued on the occasion of the Workshop on
METHODOLOGY FOR FISH AND SHELLFISH NUTRITION
organised by
The Centre of Advanced Studies in Mariculture,
Central Marine Fisheries Research Institute,
held at Cochin from 11 - 16 January 1982

Published by: **E. G. SILAS**
Director
Central Marine Fisheries Research Institute
COCHIN

PREFACE

The Centre of Advanced Studies in Mariculture established at the Central Marine Fisheries Research Institute has been conducting Workshops in Research Methodologies on specialised disciplines with a view to enhance the competence of the scientific workers specialising in researches connected with mariculture. The main emphasis in mariculture research has been directed towards the development of economically viable culture techniques for culturable species of fish and shellfish, with a view to augmenting the fish and shellfish production of the country. In order to develop low-cost technologies the essential operational inputs have to be rationally utilized.

It has been well established that feeding constitutes the major cost of production, often exceeding 50 per cent of the operating costs in intensive aquaculture operations. Two main factors affecting the cost of feeding are composition of the diet and efficiency of feed conversion. In order to develop least-cost formula diets of high conversion efficiency, knowledge of the nutritional requirements of the different species during the different phases of the life cycle and the nutritive value of the complex feed ingredients available in the country to the candidate species is a prerequisite.

The existing information on the nutritional requirements of cultivated species of fish and shellfish in India, is meagre and recently research has been intensified in this area. If researches on this field could be carried out using standardised experimental procedures, the data obtained on the nutritional requirements of the different species could be stored in a fish and shellfish nutrition data bank, from where data could be disseminated to the users such as feed manufacturers, farmers, extension workers and research workers as and when required. It is also necessary that the data collected on the chemical composition of the feed ingredients and their nutritive value for the species should be based on standard chemical methods and experimental procedures so that the data could be stored in

the data bank which eventually could become a National Fish Feed Information Centre.

To undertake studies on the above lines, especially by the technicians and research workers entering afresh into the field, the need of practical guides describing the research techniques and methods, planning of investigations, collection of data and their interpretation need not be emphasized. Keeping this in view, the present manual on Research Methods in Fish and Shellfish Nutrition is issued by the Centre of Advanced Studies in Mariculture on the occasion of the Workshop on Methodology of Fish and Shellfish Nutrition.

Dr. Akio Kanazawa, Professor of Nutritional Chemistry, University of Kagoshima, Japan and Consultant in Fish and Shellfish Nutrition at the CAS in Mariculture, has been kind enough to cooperate with the Scientists of CAS in Mariculture of the Central Marine Fisheries Research Institute in the preparation of this manual. There are chapters in this manual covering various methods on composition analysis of feeds, including growth inhibitors and toxins; determination of digestibility coefficient; protein evaluation; bioenergetics; determination of essential amino acid requirements using radioisotope method; research test diets for fishes and prawns; feed formulation methods; experimental design, etc. Methods of preparation of microparticulate diets, phytoplankton and zooplankton culture methods, etc. are also included to facilitate larval nutrition studies. Many of the methods given in the manual have been standardized for fish and shellfish nutrition studies in India and abroad. The users can also gain maximum benefit by suitable modifications of other methods which are given as guidelines.

I would like to thank all the scientific and technical staff especially Shri S. Ahamed Ali, Dr. K. Alagarswami, Shri D.C.V. Easterson, Shri C.P. Gopinathan, Shri T. Jacob, Shri M.S. Muthu, Dr. R. Paul Raj, Dr. A.G. Ponniah and

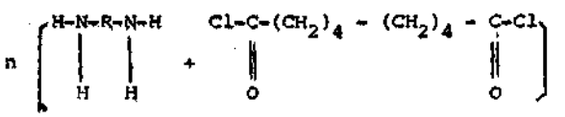
Dr. P. Vedavyasa Rao who have rendered assistance during the preparation of this manual. Thanks are also due to Shri Johnson, Librarian and Shri Kambadkar, Technical Assistant, Central Marine Fisheries Research Institute, for the help rendered by them in printing this manual.

(E.G. Silas)
Director, CMFRI,
Sub-Project Coordinator,
Centre of Advanced Studies in Mariculture

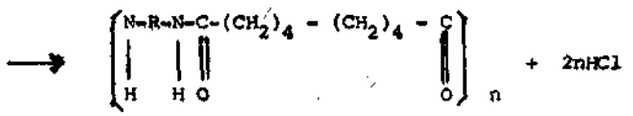
CHAPTER 15

PREPARATION OF MICROENCAPSULATED DIET*

1 Principle



Diamine Sebacoyl chloride



Wall structure

2 Reagent

(a) Diet ....e.g. chicken egg

(b) Diaminohexane solution ....0.92g of 1, 6-diaminohexane + 20 ml of 0.45 M $NaHCO_3$ - $NaCO_3$ buffer (pH 9.8)

(c) Mixed solvent solution ..... chloroform - cyclohexane (1 : 4)

(d) Span 85

(e) Sebacoyl chloride

(f) Sucrose monolaurate

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* Prepared by Akio Kanazawa, Professor of Nutritional Chemistry, Kagoshima University, Japan.

3 Procedure

Mixed solvent solution (25 ml) + Span 85 (0.5 ml)

↓

+ Diamine soln. (0.5 ml) + Diet Soln. (2.5 ml)
Emulsification for 3 min. by homogenizer
+ Mixed solvent soln. (10 ml) + Sebacoyl chloride (0.2 ml)
+ Mixed solvent soln. (30 ml)

Precipitate (Microencapsulated diet)

↓

Washed with mixed solvent soln. (100 ml)
2-3 times by decantation

Precipitate

↓

+ 7 ml sucrose monolaurate
stirring for 10 min.
Washing for 24 hr. in water (2 litre)
Filtration with cloth sack of 10 μm mesh
Wash with water (2 litre)
2 times by filtration

↓

Microencapsulated diet

store in 1 mol NaCl