77/17

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

FINAL REPORT OF PROJECT

EVALUATION OF THE FUNCTIONAL PROPERTIES OF FISH MUSCLE PROTEIN FOR SUBSEQUENT USE IN FOOD SYSTEMS

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ACKNOWLEDGEMENTS

The project investigators would like to thank Lindsay Gullifer for carrying out the technical and laboratory work on the project, and the Manager of the Food Science Pilot Plant, the technical officer and staff of the School of Food Sciences for their co-operation.

We would also like to thank Carole Pfeiffer and Connie Haggie for their assistance in typing and compiling the reports.

A. F. D'Mello,
Associate Investigator.

P. A. Baumgartner,
Principal Investigator.

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

FINAL REPORT OF PROJECT

1. Title: Evaluation of the functional properties of fish muscle protein for subsequent use in food systems.

2. Name of Institution: School of Food Sciences, Hawkesbury Agricultural College, Richmond N.S.W. 2753

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4. Date of Report: 10th February, 1981

5. Date Project Commenced: 1st July, 1977

6. Date Project Completed: 30th June, 1980

7. Funds Granted:

	1977/78	1978/79	1979/80
Total salaries and wages	$10,156	$9,890	$10,393
Total operating expenses	2,200	2,000	1,500
Total capital expense	-	-	-
	$12,356	$11,890	$11,893
Amount received	12,356	9,406.37	7,766.61

Total funds granted for 1977/1980 = $36,139
Funds actually received = $29,529

1. BACKGROUND INFORMATION

The seafood industry is a rapidly developing industry. Mechanised operations, increasing cost of raw materials, labour and equipment, and the rising standard of consumer living have led to greater and more efficient utilisation of primary production.

In Australia and to this date, very little fundamental, scientific or technical data is available, particularly the composition of Australian species of seafoods, their intrinsic and extrinsic qualities, which could be utilised by industry.

This project has set out to initiate, and indicate the type of work and information urgently required for the development of the seafood industry, particularly the efficient use of proteins from underutilised seafood species or recovered muscle protein from processing operations.

2. AIMS OF THE PROJECT

1. To collect fundamental data, essential for future research and industrial application, particularly data on the composition of Australian seafood species.

2. To develop methods, and equipment that could be used by industry for assessing the quality of fish muscle protein in food systems.

3. To evaluate the functional properties of seafood muscle protein.

4. To investigate the production of protein isolates for use in various food systems.

The importance of this concept being that recovered seafood muscle protein, from underutilised species or from waste obtained from processing operations, could be modified for use in general food systems.

3. SUMMARY OF RESEARCH FINDINGS

Part A The proximate composition for some of the Australian seafood species was evaluated. The species used were: gemfish, latchet, Nanaghai, Morwong, Mullet and prawns. Unlike the Northern Hemisphere fish species, the composition was not significantly different between species.

Part B Methods for evaluating emulsion capacity, nitrogen solubility index, viscosity (gelling) were standardised.

Part C Limited investigations were carried out to evaluate textural properties. An instrument for measuring texture was used for profile analysis. Various products were prepared and sensory tests and properties related to binding, gelling and foaming were evaluated.

Part D Protein isolates were prepared by various methods, which included freeze drying, calcium co-precipitation, alkaline treatment, and acid treatment. Of these, acid treatment was found to be the most suitable. Although the dry powder isolate lost some of the functional properties, particularly gelling and binding, the emulsion capacity and nitrogen solubility were still retained.

Part E The results from the nitrogen solubility index for the fish species examined, will be very useful, particularly for blending fish muscle and production of fish protein isolates. This is, perhaps, the first valuable information now available for these Australian species.

4. INDUSTRY SIGNIFICANCE OF THE PROJECT

1. The availability of data related to the proximate composition (percent moisture, fat, protein and mineral salts) will in the future be useful for the development and recommendation of procedures for handling and processing of seafoods.

2. The nitrogen solubility index will provide very useful information, particularly in the extraction of fish muscle protein, and production of fish protein isolates.

3. The data from the viscosity and emulsion capacity of fish muscle can be useful and applied to blending fish muscle of different species, and to predict the effect on viscosity and gelling particularly for the production of "Kamaboko" type products, or fish sausages.

4. Underutilised seafood species can be effectively used and blended with recovered muscle portions if the functional properties could be predicted. This is possible although to a limited extent through this research project.

5. Production of spray dried protein isolates by acid treatment will permit collection and storage of fish homogenates, under ambient temperatures and thus facilitate handling and transport from various fishing ports, and also allow for a centralised spray drying unit.

5. RESEARCH AREAS REQUIRING FURTHER RESEARCH

From the research work done for this project a few important findings indicate:

(a) the proximate composition of the species so far examined appears to show very small differences in the moisture, protein and fat content. These findings indicate that there is a need for the evaluation of the proximate composition, at different seasons and various stages of maturity.

(b) it would be useful to the processing industry if the nitrogen solubility index for the commercial seafood species is evaluated in relation to the proximate composition and maturity of the species.

(c) The Instron, Braebender Viscograph and the Internal Quality Analyser is equipment that could be used to evaluate and assess the textural and functional properties. Further investigations should be carried out not only to standardise methods but to study and assess the rheological characteristics of various species under different conditions.

(d) An important finding from this project is that hydrolysed fish slurries can be spray-dried, however, there are pre-drying problems, of which the most important is concentration of the slurry. Because of heat coagulation or freeze denaturation of proteins, it appears that membrane concentration might be the most suitable and effective method. Laboratory type experiments carried out on a limited scale, indicate that membrane concentration could be used. More research has to be done is this field.

6. EXPERIMENTAL WORK

Proximate Composition of Available Species

The proximate composition of seafood species under investigation is reported in Table 1. The edible muscle was used for the determination of moisture, protein, fat and ash. The composition was also evaluated for the species used for the determination of the nitrogen solubility index, emulsion capacity, viscosity and for the species used in the production of protein isolates.

The moisture content was determined by drying representative samples to constant weight at 105°C in an air oven (A.O.A.C. 1975).

The protein content was determined by macrokjeldahl using selenium as catalyst and a mixed indicator (Bromo-cresol green and methyl red). The protein calculated as N x 6.25 (A.O.A.C. 1975).

The fat determinations were carried out by Soxhlet using petroleum spirit (B.P. 60°C-80°C) as a solvent (A.O.A.C. 1975).

The ash content determined by ashing in a muffle furnace at 550°C for 16 hours (A.O.A.C. 1975).

Table 1 The proximate compositions of seafood species under investigation (edible muscle)

Seafood Species	Range/high-low ratio			
	Moisture %	Protein %	Fat %	Ash %
Gem fish	78-80	20-16	1.0-4	1.0-1.5
Flathead	77-79	19-21	0.1-0.5	1.0-1.5
Latchet	76-78	19-21	0.1.2	1.5
Silver Bream	77-79	19-21	0.5-1.0	1.0
Nanaghai	77-82	16-21	0.5-1.0	1.0
Morwong	79-80	18-20	0.5-1.0	1.0
Mullet	70-74	19-21	3.0-10.0	1.0
Prawns (Banana)	72-74	22-24	1.0	2.0

Edible muscle is portion of seafood muscle with skin and bones removed.

Method for Determination of the Emulsion Capacity

There has been an increased interest in the possible utilisation of fish muscle for the production and development of fish products, however, not much data is available on the technological suitability of fish muscle in regard to the physico-chemical behaviour of the protein system. An important factor in the production and processing of meat products has been the emulsification capacity.

The relative ability of a protein to combine (emulsify) with fat is considered to be a measure of the value of the protein for use in emulsified food products (Marshall et al. 1975).

Although the emulsion capacity may not necessarily indicate the stability of emulsions during subsequent processing, measurement of the emulsion capacity over a range of pH and salt concentration will provide information as to the suitability of certain muscle proteins for technological utilisation, and also give a visual description of the characteristics of the protein in these areas.

Several methods using resistance and conductivity techniques have been developed, particularly to measure the end-point, a point which indicates the completion of the emulsion.

For this work the method used for the measurement of the emulsion capacity (E.C.) was a method developed by this department for measuring the E.C. of meat sausages, a modification of the method used by Webb et al. (1970) in which the breaking point was measured by the principal of electrical conductivity instead of electrical resistance.

The solution of the protein in water has a zero resistance and high conductivity, however, after the formation of the emulsion, and the break point the resistance increases, while the conductivity is almost zero. The oil is added at a calculated speed to a known value of the protein, so that the emulsion capacity is measured by the amount of oil taken up and expressed as millilitres of oil per gram of protein.

Preparation of the fish homogenate

Minced samples of fish muscle were prepared using a food mincer (¼" plate), and stored at 4°C for use during the tests.

The protein content was determined for each sample.

Method

One gram of the homogenised fish muscle was dispersed in distilled water. The NaOH and HCl were used to adjust the pH levels to pH 2, 3, 4, 6.2 to 6.8, 8, 9 and pH 11. NaCl was added to make up 0%, 1% and 5% NaCl solution. The protein content being 0.4% w/w.

The dispersed solid was blended at high speed for 1 minute in a waring blender. A 50 ml aliquot of the blended sample (approximately 0.2% protein w/w) was transfered to a 250 ml beaker, and after standing for 30 minutes, the beaker with contents was placed under a Sorval Omni

mixer with a setting of 3.8. The emulsion meter was set to read a conductivity of 20 to 30 uA. Oil was added at an approximate speed of 1 ml per second, and the break point in the emulsion recorded when the conductivity of the meter dropped to zero. The amount of oil added was noted, and the emulsion capacity calculated as mls of oil per gram of protein.

The emulsion capacity expressed as mls of oil per gram protein plotted against pH, 0% NaCl, 1% NaCl, 5% NaCl are shown in the graphs.

The results were analysed statistically, using the Analysis of Variance, the level of significance set at 95%. The results examined were the maximum at the high pH level, and maximum at low pH, and at 0% NaCl and 1% and 5% NaCl level a similar trend was indicated.

The results of the statistical analysis are shown in Table 4.

Results and Discussion

The general trend as indicated by the graphs are similar to those reported by overseas research.

These results could be discussed with respect to the:

(a) Effect of pH
(b) Effect of pH and NaCl

(a) Effect of pH

The E.C. as indicated by the results is maximum at pH 2.4 and pH 9-11, while it is minimum at pH 6.2 to 6.8. The E.C. appears to be higher in the high pH range than the low pH. The effect of spray drying in the preparation of protein isolates from nanagai has had only a slight influence on the E.C. of the spray dried powder when compared with the fresh fish.

(b) Effect of NaCl Concentration

The maximum E.C. appears to be higher in the high pH range than the low pH in both the 1% and 5% NaCl solutions.

The effect of the salt levels 1% and 5% are not much different as indicated by the shape of the plots.

Spray drying has not significantly effected the emulsion capacity of the spray dried gem fish and nanagai isolates.

At the iso-electric point the effect is virtually the same for all 0% NaCl, 1% NaCl and 5% NaCl salt levels.

The results indicate that the difference in emulsion capacity between species is small, this is of advantage if fish muscle of various species was blended.

EMULSION CAPACITY

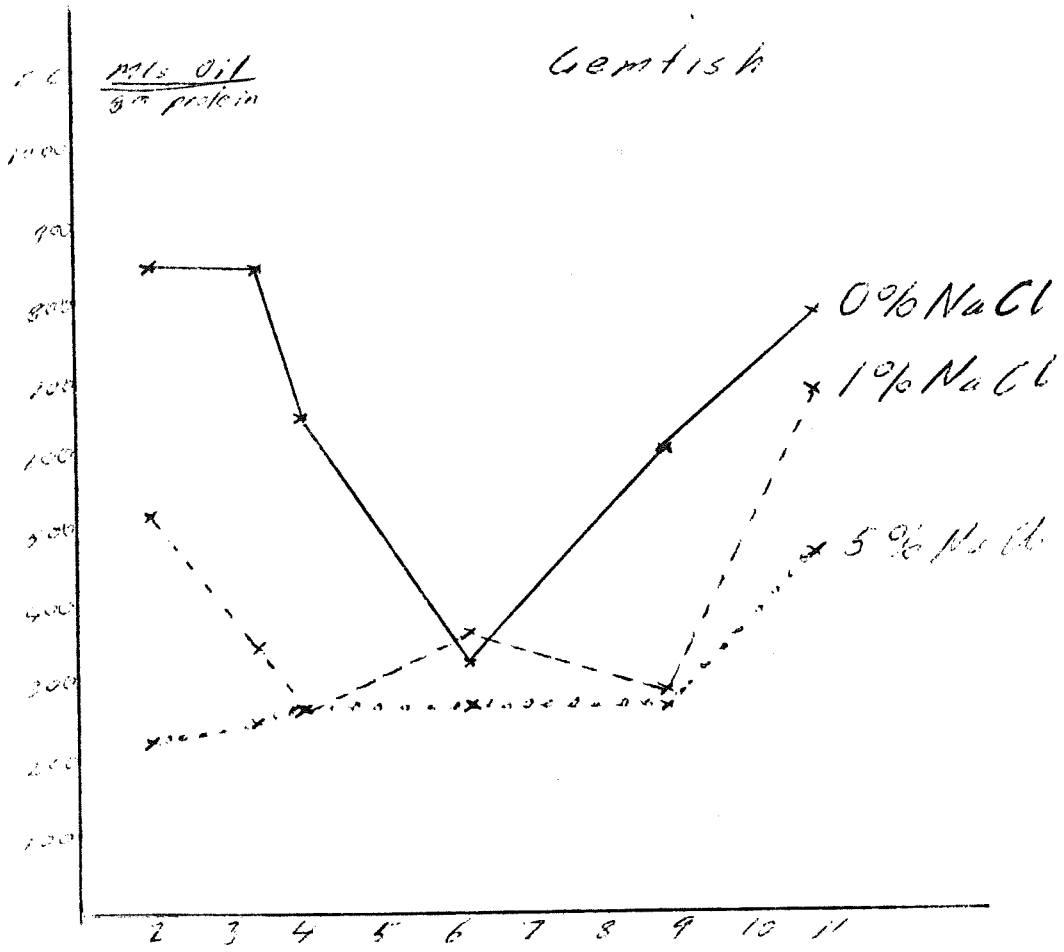


Figure 1

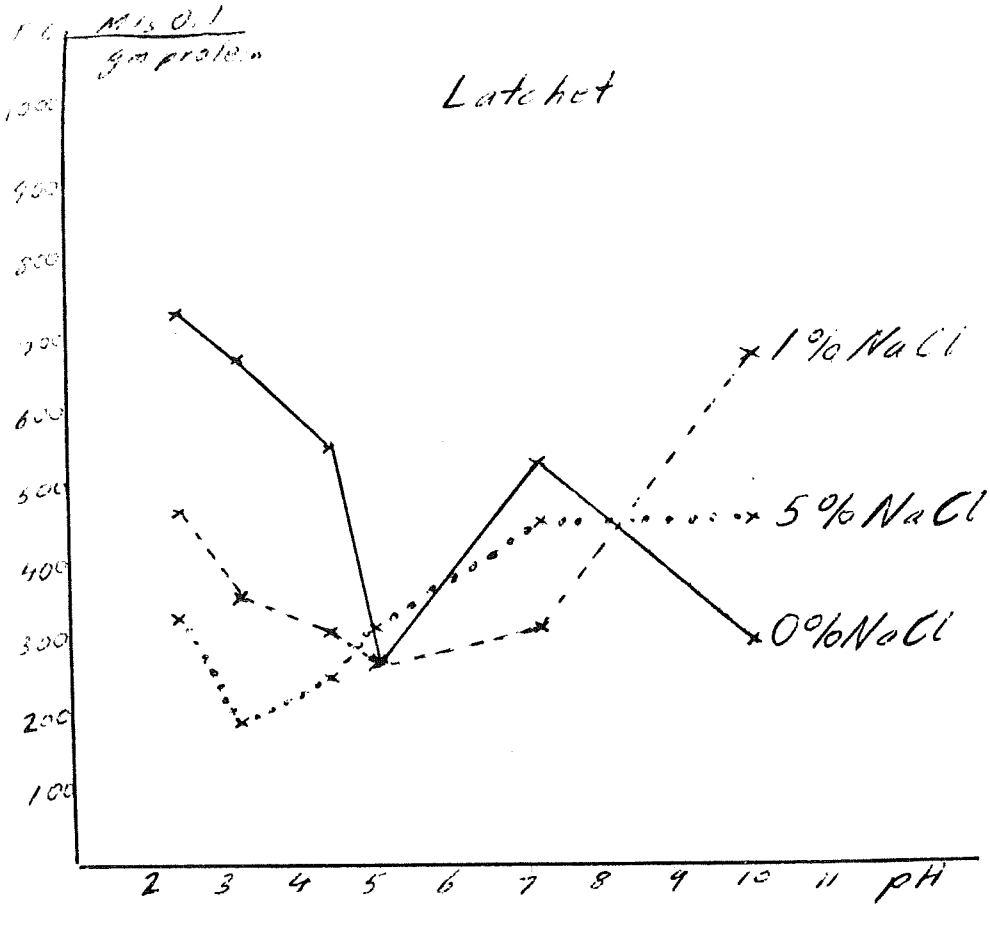
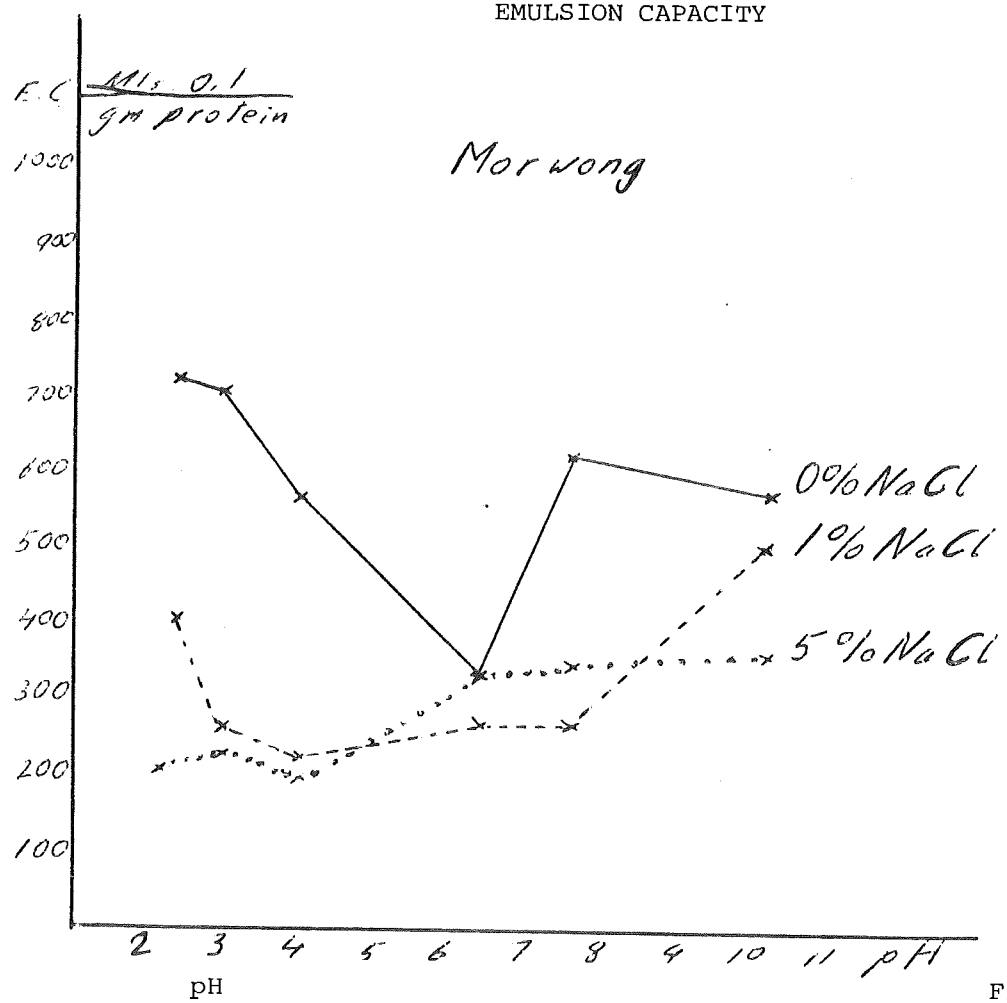


Figure 2

EMULSION CAPACITY



pH

Figure 3

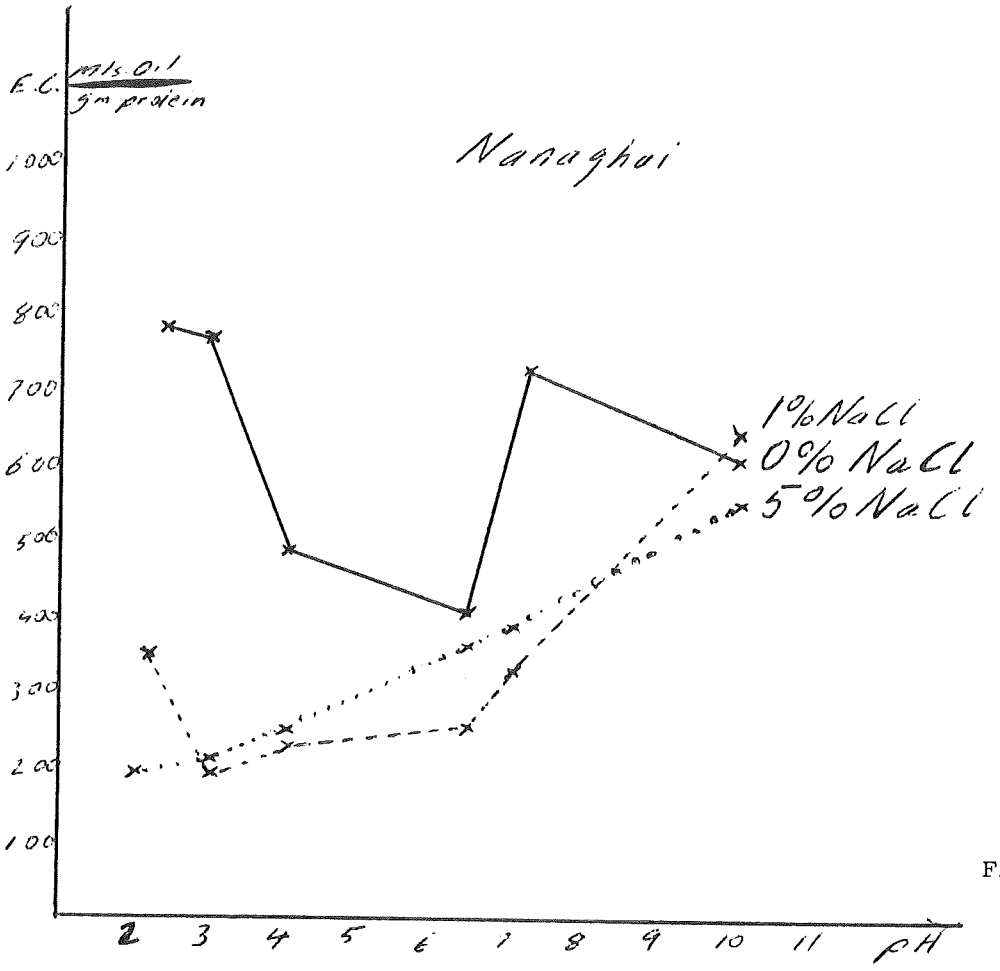
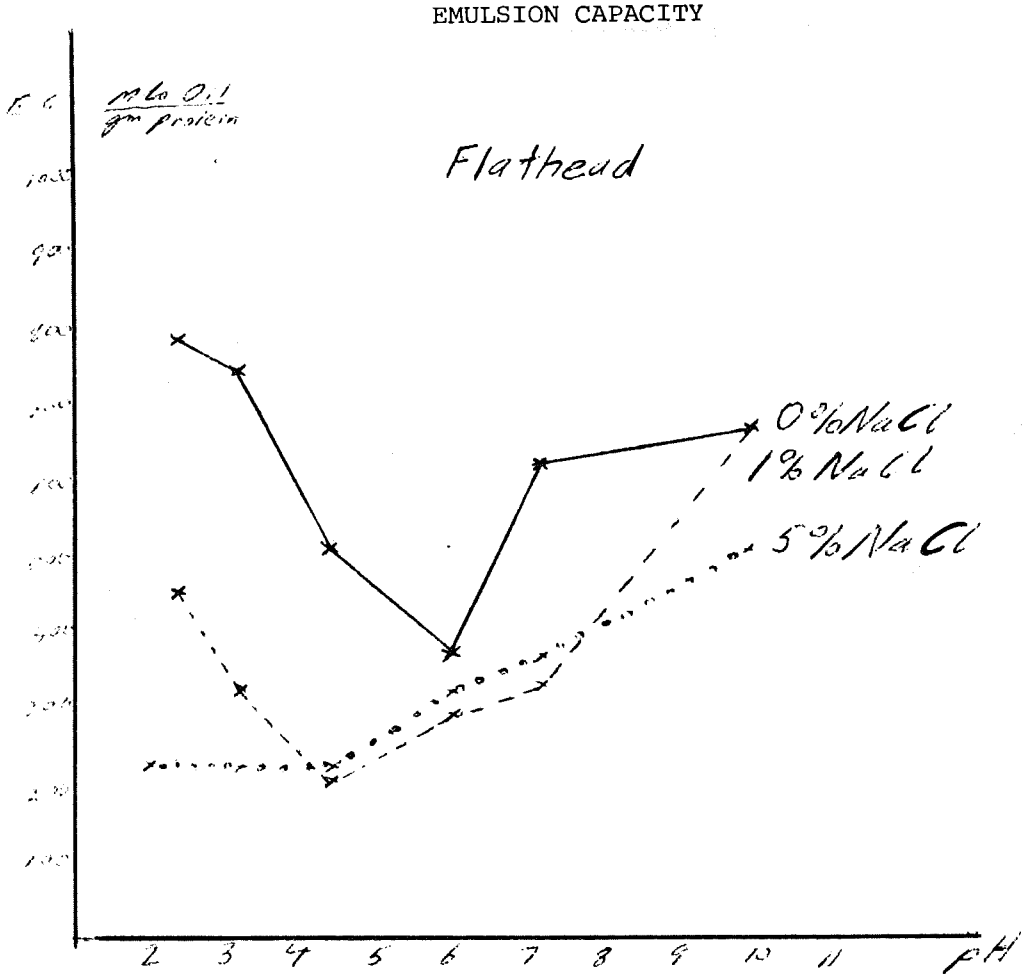


Figure 4

EMULSION CAPACITY



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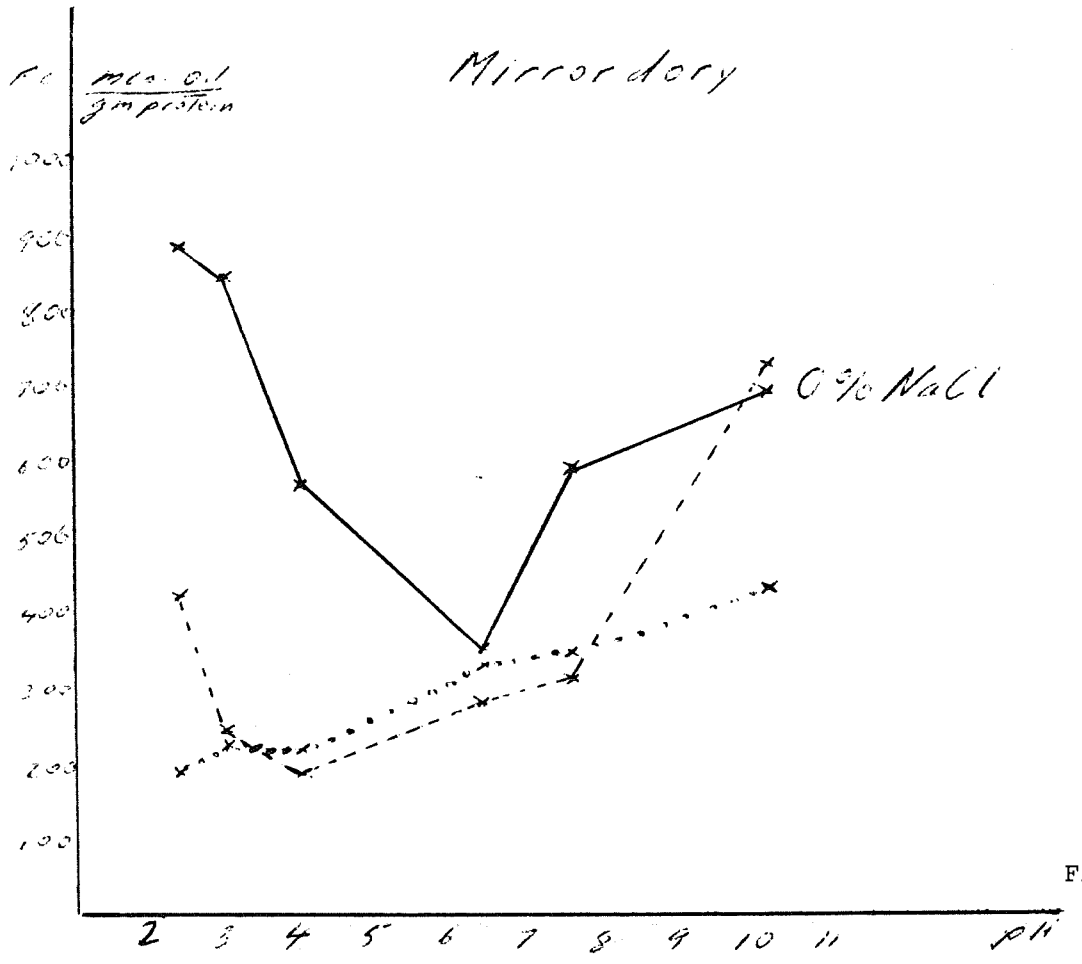
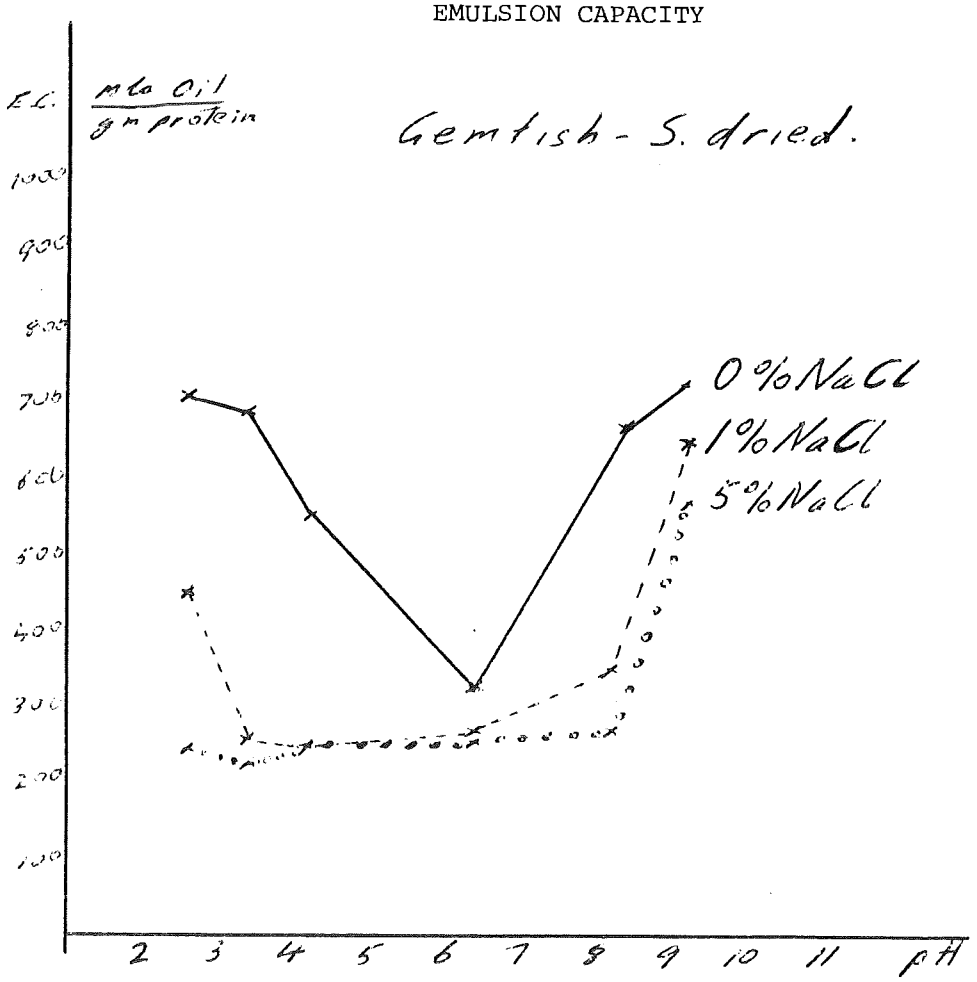


Figure 6

EMULSION CAPACITY



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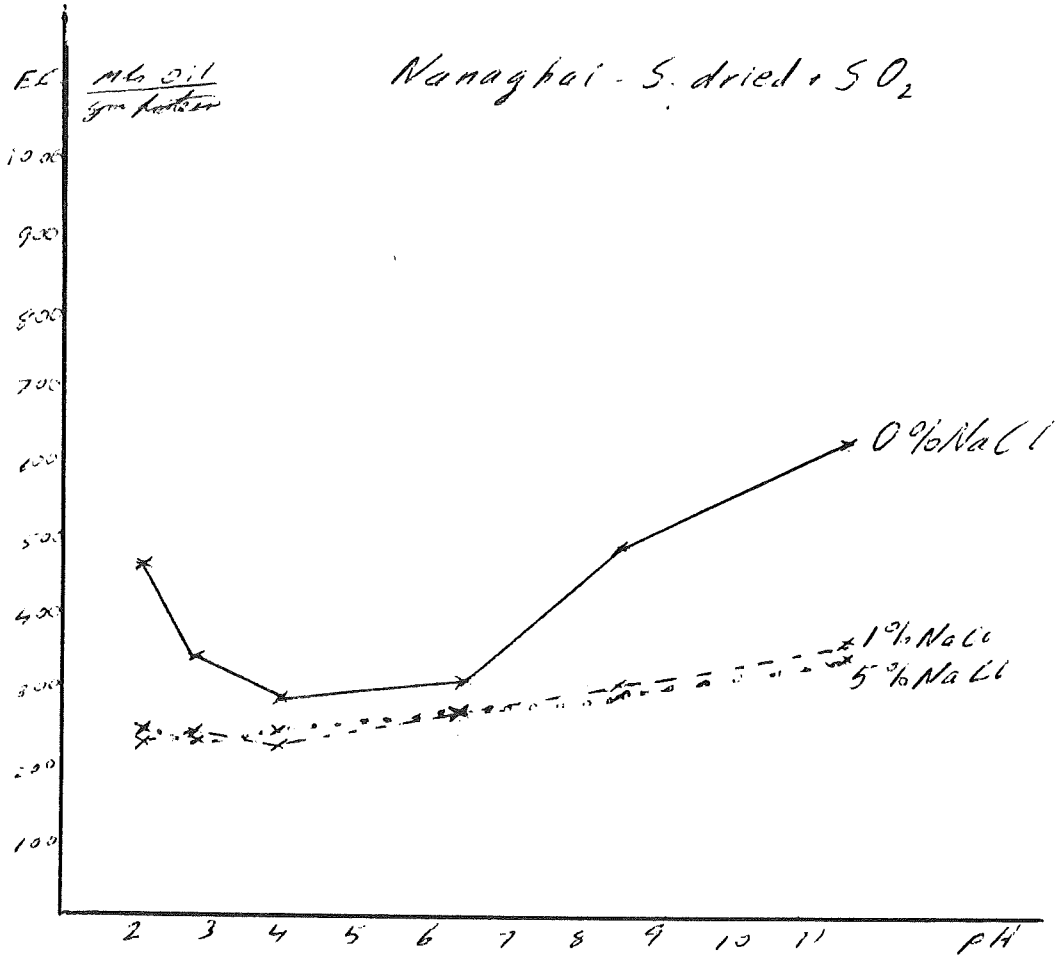


Figure 8

EMULSION CAPACITY

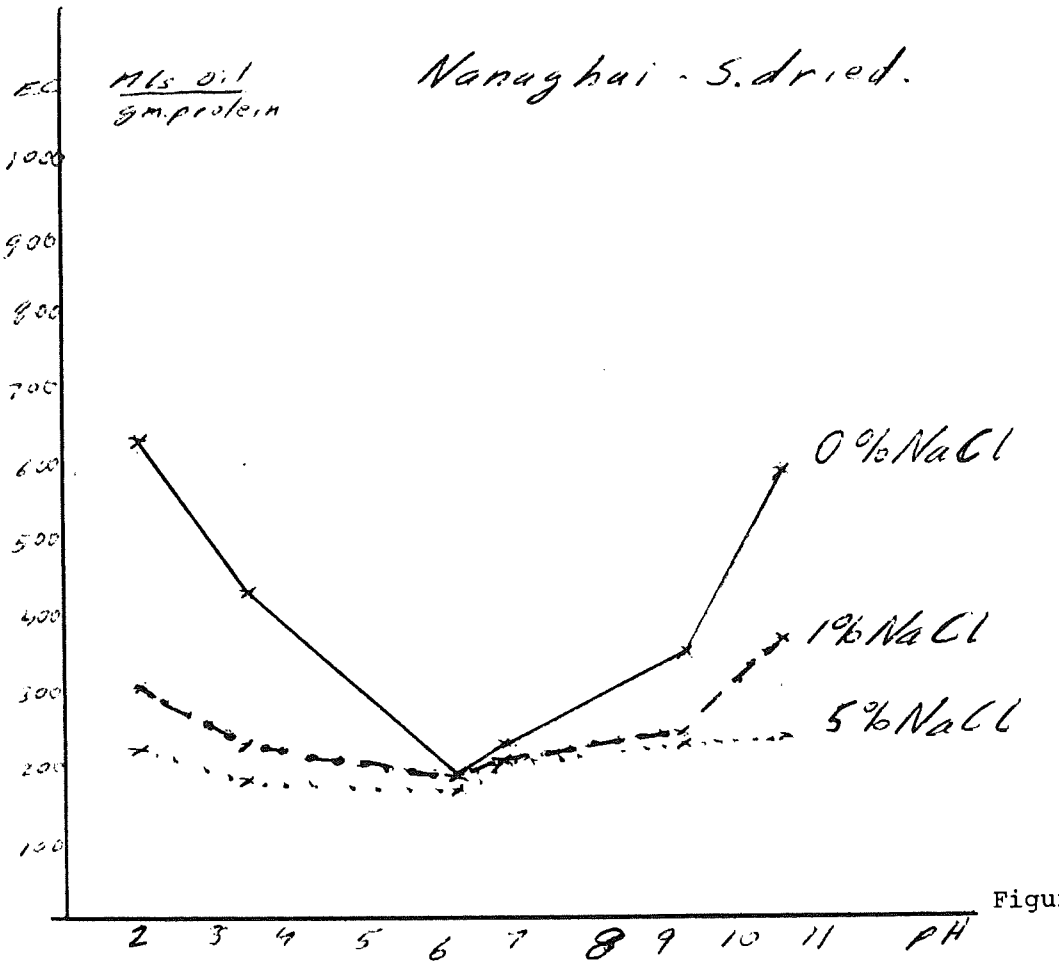


Figure 9

Nitrogen Solubility Index (N.S.I.)

The solubility profile of fish muscle protein can be represented by the nitrogen solubility index (N.S.I.). The N.S.I. indicates the protein (N x 6.25) soluble in varying conditions of pH and salt concentrations, and like the emulsion capacity, the N.S.I. also is related to the functional property of the protein.

While the emulsion capacity is important for products like sausages or gel-type products, the N.S.I. is a useful index for the extraction of protein in the production of pure protein isolates, and preparation of modified protein products.

Meinke and co-workers (1972) have published data on the N.S.I. for some species of fish, caught in the American fishing grounds. The method used in this project was slightly modified, however, the N.S.I. values determined by the modified method indicated a similar trend to the U.S.A. data published.

Method

Two gms of fish muscle weighed in a 50 ml polypropylene centrifuge tube, was made up to 20 gms with sodium chloride and solutions of 0.1N HCl or 0.1N NaOH to make up solutions of varying salt levels (0%, 1% and 5% NaCl) and pH levels of 2, 3, 4, 6.2, 6.8, 8 and 11.

The tubes were mechanically shaken for 30 minutes on a Dumax flask shaker and then centrifuged at 1000 g for 20 minutes in a MSEGF8 centrifuge.

The supernatent was strained through fine gauze to remove the top skin layer. The protein nitrogen values were then obtained using 10 ml aliquots of the strained liquid, by macrokjeldahl digestion and distillation.

The percent solubility nitrogen then is plotted against pH values for 0% NaCl, 1% NaCl and 5% NaCl as indicated in the graph.

NITROGEN SOLUBILITY INDEX

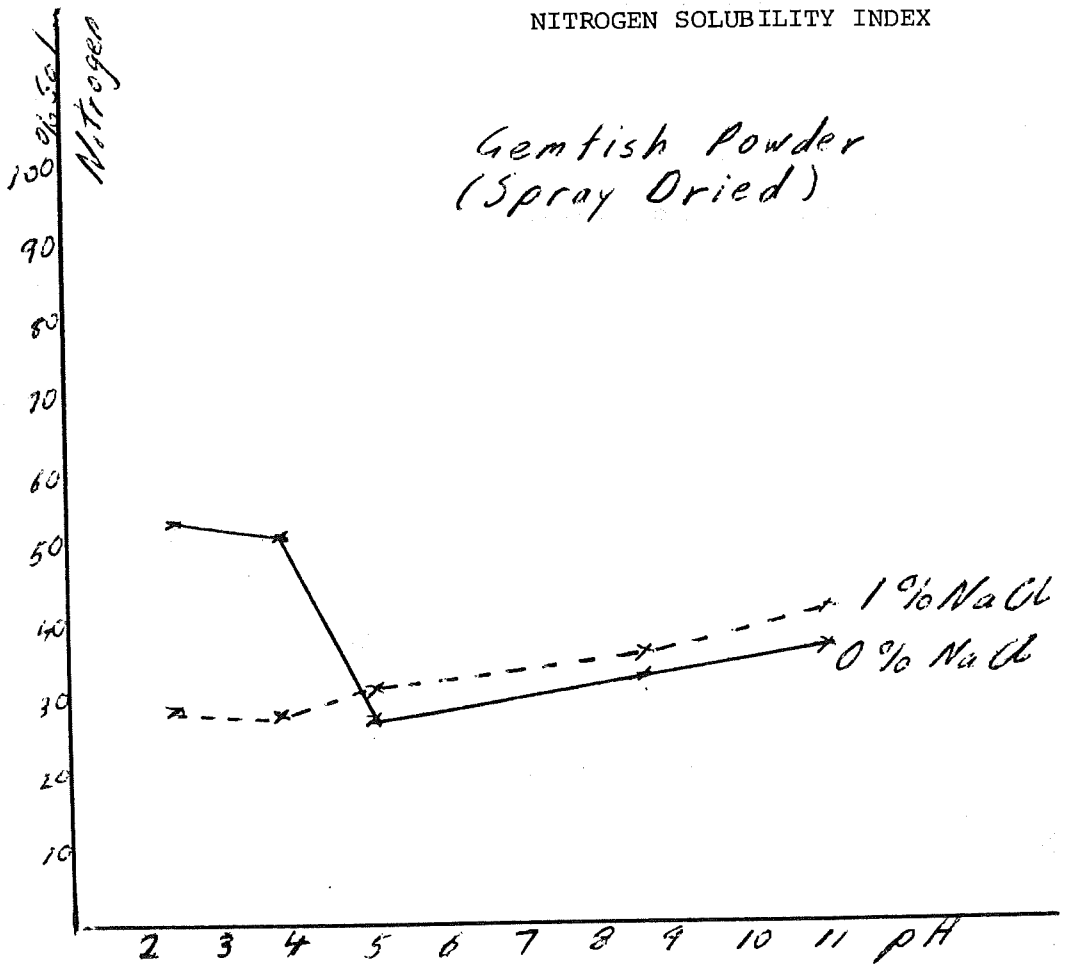


Figure 10

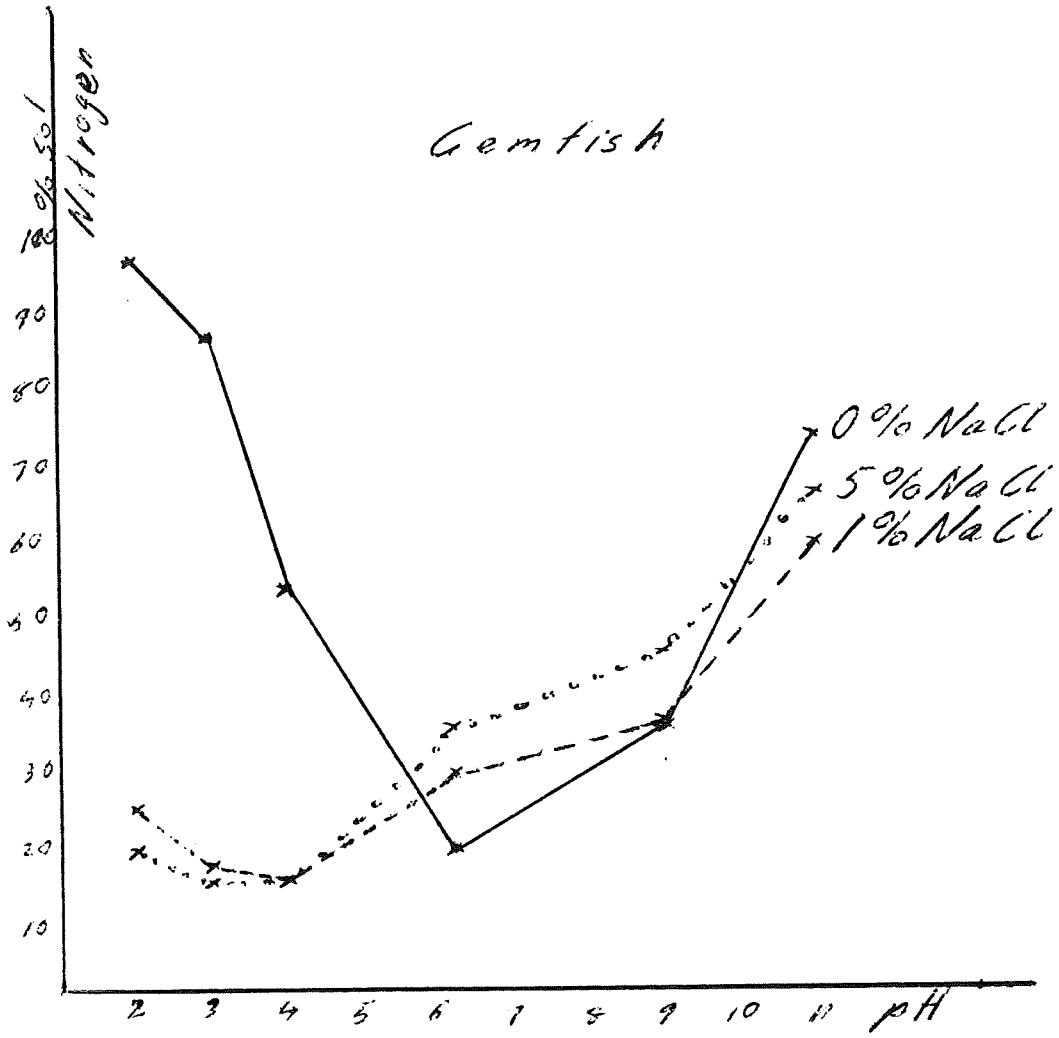


Figure 11

NITROGEN SOLUBILITY INDEX

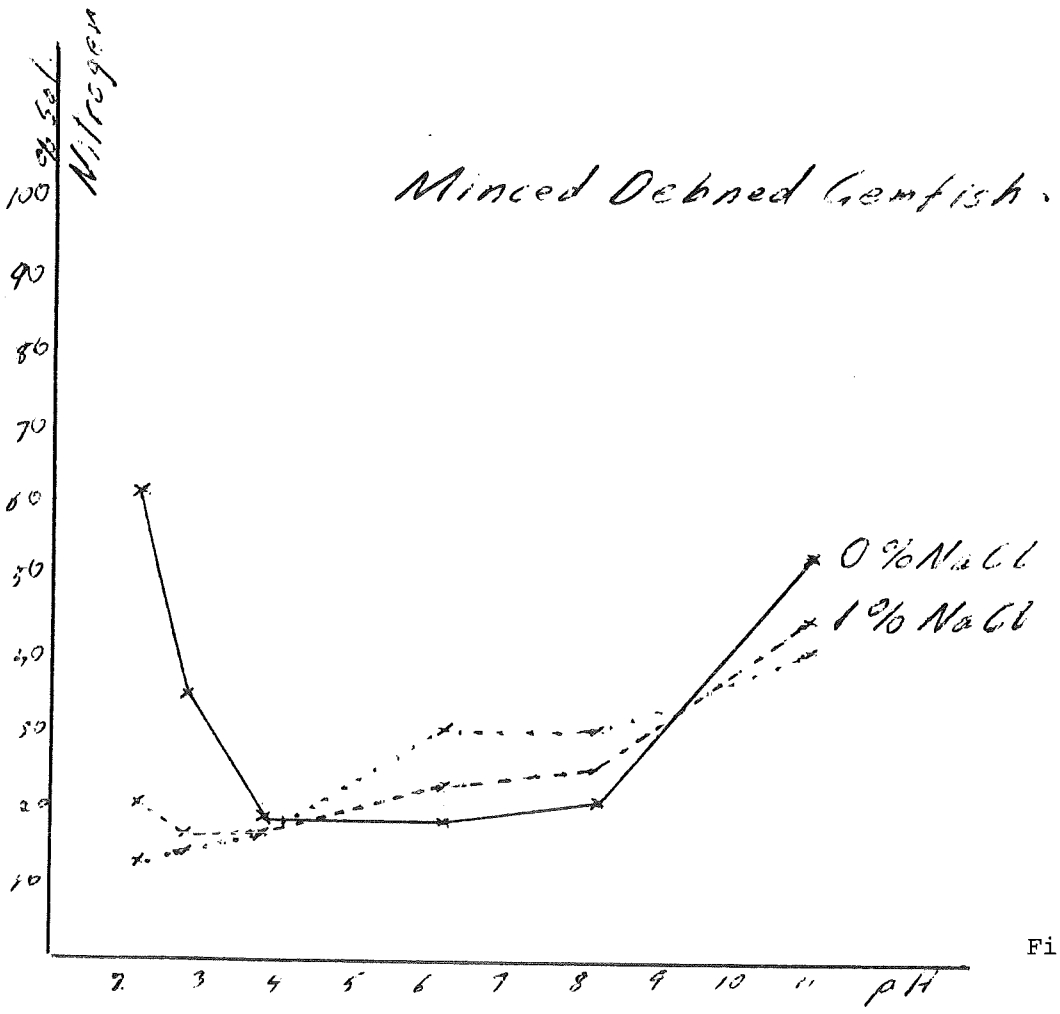


Figure 12

NITROGEN SOLUBILITY INDEX

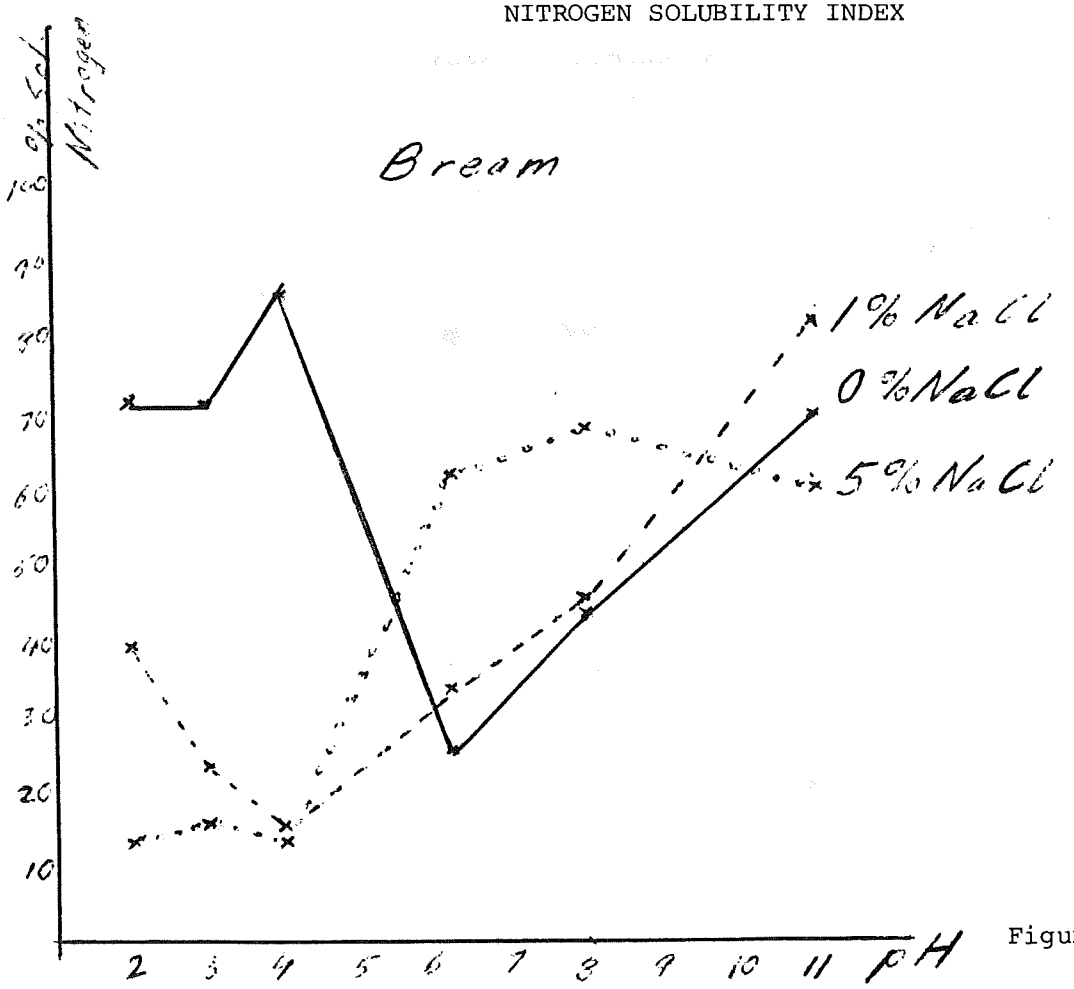


Figure 13

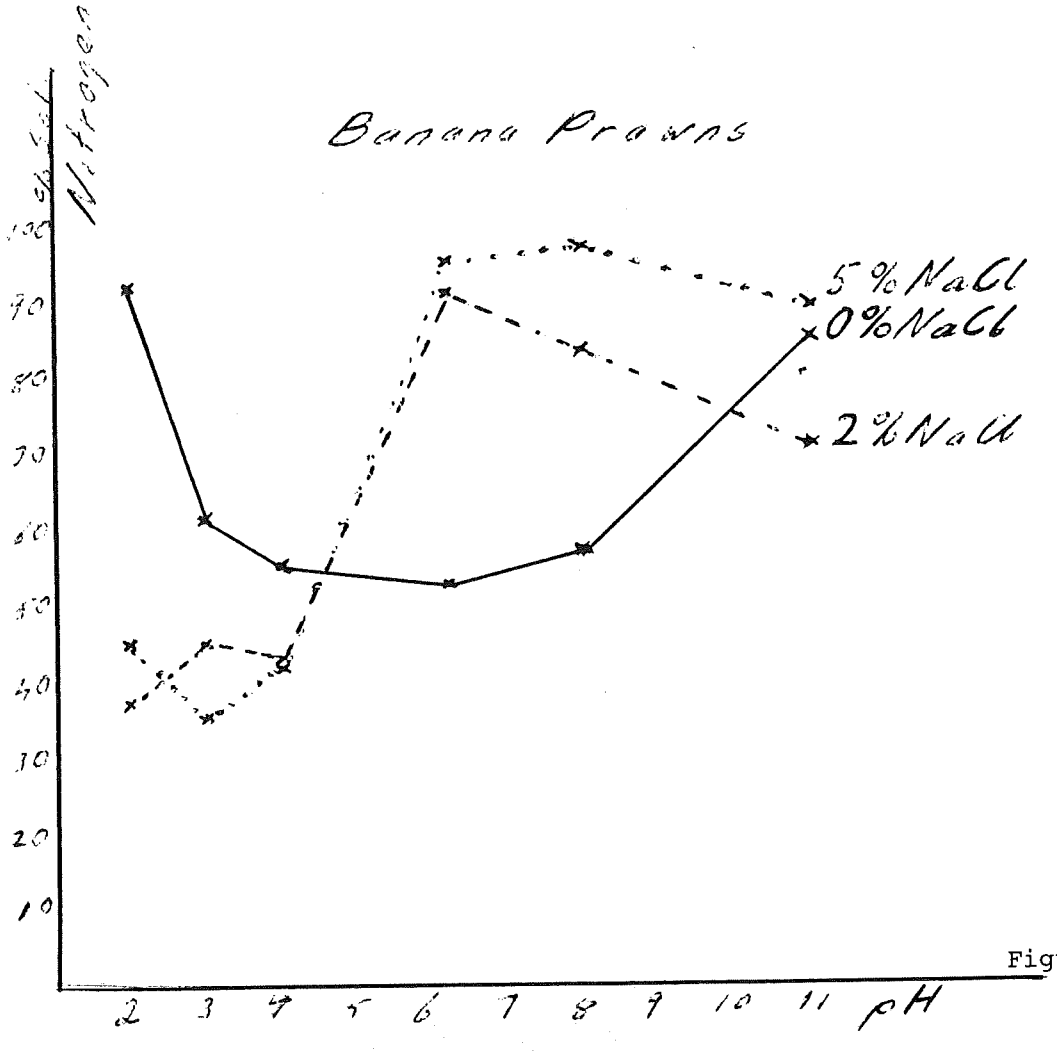


Figure 14

NITROGEN SOLUBILITY INDEX

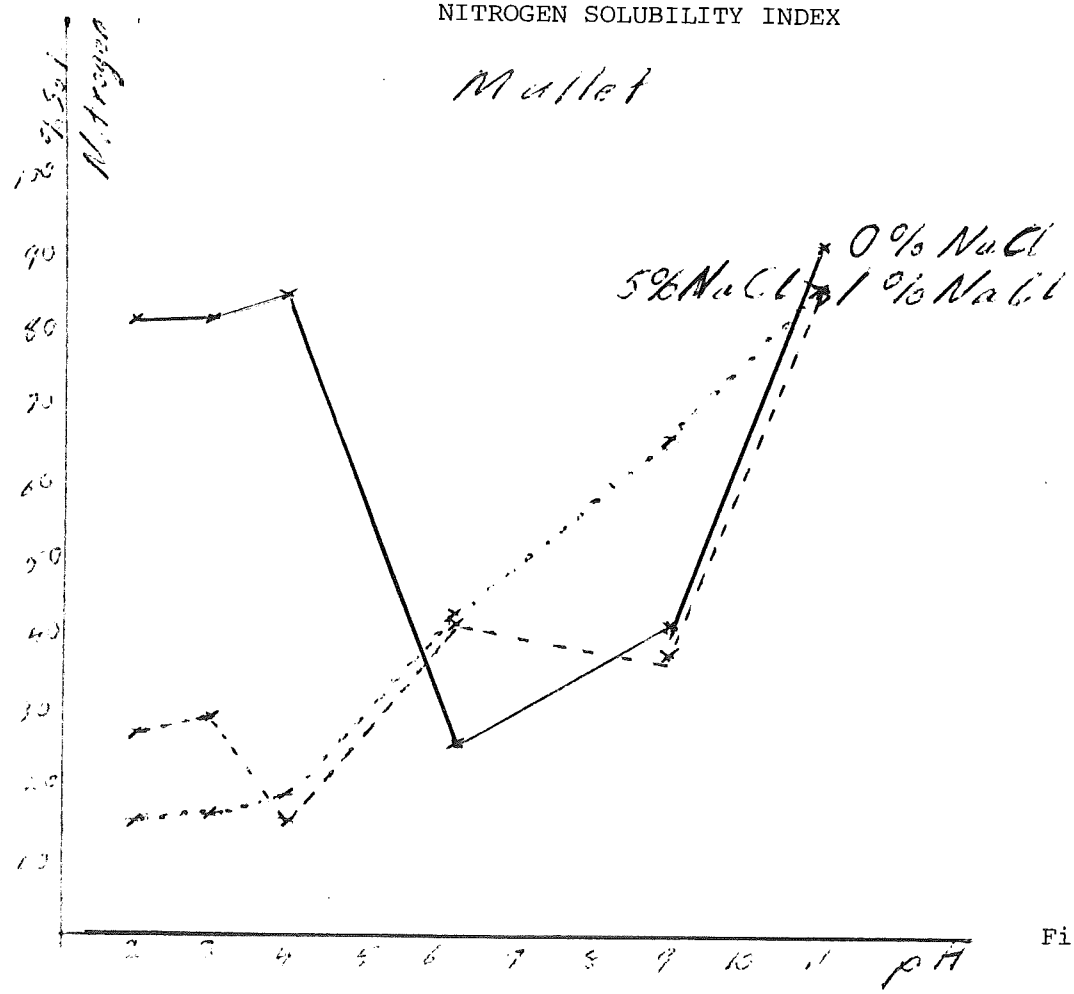


Figure 15

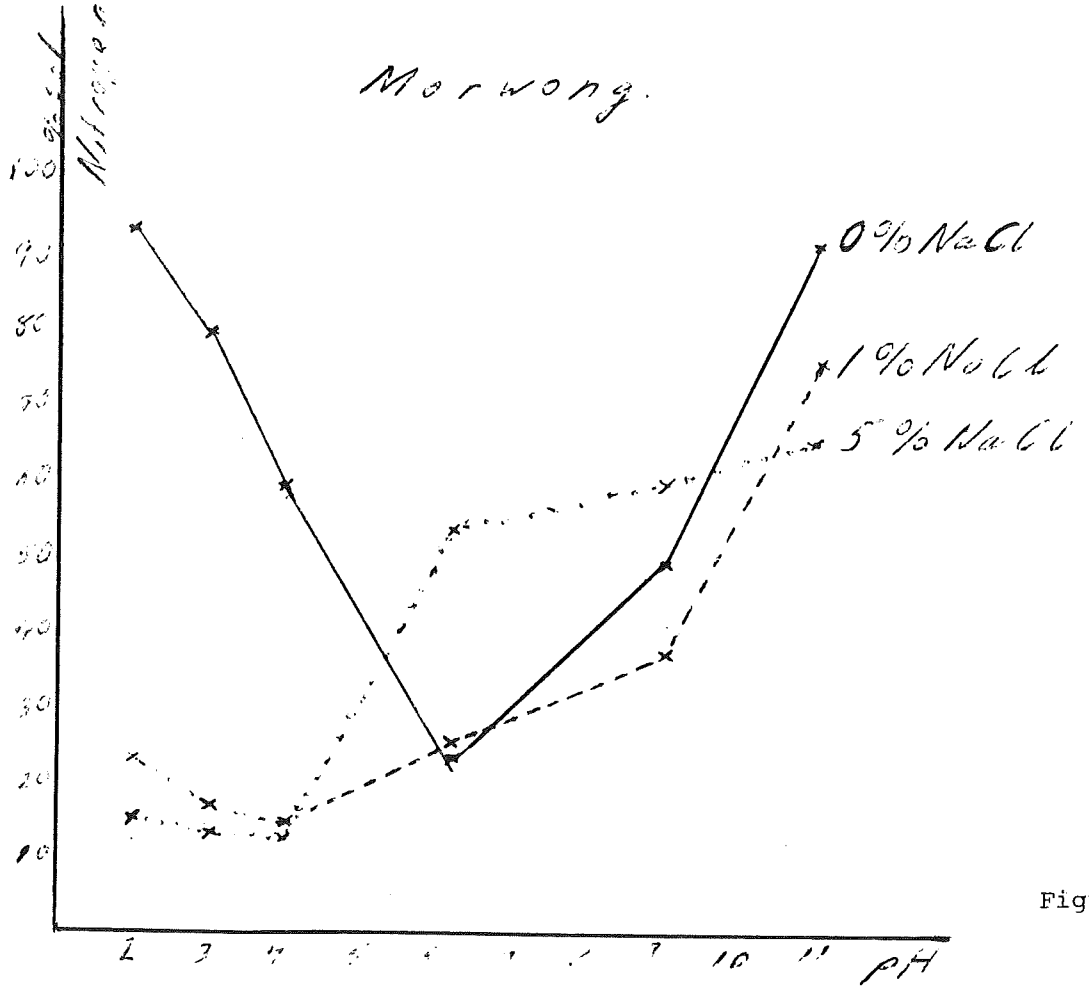
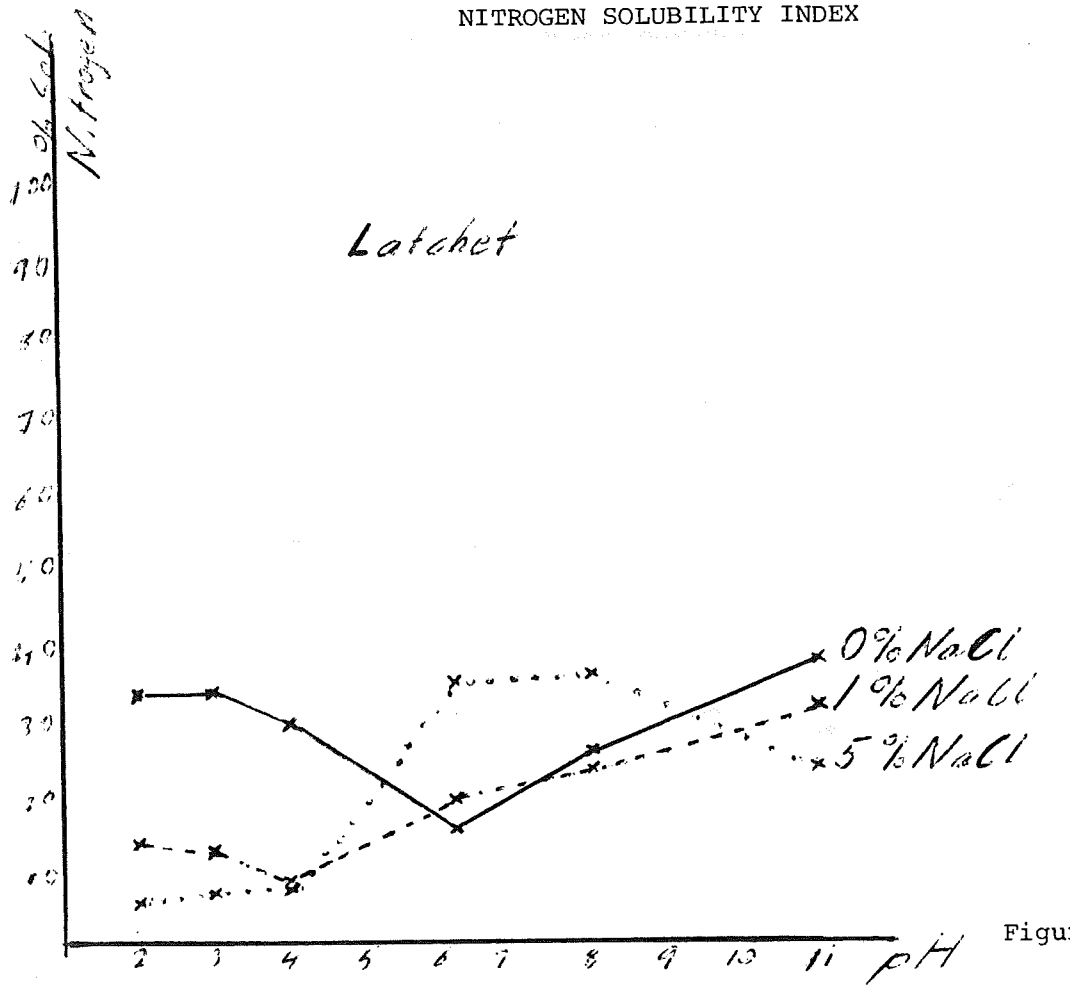


Figure 16

NITROGEN SOLUBILITY INDEX



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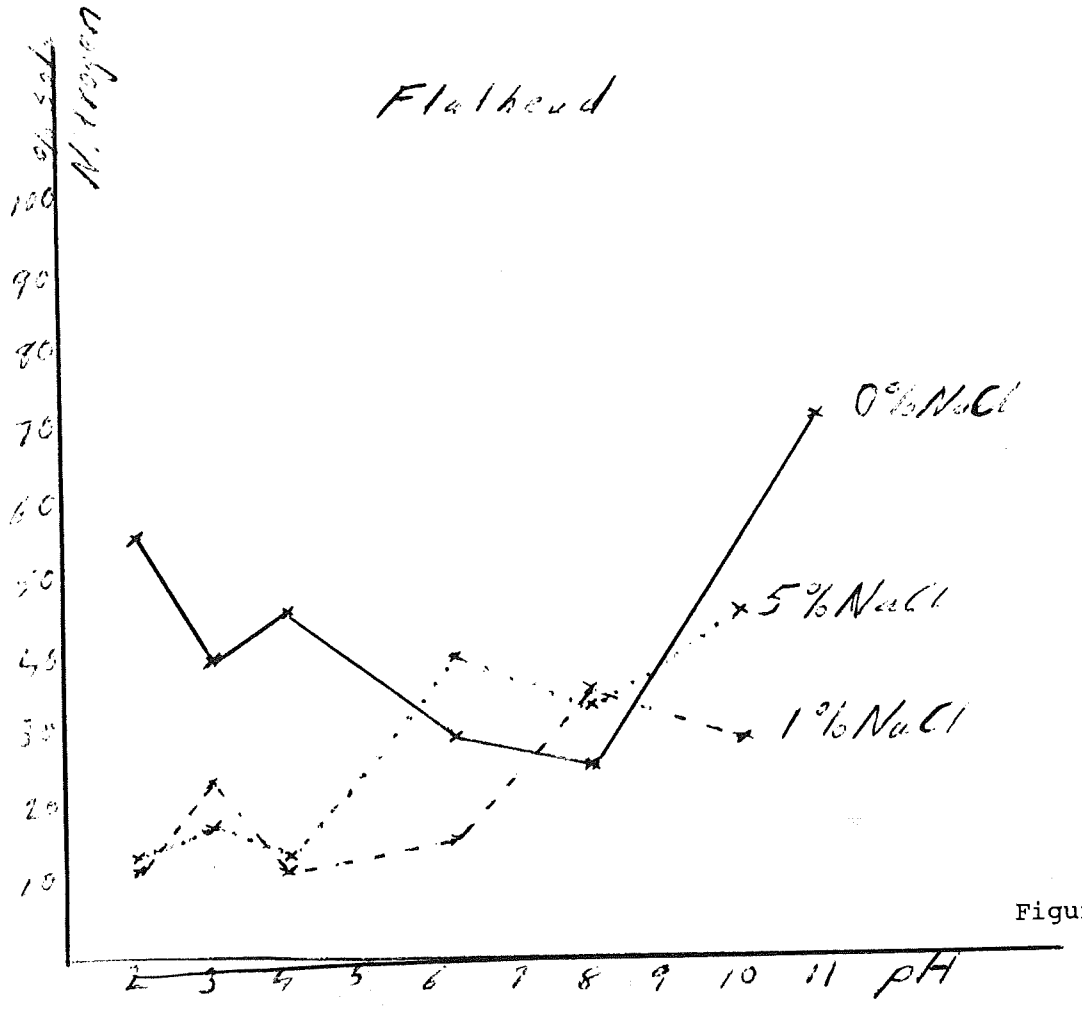

Figure 18

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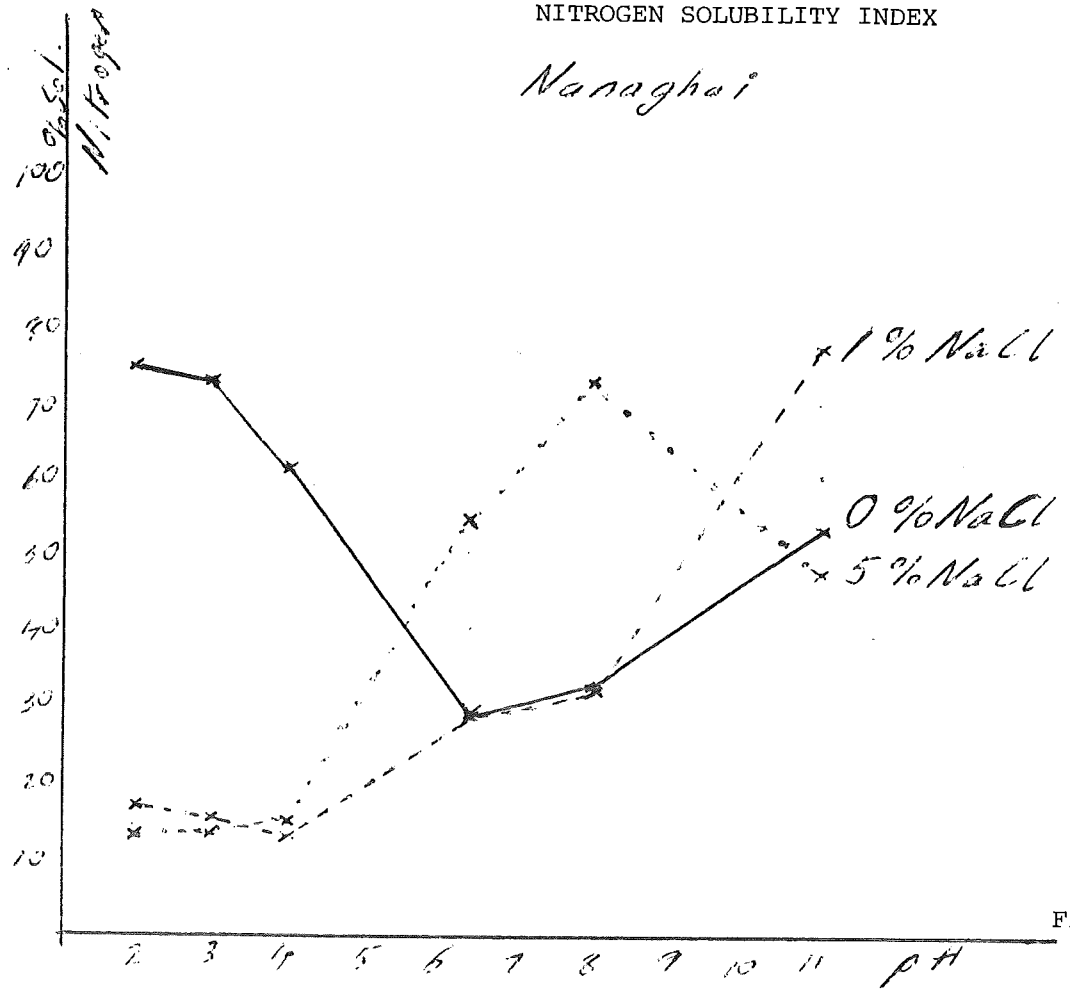

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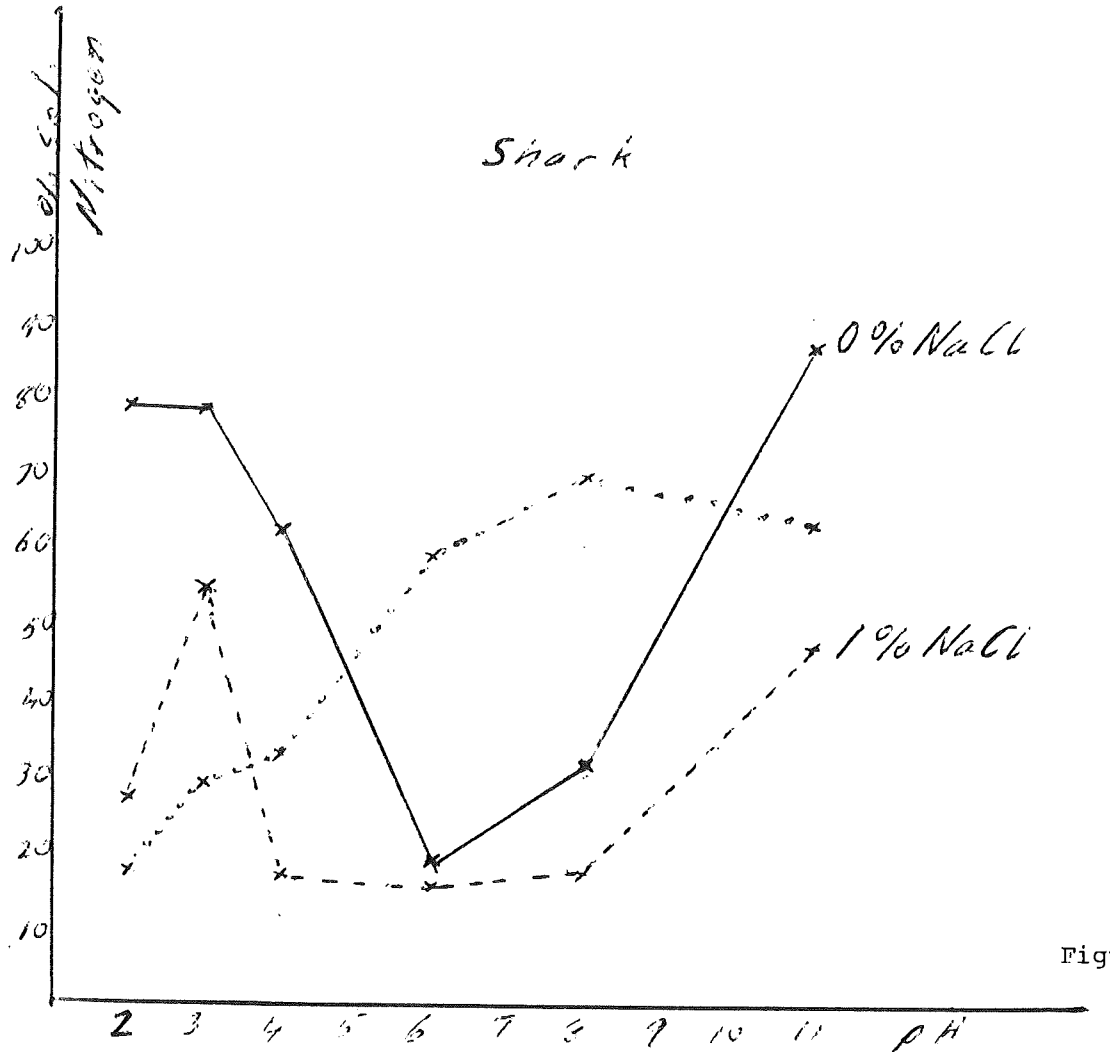


Figure 20

NITROGEN SOLUBILITY INDEX

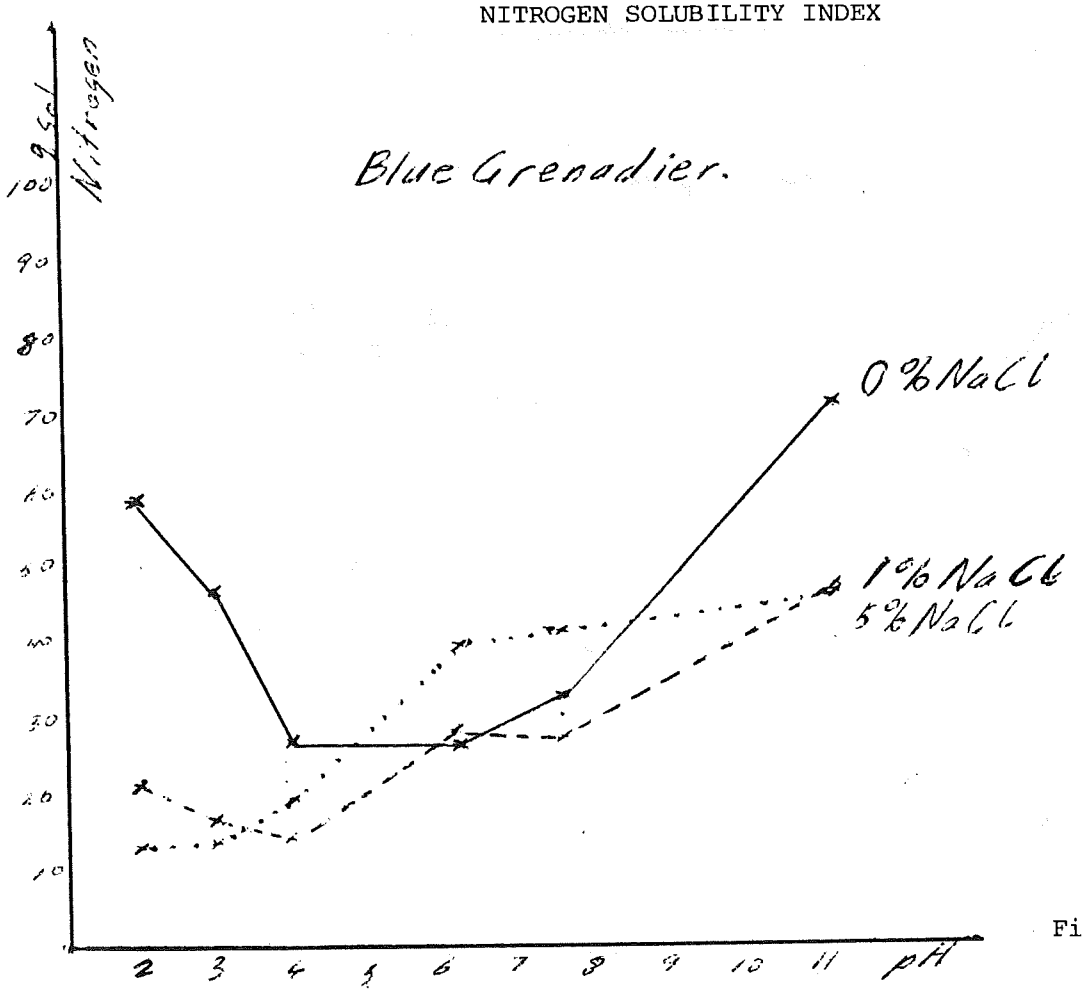
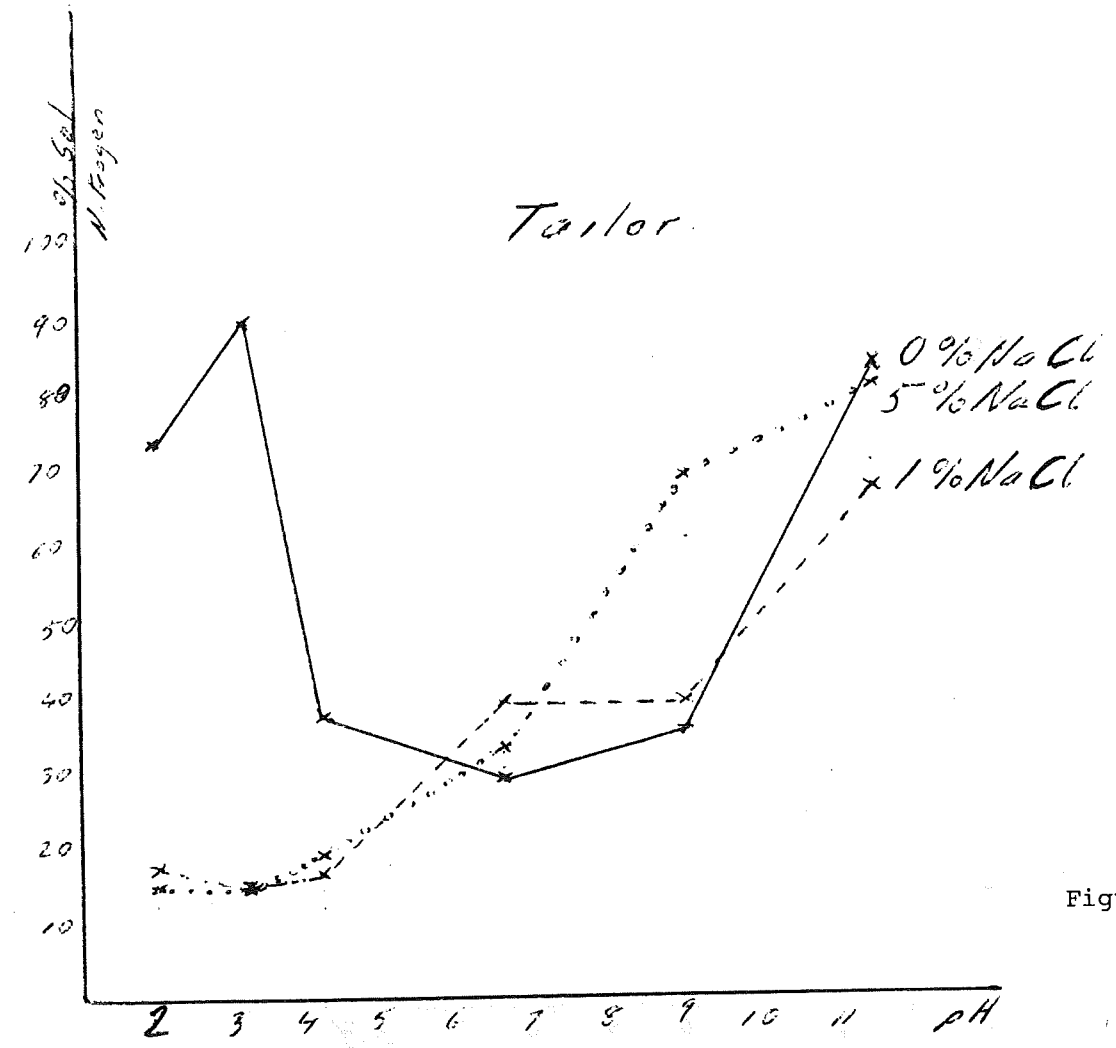


Figure 21



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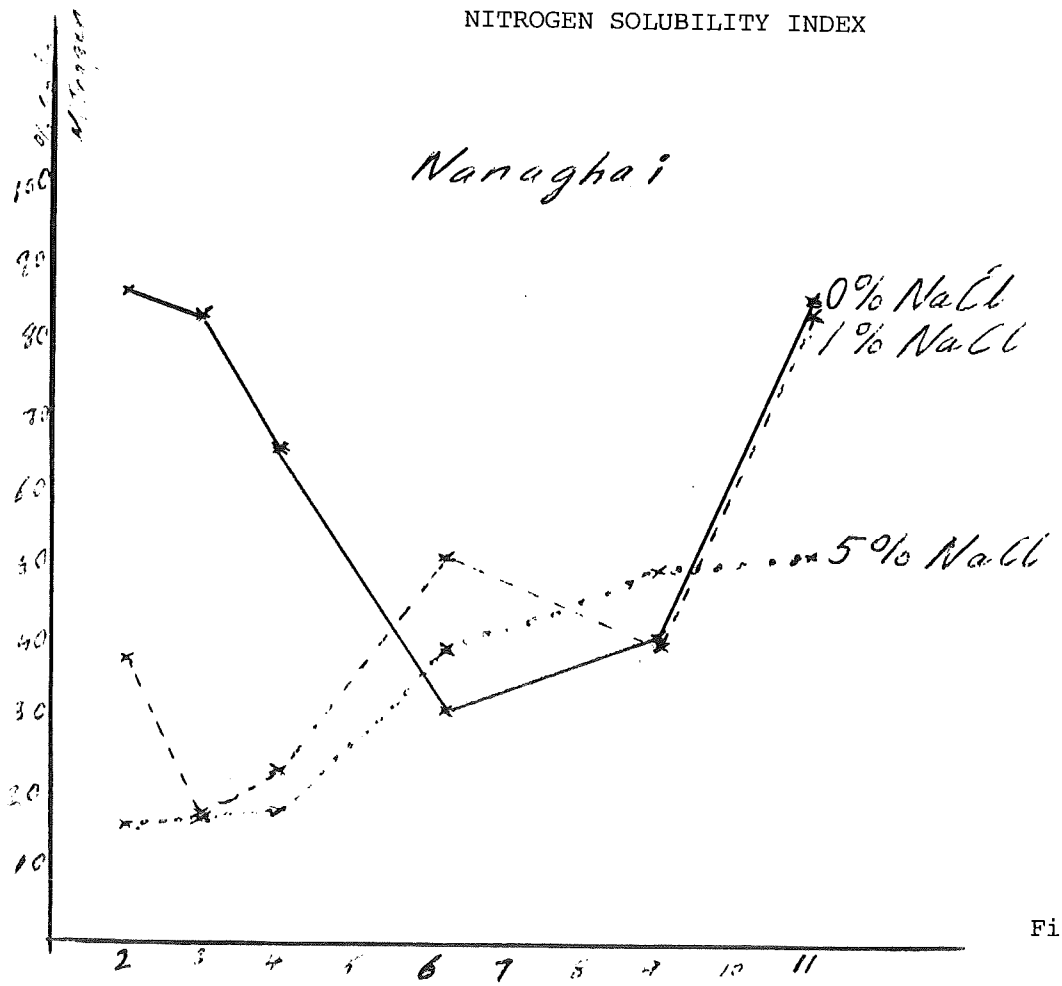

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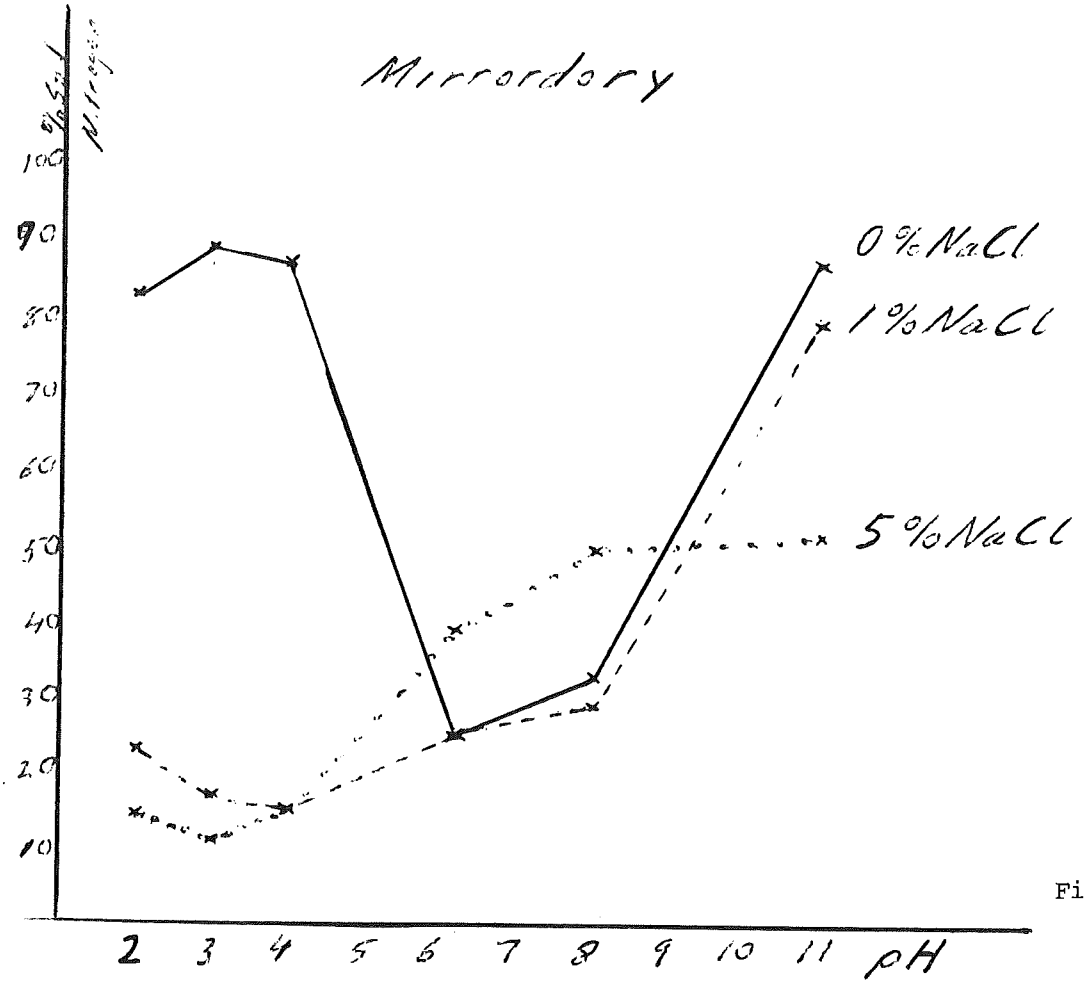


Figure 24

Statistical Analysis (E.C. and N.S.I.)

Analysis of variance was applied to the values of N.S.I. and E.C. at low and high pH for various fish species and salt concentrations, with these results shown in Tables 2 and 3.

Using step-wise multiple linear regression, models were developed for the effect of pH and salt concentration on E.C. and N.S.I. Tables 4 and 5 give the models for the effect of pH on E.C. and N.S.I. respectively at 0% NaCl, with these models taking the form:

$$Y = b_0 + b_1X + b_2X^2$$

where Y = N.S.I. or E.C.
X = pH
and b_0, b_1, b_2 = regression coefficients

Tables 6 and 7 give the models for the effect of pH and % NaCl on E.C. and N.S.I. respectively, with these models taking the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_{12}X_1X_2$$

where Y = E.C. or N.S.I.
X_1 = % NaCl
X_2 = pH

and b_0, b_1, b_2, b_{11}, b_{22}, b_{12} = regression coefficients

Table 4 - Regression models for effect of pH on E.C. at 0% NaCl

Species	R^2	b_0	b_1	b_2
Flathead	.69	1272.9	-243.8	18.6
Spray dried Gemfish powder	.79	1567.6	-388.5	32.6
Spray dried Nanaghai powder	.98	1312.1	-337.1	25.6
Nanaghai spray dried and SO_2	.82	553.0	-85.6	8.3
Gemfish	.72	1422.9	-284.1	20.9
Latchet	.70	1025.0	-142.3	7.2
Mirrordory	.82	1517.3	-316.2	23.4
Morwong	.56	1082.5	-175.2	12.5
Nanaghai	.35	1087.2	-165.7	12.2

Table 5 - Regression models for effect of pH on N.S.I. at 0% NaCl

Fish Species	R^2	b_o	b_1	b_2
Gemfish	.81	174.7	-38.8	2.7
Flathead	.85	92.8	-21.3	1.7
Nanaghai	.89	124.8	-24.3	1.6
Minced deboned gemfish	.85	101.8	-27.4	2.1
Blue Grenedier	.95	96.9	-23.0	1.9
Tailor	.71	144.1	-33.1	2.4
Mullet	.90	152.0	-36.3	2.8
Shark	.86	157.3	-38.7	2.9
Mirrordory	.66	163.0	-36.0	2.6
Morwong	.91	161.1	-38.2	2.9
Bream	.59	121.5	-22.3	1.6
Banana Prawns	.89	129.1	-26.1	2.0
Latchet	.76	53.5	-10.3	0.8
Nanaghai (frozen)	.90	153.8	-34.4	2.6

Table 6 Regression models for effect of pH and % NaCl on E.C.

Species	R^2	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
Flathead	.82	1042.5	-323.5	-173.3	42.9	14.3	8.4
Spray dried Gemfish	.85	1297.9	-317.0	-292.8	44.5	25.4	5.6
Spray dried Nanaghai	.72	803.9	-187.8	-150.4	28.0	11.6	1.3
Nanaghai spray dried and SO_2	.82	412.2	-170.7	-23.3	30.48	3.3	-2.1
Gemfish	.80	1125.8	-342.7	-180.2	46.9	13.8	5.4
Latchet	.54	941.7	-194.3	-145.0	18.3	9.8	12.3
Mirrordory	.78	1114.9	-344.4	-187.1	55.5	15.2	7.6
Morwong	.80	873.3	-351.0	-107.0	50.6	8.1	6.5
Nanaghai	.78	897.3	-411.4	-115.3	59.7	9.9	9.5

Table 7 - Regression models for effect of pH and % NaCl on N.S.I.

Species	R^2	b_0	b_1	b_2	b_{11}	b_{22}	b_{12}
Gemfish	.67	107.9	-49.29	-15.73	6.74	1.12	1.58
Flathead	.64	48.1	-22.89	-3.31	4.30	.36	.52
Nanaghai (spray dried)	.49	67.1	-35.75	-5.81	4.91	.47	1.32
Minced deboned gemfish	.59	55.2	-15.26	-9.63	.53	.82	2.00
Blue Grenedier	.69	46.1	-26.41	-3.56	4.14	.44	.46
Tailor	.73	86.0	-40.30	-12.89	5.54	1.05	1.51
Mullet	.80	90.0	-39.29	-17.50	5.31	1.50	1.48
Shark	.57	98.6	-43.94	-14.72	6.64	1.06	1.35
Mirrordory	.63	98.5	-46.78	-14.07	6.74	1.01	1.01
Morwong	.66	87.2	-41.90	-11.31	5.86	1.01	1.23
Bream	.48	67.4	-26.91	-3.98	3.01	.37	1.31
Banana Prawns	.51	71.7	-26.79	44.22	4.02	.43	1.23
Latchet	.56	26.0	-16.47	-	2.37	$.64 \times 10^{-1}$	.46
Nanaghai (fresh)	.63	91.0	-43.16	-11.84	6.02	.99	1.70

Results and Discussion

The results indicate that the N.S.I's are higher at the higher pH value (between pH 10 and 11) than the low pH value (between pH 2 to 3), and at 0% NaCl concentration; the minimum at pH 6 to 6.8.

The N.S.I. values for the latchet is very low both in the high pH value and the low pH vlaue, while the N.S.I. for Banana Prawns and Shark, are higher than the remaining species.

Viscosity Measurements of Fish Homogenates

The Brabender Viscograph is normally for evaluating viscosity and gelling characteristics of starch solutions.

The Brabender Viscograph was used in this project to evaluate the flow behaviour and viscosity characteristics of fish homogenates.

Research reports on the gelling characteristics of fish muscle indicate that salt soluble proteins play an important role in the gelling phenomena.

The method used was formulated after examining the method suggested for evaluation of starch gels. Initially fish gels were prepared and examined for consistency using varying salt concentration and fish muscle content and from these observations, the fish homogenate composition was formulated.

Preparation of the Fish Homogenate

The minced edible portion of fish muscle was used in these experiments. Fresh minced muscle besides being used for the measurement of the viscosity of the fresh muscle homogenate, a portion of the mince was also frozen, and viscosity measurements made on the frozen muscle as well.

The composition of the homogenate was 100 gms minced muscle in 300 mls 5% NaCl solution to which 100 gms of ice was added and the solution homogenised in a waring blender first at low speed for 20 seconds and then at high speed for 30 seconds. The homogenate was then transferred to the Viscograph bowl. The bowl rotated at 75 r.p.m. for 30 minutes. The curve recorded in Brabender units against time in minutes.

The Brabender Viscograph

The Brabender Viscograph is characterised by using radiant heat to heat the bowl containing the homogenate. The heating is thermostatically controlled so that the viscosity characteristics can be evaluated at preset temperatures. The measurements were made at $25^{o}C$ and, in order to maintain this temperature, cooling water was circulated through jacketed portion of the stirrer agitator fitted into the bowl.

Pins suspended in the bowl divide the cup into three annular regions:

(a) the space near the edge of the bowl centred 6 mm from the edge;

(b) an intermediate space 21 mm from the edge of the cup;

(c) a central region which is circular and about 50 mm diameter.

The temperature sensing device (a thermoregulator), a cooling bar and spindle pins which measure 55% of the viscosity are in the first region.

Spindle pins which measure 45% of the viscosity are in the intermediate regions.

The central region has no provision for mixing but depends on the turbulence of the slurry in the bowl. The viscosity is measured by the torque exacted on the spindle as the bowl rotates.

The spindle also serves to mix and shear the sample.

The viscosity measurements were made using different concentration of the fish muscle. The following plots of Brabender units against time in minutes are shown in Figure 25.

The Brabender units express the torque. For more viscous mixtures the Brabender units are higher but fall for mixtures of low viscosity. For solutions in which the solute is completely soluble, the Brabender units are nearer the zero point.

From the plot of the Brabender units against time, although initially there is rise or fall in the Brabender units, there is a stage where the homogenate almost stabilises as a sol or gel. In this project this stabilisation point has been taken as a point to indicate the viscosity of the mixture. Further work may be necessary for the proper interpretation of these results, however, the plots do indicate a relation between the fish muscle concentration of the homogenate, and the Brabender units, which gradually decrease with the lowering in the concentration of the fish muscle homogenate.

It may perhaps be more appropriate to express these results in relation to the protein content. However, since the protein content of the fish species examined is not much different the results are expressed in this report as a ratio of Brabender units per gram of fish muscle, instead of per gram of the protein.

The following Table 8 indicates this ratio for the various species of fish examined, which is represented by the bar graph Figure 26.

Table 8 Viscosity of Fish Homogenate expressed as Brabender units per gram of muscle at the point of stabilisation

Species	B. U./gm muscle
Gemfish	11.5
Flathead	10.0
Tailor	8.5
Morwong	8.1
Latchet	7.8
Nanagai	7.5
Mullet	6.5
Blue grenedier	-4.8*

* The -4.8 indicates that the Brabender units kept on decreasing. All trials were programmed for a 30 minute cycle, it is expected that the units could fall significantly indicating that this fish under these conditions lacks the properties required for gelling or forming viscous solutions. Poor textural properties have been previously reported for Blue grenedier (Brennan 1980).

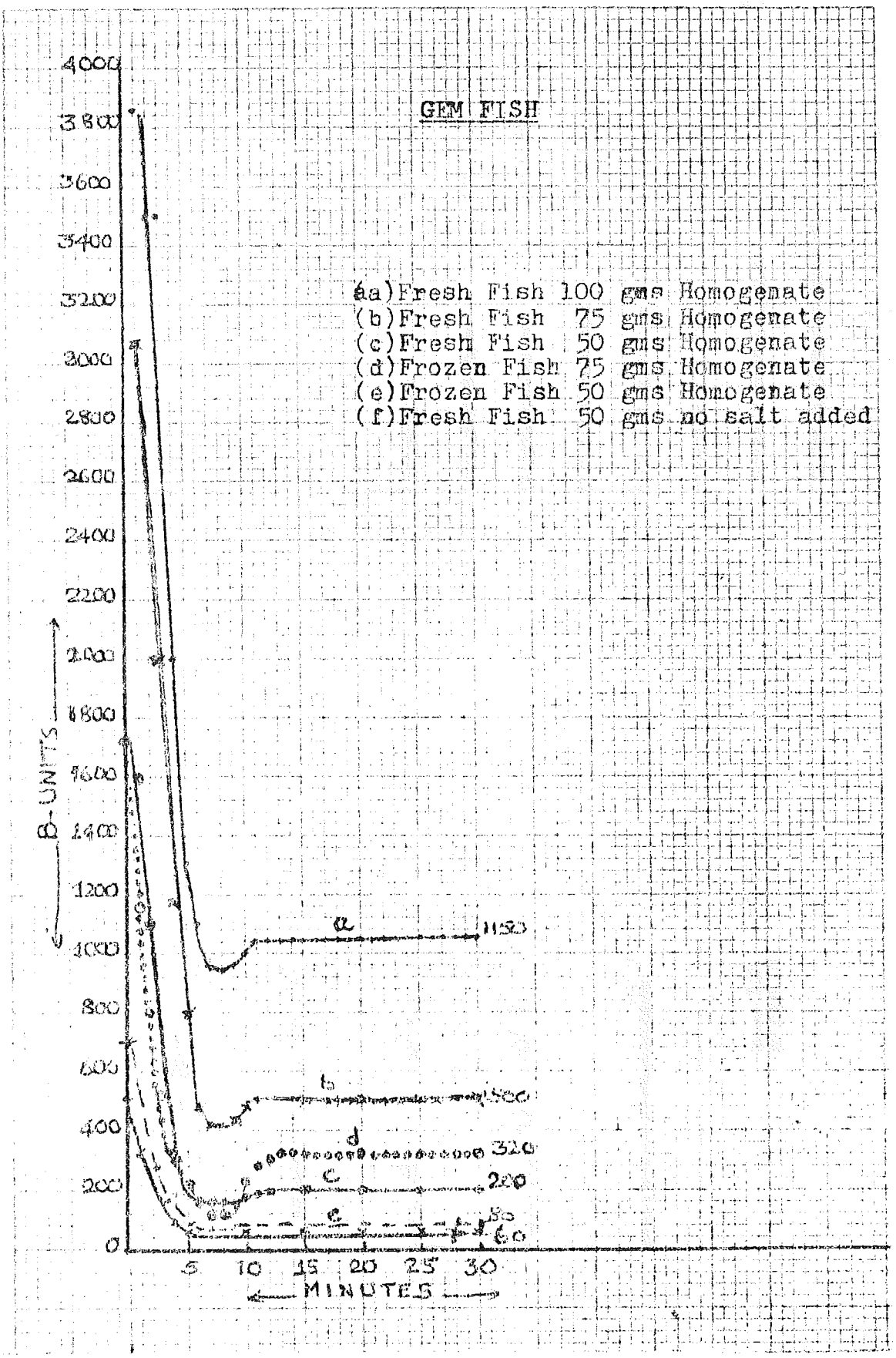

GEM FISH
(a)Fresh Fish 100 gms Homogenate
(b)Fresh Fish 75 gms Homogenate
(c)Fresh Fish 50 gms Homogenate
(d)Frozen Fish 75 gms Homogenate
(e)Frozen Fish 50 gms Homogenate
(f)Fresh Fish 50 gms no salt added
4000
3800
3600
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Q-UNITS
a
1150
b
580
d
320
c
220
e
80
f
60
5
10
15
20
25
30
MINUTES

Figure 25

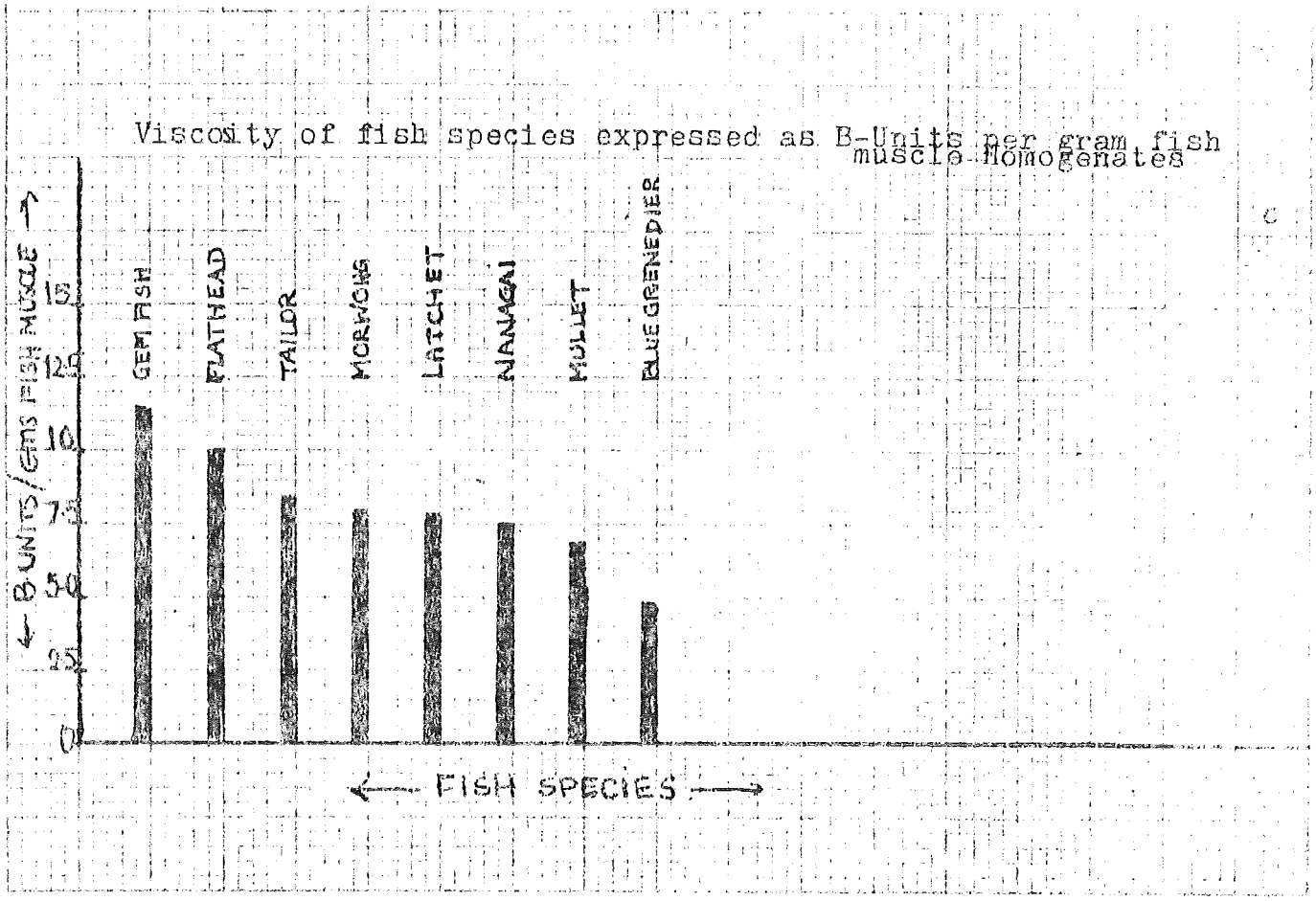


Figure 26

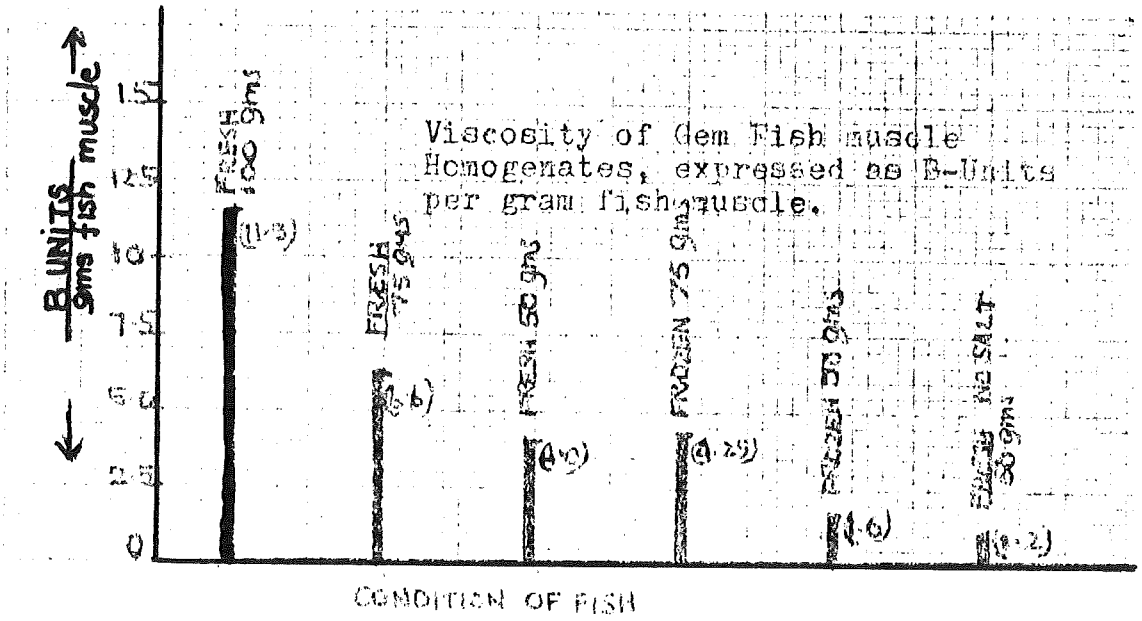
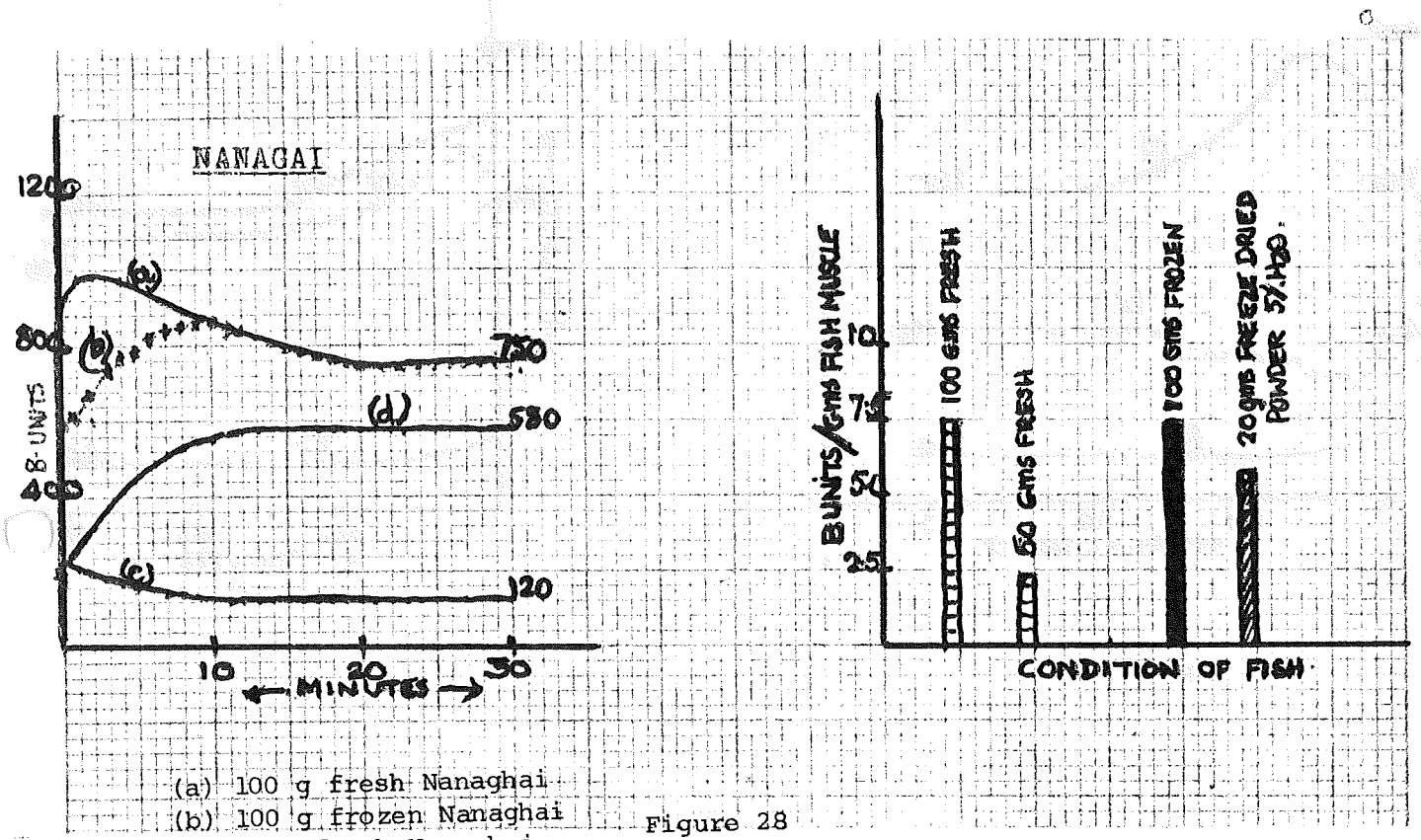


Figure 27



(a) 100 g fresh Nanaghai
(b) 100 g frozen Nanaghai
(c) 50 g fresh Nanaghai
(d) 20 g freeze dried powder

Figure 28

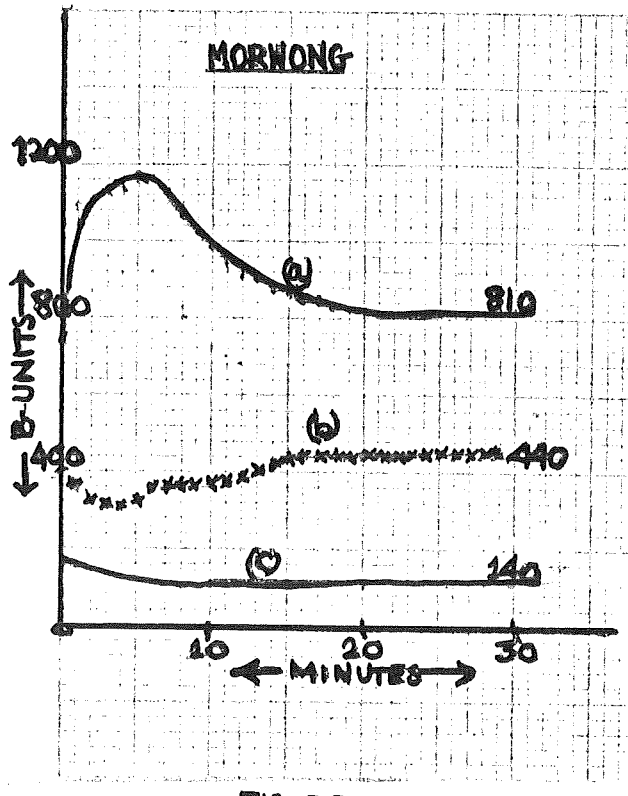


FIG-29

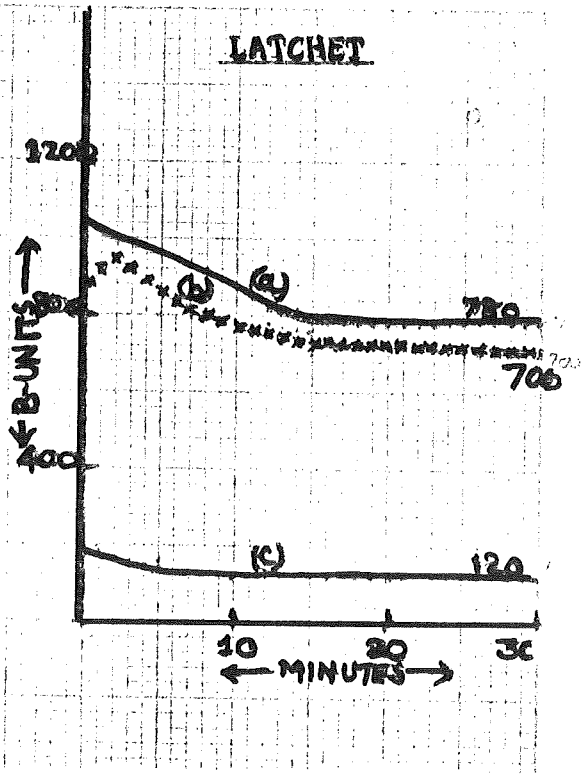


FIG-30

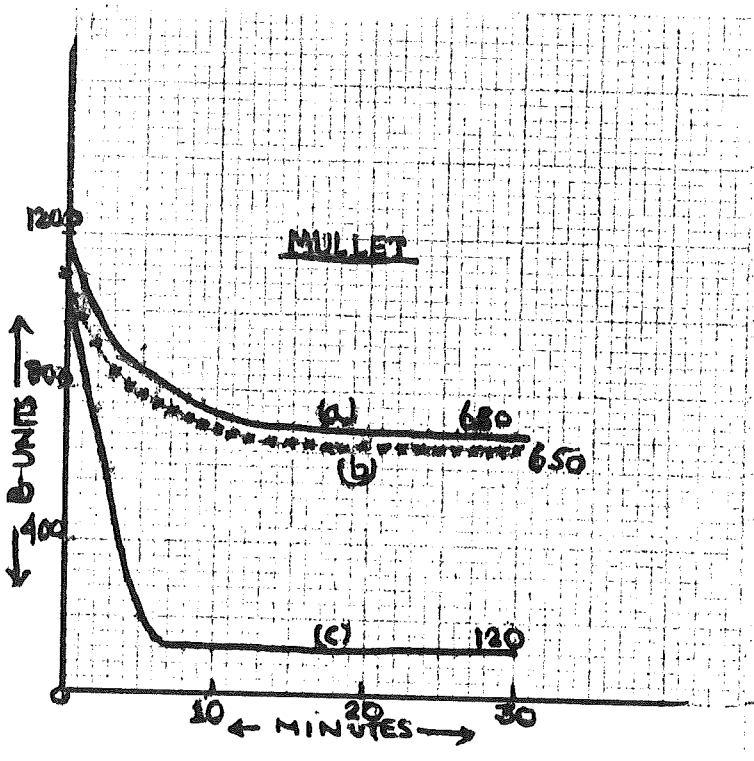


FIG- 31

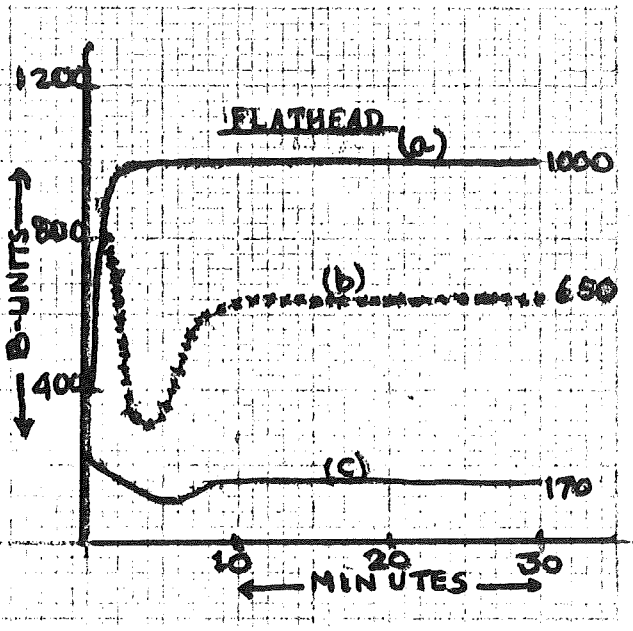


FIG-32

In the above graphs:
(a) 100 g fresh fish homogenate
(b) 100 g frozen fish homogenate
(c) 50 g fresh fish

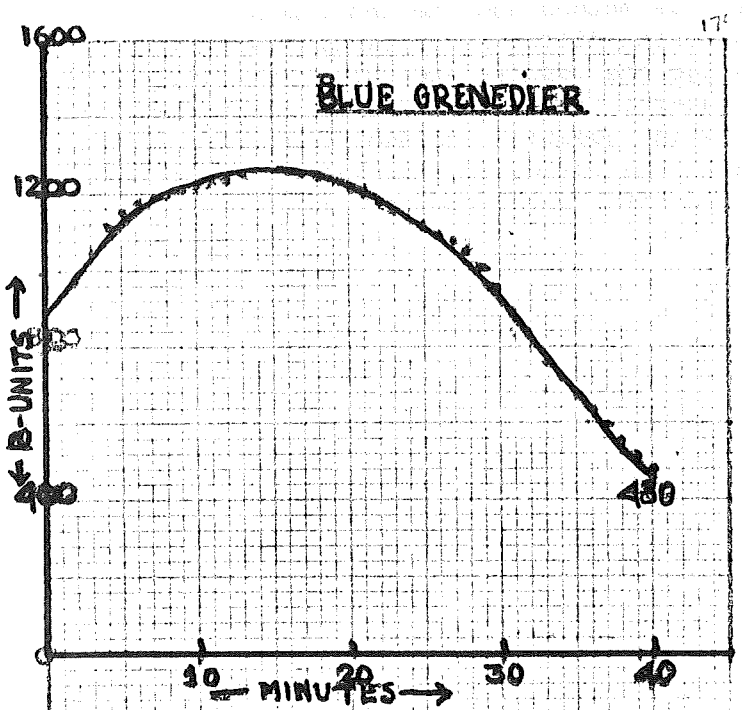

BLUE GRENEDIER
1600
1200
800
400
0
B-UNITS
10
20
30
40
MINUTES

FIG-33

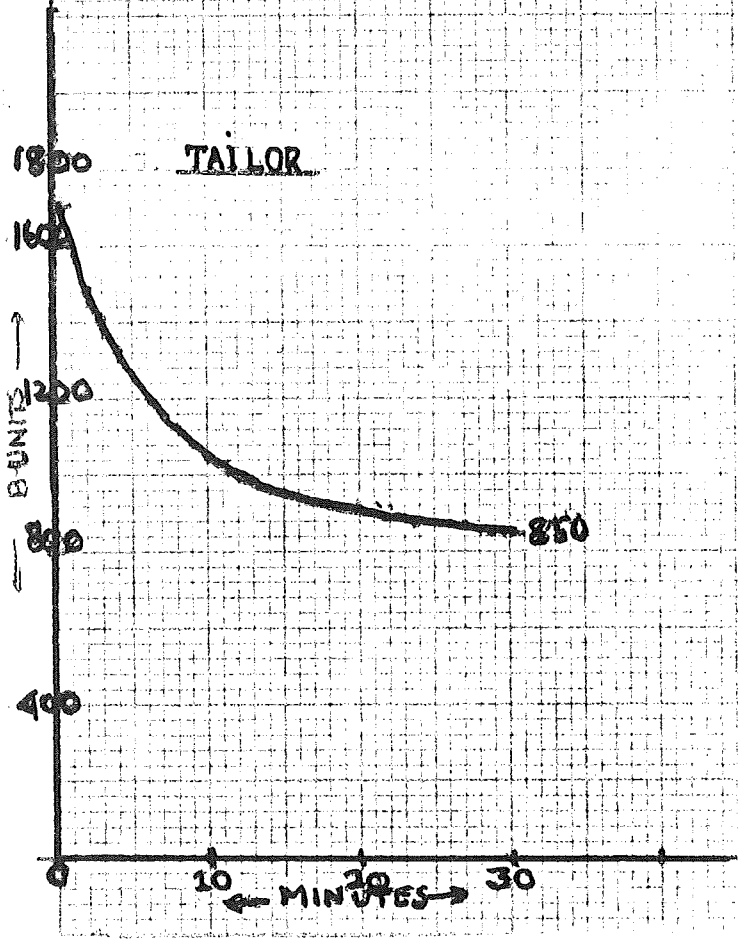

TAILOR
1800
1600
1200
800
400
0
B-UNITS
10
20
30
MINUTES
850

FIG-34

Investigations were also carried out to evaluate the effect of freezing in which a significant lowering of the Brabender units was observed. It would seem that this method and the instrument could serve as a suitable tool to evaluate the difference between frozen and unfrozen fish, however, at this stage the results are only comparative and it is necessary, and would be useful if the opportunity for investigational work could be provided. An interesting question unanswered because of the lack of research, is the question on the effect of viscosity of fish homogenates prepared from varying quality of the fish, i.e. from the fresh stage to spoilt phase.

Production of Protein Isolates

William W. Neinke, Rahman and Mattel in their paper "Some Factors Influencing the Production of Protein Isolates from Whole Fish" defined fish protein concentrate (F.P.C.) as a nutritious, wholesome, protein supplement prepared by solvent extraction procedures, yet "the denatured protein supplement is lacking in such functional properties as solubility, water-binding and holding characteristics and whippability. The absence of these properties, so desired by food formulating industries essentially precludes the use of the solvent extracted F.P.C. for functional properties in beverages, sausages and luncheon meat formulations and for whipped toppings for food.

The above mentioned workers have described the basic approach to protein isolate as involving four essential steps, namely:

1. Solution of the protein in aqueous medium with proper pH and/or salt conditions;

2. Removal of the undissolved residue (bones, grit, scales, etc) from the protein solution;

3. Recovery of the protein solution as a curd by proper pH adjustments or dilutions; and

4. Purification and drying of the protein fraction.

The objective of this section of the work was to produce an isolate from fish muscle, making use of:

(a) the data obtained from the nitrogen solubility index;

(b) the extraction of the protein, and

(c) processing the extracted protein as a dry powder.

The real purpose in the production of the protein isolates is to provide an outlet for the utilisation of waste recovered fish muscle or the utilisation of low commercial value fish muscle. The protein isolate thus produced to be used in food systems in relation to the evaluated functional properties.

Experimental Work

The methods for the production of the isolate investigated were:

(a) calcium co-precipitate freeze dried

(b) low pH extraction of soluble protein (spray dried and freeze dried)

(c) high pH extraction of soluble protein (spray dried and freeze dried).

The type of fish species used were gemfish, mullet, to represent the medium to high fat fish, and nanagai to represent non-fatty to low fat fish.

Calcium co-precipitate type protein isolate was prepared with a view to producing a product similar to milk co-precipitate.

This was prepared by blending fish muscle and water in the ratio of 1:7 at a temperature of 40^{o}C with constant stirring. To this a saturated solution of calcium carbonate was added until a precipitate was formed. The stirring stopped, the solution allowed to stand, and the aqueous layer decanted. The solids were then freeze dried. The reason why freeze drying was done was because of the particle size which was unsuitable for spray drying. The protein content of these isolates were between 75% and 77%.

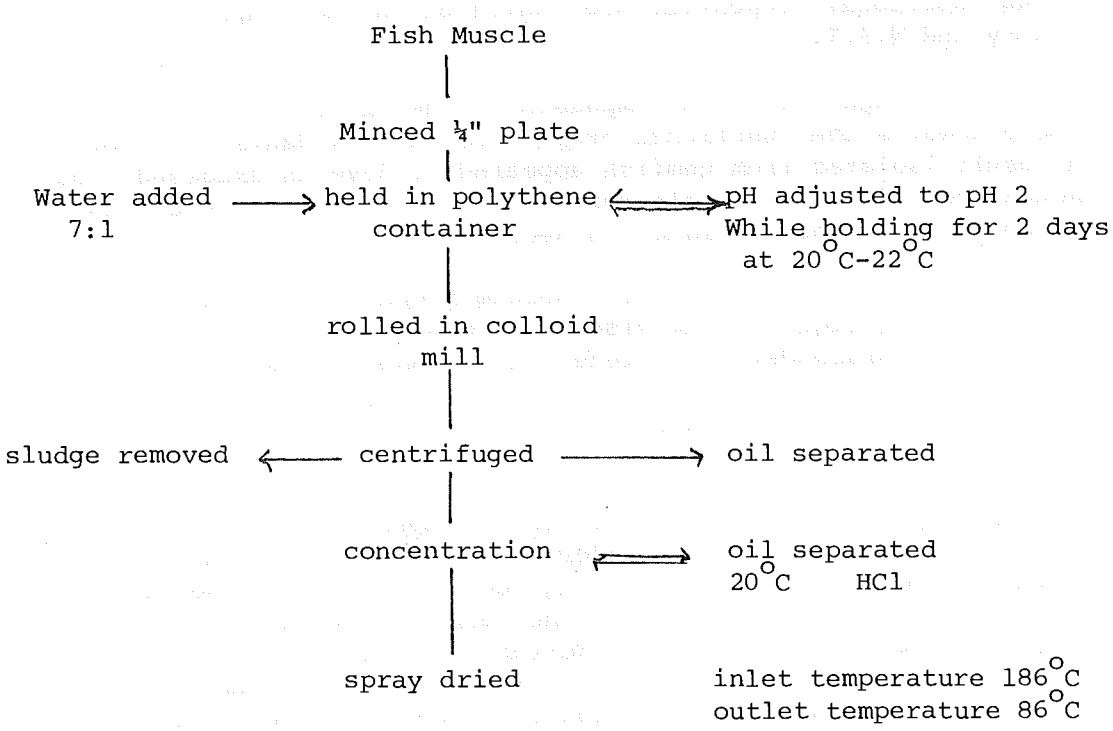
In the second method, the protein extraction was carried out by extracting at low pH using hydrochloric acid, the pH adjusted to pH 2. The fish was blended in a ratio of 1:7 with water and adjsuted to pH 2. The solution was kept for 2 days with continuous pH adjustment. The mixture then centrifuged to remove the oil and sludge (scales, bones, etc.) and the centrifuged liquor then freeze dried. Similar extractions were made at pH levels of pH 2, pH 4 and pH 9.

The advantage in maintaining and drying the slurry at pH 2, is that this slurry could be held or stored for several days without any breakdown, and this would be an ideal situation for commercial operations, where fish waste could be collected from remote areas and hydrolysed, and then transported to a centralised processing and drying operation.

Freeze drying is not a commercial feasibility because of the large expense in removing water, and the next step was to investigate a drying operation which would be more adaptable and economic to current industrial situations. In order to be able to spray dry the mixture, the particle size had to be reduced considerably and this was done by the use of a colloid mill, after the adjustment to the desired pH. A self-desludging centrifuge was used to remove oil and sludge, and then the desludged mixture spraydried. The pH range appears to be critical, in that spray drying became difficult beyond pH 3.5 because gelling in the atomiser feed tube.

The low acid hydrolysed spray dried powder indicated better functional properties, particularly solubility and emulsion capacity, over the calcium co-precipitate, but the problems in the particle size again made spray drying operations difficult.

The actual process can be represented by the flow diagram.



The concentration was a problem, as heat caused gelling beyond 6% to 7% solids. Ultrafiltration was tried on a laboratory scale, which shows some promise, however, at this stage the information on the size and capacity of filters for a pilot plant or commercial operation is not available, and information is still awaited.

The powders produced were of a good light colour and fine particle size. However, since no antioxidants were used, the gemfish isolate quickly oxidised, while the powder produced from nanagai was stable. Powders produced from homogenates to which metabisulphite was added were lighter in colour and retained better functional properties.

An interesting observation in these trials were that the dry isolate powders at pH 2 exhibited a light fishy smell on reconstitution but as the pH was gradually increased the fishy odour was more noticeable at pH 4 and very pronounced at pH 7.

The proximate analysis of the spray powder is shown in the following table.

	Dry isolate from	
	Gemfish	Nanagai
Moisture %	7.0	6.8
Protein (N x 6.25)%	80.8	87.0
Fat	4.5	0.7
Ash	5	5.6

The functional properties are indicated in the graphs for E.C., viscosity and N.S.I.

At this stage, since the emphasis on the production of the isolate was more towards the functional properties, antioxidants were not used. As a result isolates from gemfish appeared to have an oxidised odour, although the colour was a pale white colour and further investigations into the stability of these isolates is necessary.

The viscosity of the protein isolate prepared from nanagai is shown in the following figures, the viscosity measured at pH 2, pH 4, pH 6 and pH 8.5, in Brabender units(refer Fig 35 and Fig 36).

The wettability was measured, using the method described by Rasek, viz.:

> 1 gm sample placed in a graduated cylinder. A finger was placed over the top end. The cylinder was inverted and clamped at 10 cms above a 600 ml beaker containing 400 ml of water. The finger was released and the time for the sample to become totally wet was measured with a stop watch. Wettability was classified as follows: any sample that was wet within 2 minutes or less was considered as excellent; between 2 and 5 minutes, good; between 5 and 15 minutes, fair; any sample that took more than 15 minutes to become wet was rated poor.

The samples of the protein isolate rated good, requiring between 4 to 5 minutes for complete wetting.

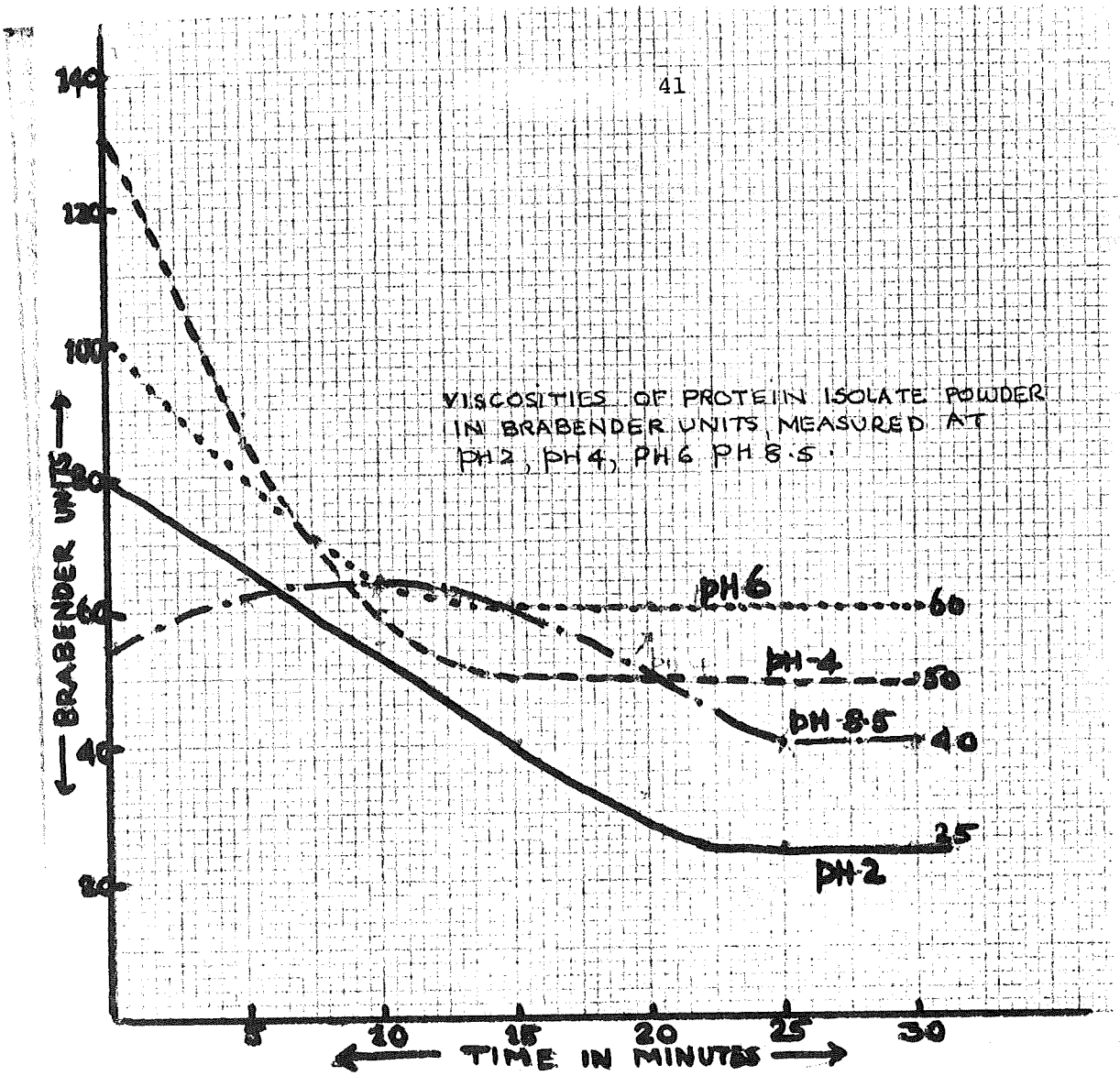


Figure 35

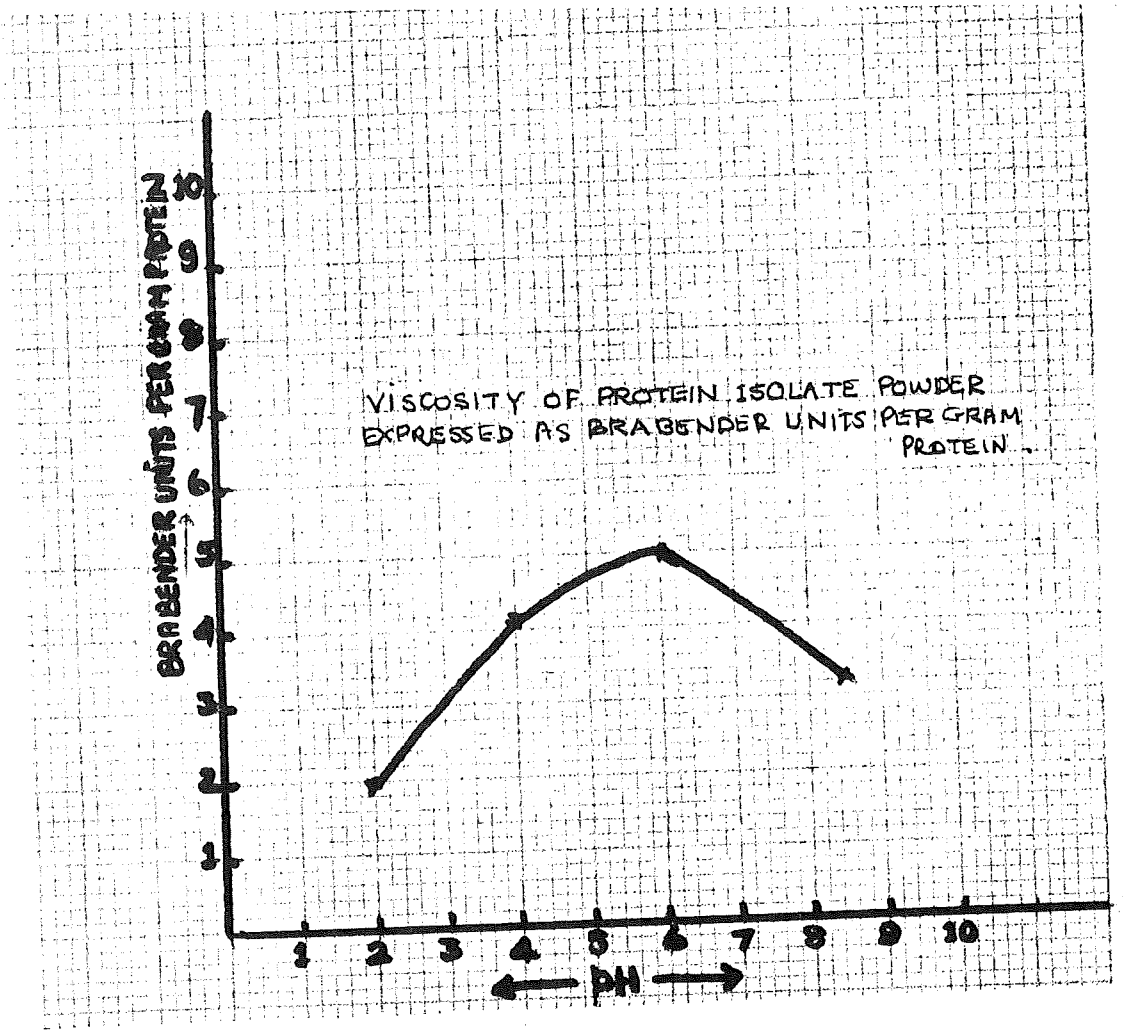


Figure 36

Texture Measurements Using the Instron 1140

The evaluation of various texture parameters using Texture Profile Analysis (Bourne 1978) should give an indication of the gell properties of the various species of fish tested.

The Instron type TM1100 Universal Testing Machine was used in this project to carry out a two bite compression test on 6 species of fish. The textural properties were evaluated by using appropriate attachment for evaluating the various textural parameters and measured in terms of force required to shear or compress specimens under standard conditions specified for the machine.

The testing was done to evaluate the suitability of the species of fish to form gels, and the textural characteristics of the gels were evaluated. The gels were therefore prepared using minced fish muscle as:

Minced fish muscle	70%
NaCl	2%
Ascorbic acid	0.25%
Sodium tripolyphosphate (TPP)	0.25%
Sugar	3%
Water and ice	24.0%
Total	100%

The above mixture was mixed in a blender at low speed, by adding the fish, NaCl, sugar, ascorbic acid and half the quantity of ice and water. The tripolyphosphate and half ice and water was then added and mixture blended at high speed for 3 minutes.

The blended fish homogenate was then filled into cylindrical plastic containers, 12 mm diameter and 12 mm high, and these containers then placed in a water bath previously heated to 100°C, and held till the gel was formed, and the temperature at the centre of the cell was 75°C. The cells were immediately cooled so that the gelled cylinders could be removed from the plastic cells.

Compression of the Sample

The compression was done by means of the crosshead, at a speed of 50 mm/minute, and chart speed of 200 mm/minute. The sample was pressed to 33% of its height, i.e. from 12 mm to 4 mm.

Evaluation of the Various Parameters

The following seven parameters were examined:

Fracturability: defined as the force at the first significant break in the curve.

Hardness: the peak force during the first compression cycle.

Cohesion: the ratio of the positive force area during the second compression to that during the first compression (A_2/A_1).

Adhesiveness: defined as the negative force area for the first bite, representing the work necessary to pull the compressing plunger away from the sample.

Springiness: defined as the height the food recovers during the time that elapses between the end of the first bite and the start of the second bite.

Gumminess: Bourne (1978) defined as the product of gumminess and springiness.

The gels were prepared to represent a "Kamoboko" type product and which is indicated in the abovementioned formula.

GENERAL DISCUSSION AND CONCLUSION

The nitrogen solubility index and the viscosity measurement by the Brabender Viscograph has provided useful data. The method for the production of the spray dried fish protein isolate was formulated from the data obtained from the nitrogen solubility determined for the species of fish under investigation.

The fish protein concentrates and fish protein isolates so far produced have involved azeotropic treatment methods to eliminate the fat from the fish with additional use of antioxidants. However, in this project the acid treatment for the production of the isolates has several advantages, except that control over oxidation of fats is through the use of permitted antioxidants.

The process outlined for the production of the isolates has the following advantages:

(a) The acidified slurry can be held for extended periods at ambient temperatures.

(b) The solubilisation of the protein fractions is best effected at pH 2.0 (for acidified treatments).

(c) If required purified proteins can be re-precipitated by increasing the pH towards the iso-electric point close to the range of pH 4.

(d) Because antioxidants were not used, the protein isolate prepared from the mullet developed rancid odours with a darkening in colour within a few days the protein isolate from the gemfish was more stable while that from the nanagai was stable for several weeks retaining its light colour and functional properties.

(e) The isolate from acidified treatments has no foul fishy odour, but on increasing the pH the fish odour began to be more pronounced, up to about pH 6, and thus would be useful if fish flavour boosters are to be produced.

(f) The fat from the acidified slurry was separated by centrifugal action and although complete separation by centrifugal action may be difficult, additional use of permitted antioxidants would assist to retain the stability of the isolates.

(g) The wettability and solubility of the powders is good.

At this stage the indications are that recovered fish muscle and underutilised low fat content muscle can be suitably used for the production of fish protein isolates with functional properties, however, there is need for more investigations, particularly the utilisation of higher fat content fish muscle and the use of antioxidants.

Utilisation of recovered fish muscle for the production of various fish products is indicated in the following figures.

In Figure 37 muscle from various fish species were examined for their viscosity and gelling capacity, using the internal quality analyser (I.Q.A.) and the viscosity before and after heat treatment, and varying concentrations of fish muscle was determined by Brabender Viscograph and visual observations.

The importance of this type of gell formation has various useful applications, particularly in the formation of "Kamaboko" type products and in the speciality seafood formulations as shown in the figures 38-43.

At this stage the data obtained through this project is not enough to fully comment on the value of the results nor to make conclusive statements, however, the preliminary work done, with the internal quality analyser, was reported earlier, and from the data so far collected it would be interesting to note that there is a trend, similarly indicated through the results of the Brabender Viscograph, that the viscosity decreases with decrease in fish muscle content and firmness of gels, could be represented by the width of the bands, the longer the width representing firmer gels (Figures 37)

From this data, it appears that research on the internal quality analyser, should be continued as this instrument indicates good potential for its use as a fish muscle functional property analyser. This instrument is currently being used for assessing the quality of fruits and vegetables, and is in demand because the samples under examination are not destroyed, but measurements are made directly on the fruits and vegetables as is.

With the availability of the deboning boning machines, an increasing amount of fish muscle is now being recovered. The research findings indicate that blending of recovered fish muscle from different fish species can be used in the production of various fish products, as indicated in figures 44-45.

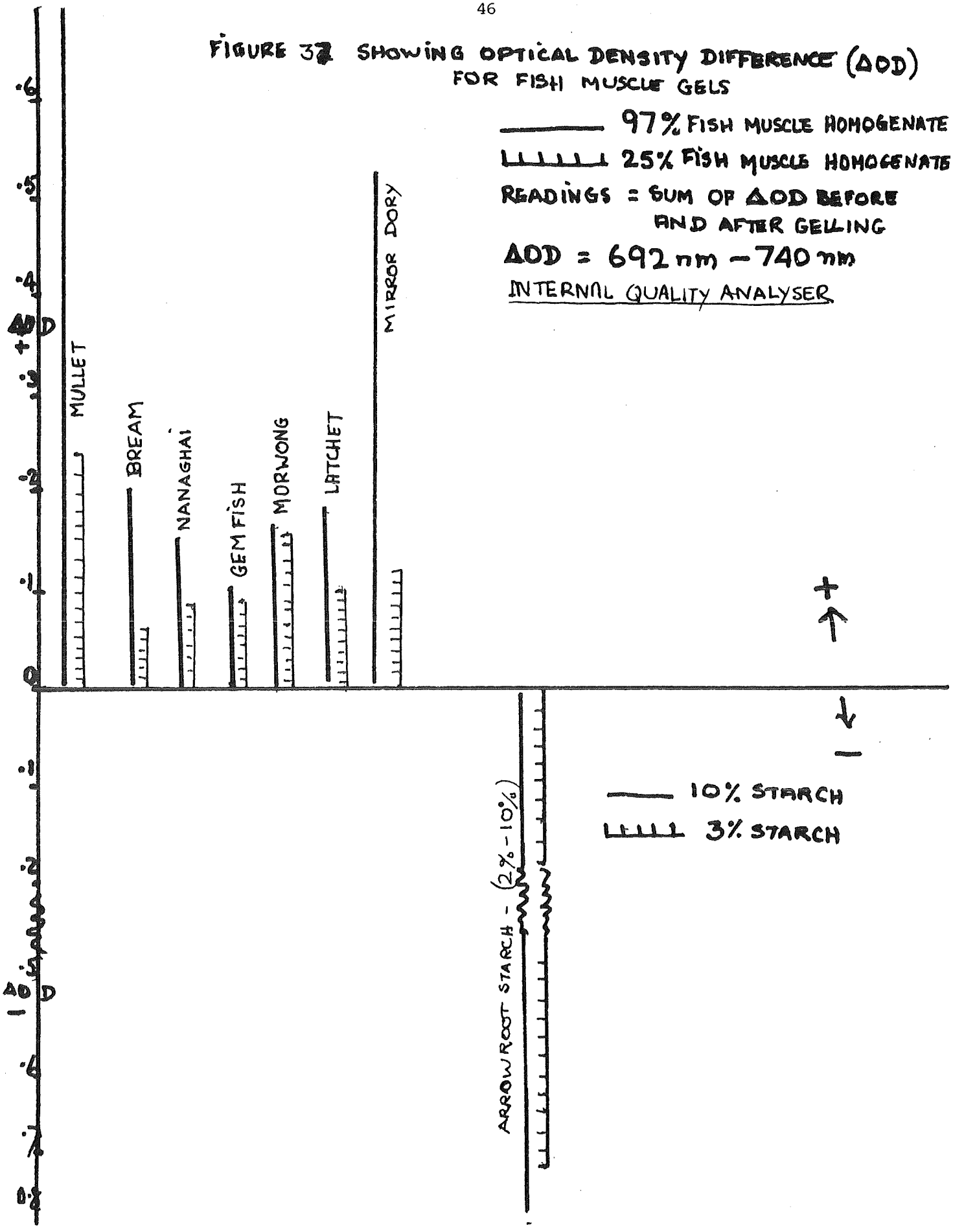
In summarising the conclusion:

1. The method for the manufacture of the fish protein isolate can be recommend, however, there is an urgent need to investigate the use of antioxidant to provide the stability of the isolate. The economy of the process could be improved if membrane concentration can be used.

2. The proximate composition and the nitrogen solubility index are providing useful information, and the determination of both the proximate composition and nitrogen solubility should be carried out for all commercial species of seafoods available in the Southern Hemisphere. This project could be undertaken jointly by various national and international organisations.

3. So far the Brabender viscograph and the internal quality analyser (I.Q.A.) has not been used for seafood products, and the future potential for the application in industry as a tool to measure the functional properties of fish muscle protein should be investigated.

FIGURE 3 SHOWING OPTICAL DENSITY DIFFERENCE (ΔOD) FOR FISH MUSCLE GELS



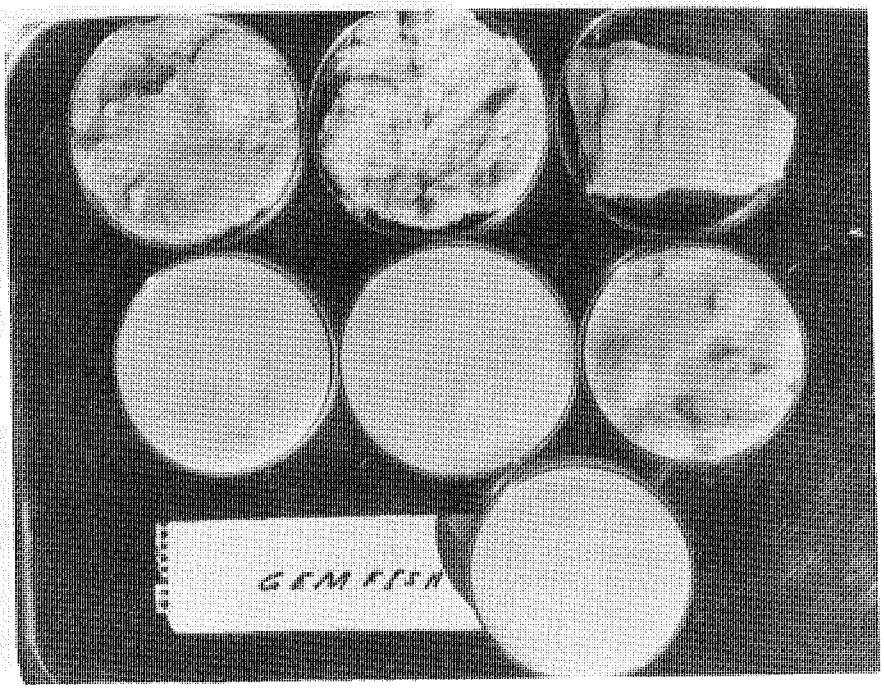


Figure 38a

Gemfish homogenate of varying concentrations before gelling

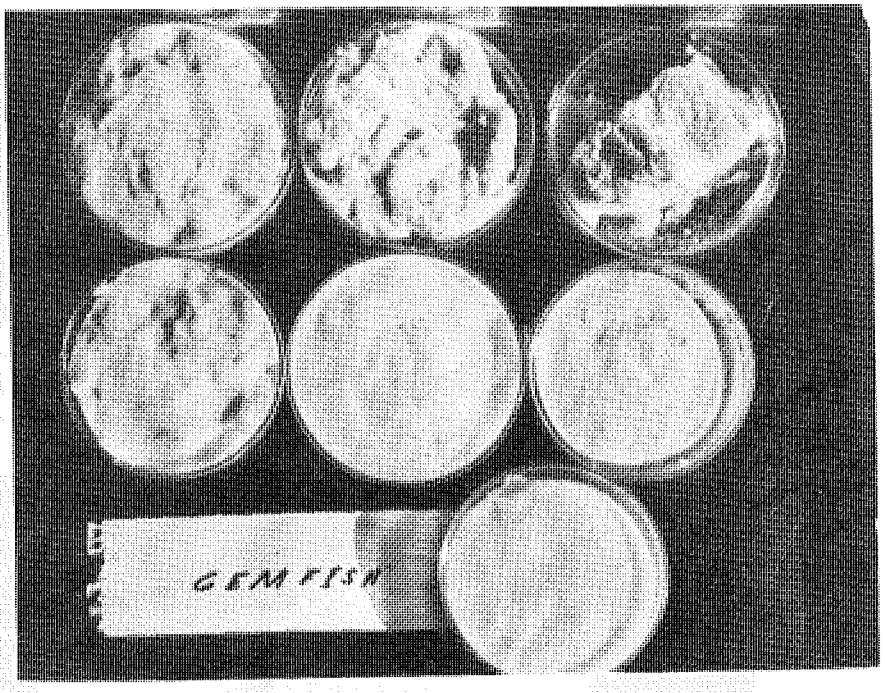


Figure 38 b

Gemfish homogeante of varying concentrations after gelling

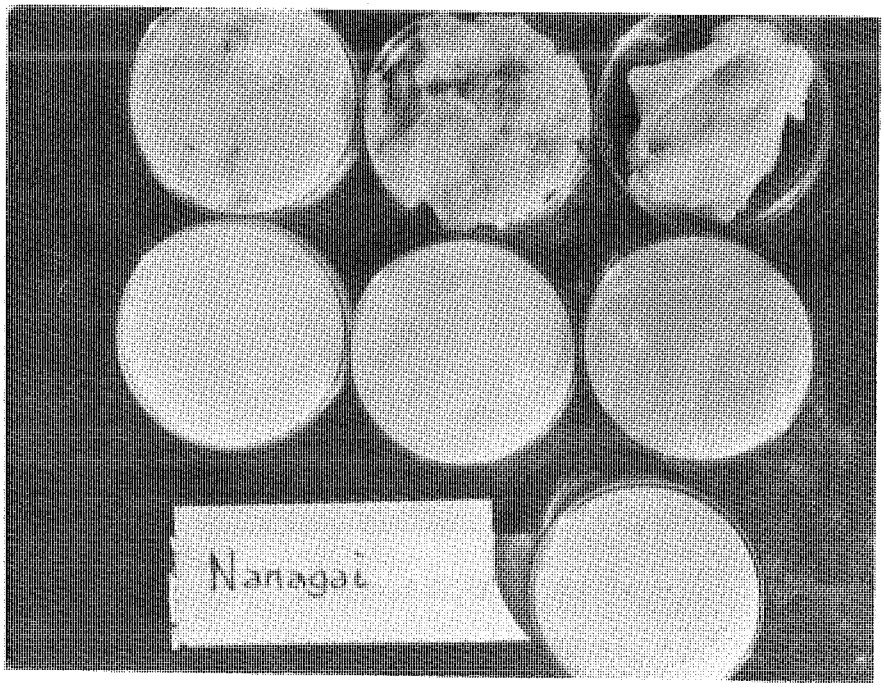


Figure 39 a

Nanagai homogenate of varying concentration before gelling

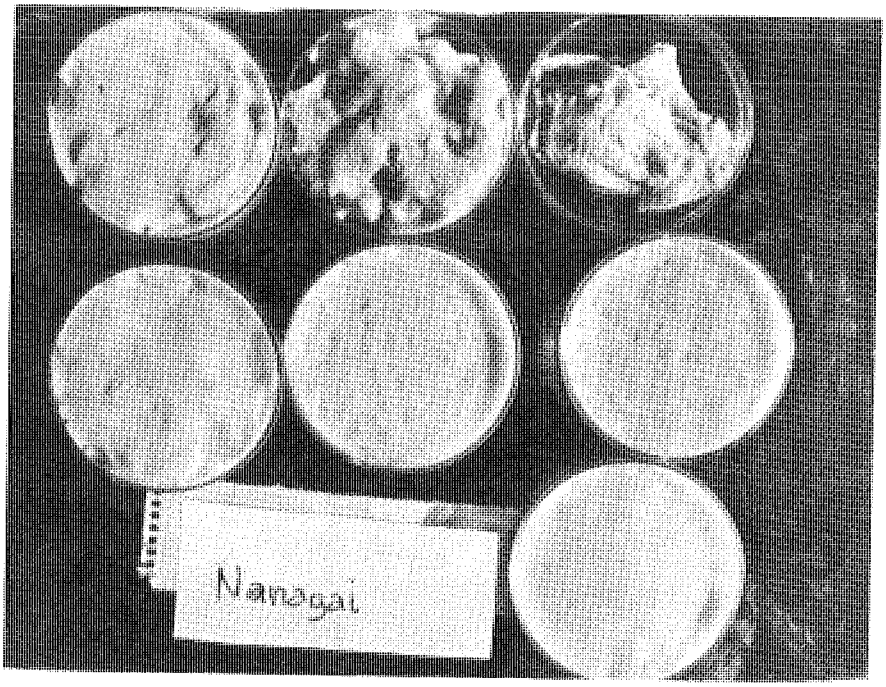


Figure 39 b

Nanagai homogenates of varying concentration after gelling

Figure 40 a

Latchet homogenate of varying concentration before gelling

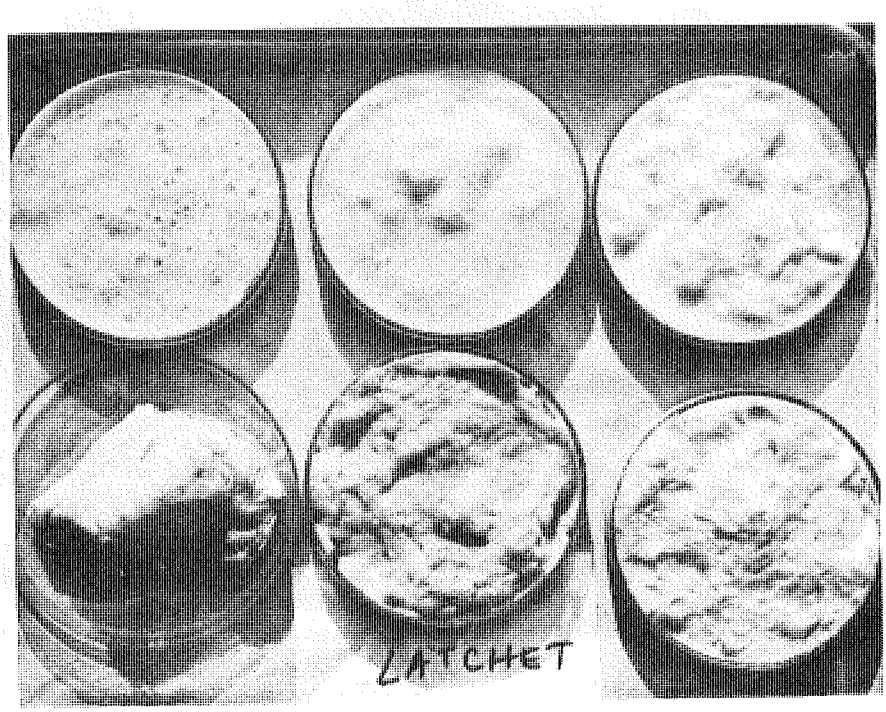


Figure 40 b
Latchet homogenate of varying concentration after gelling

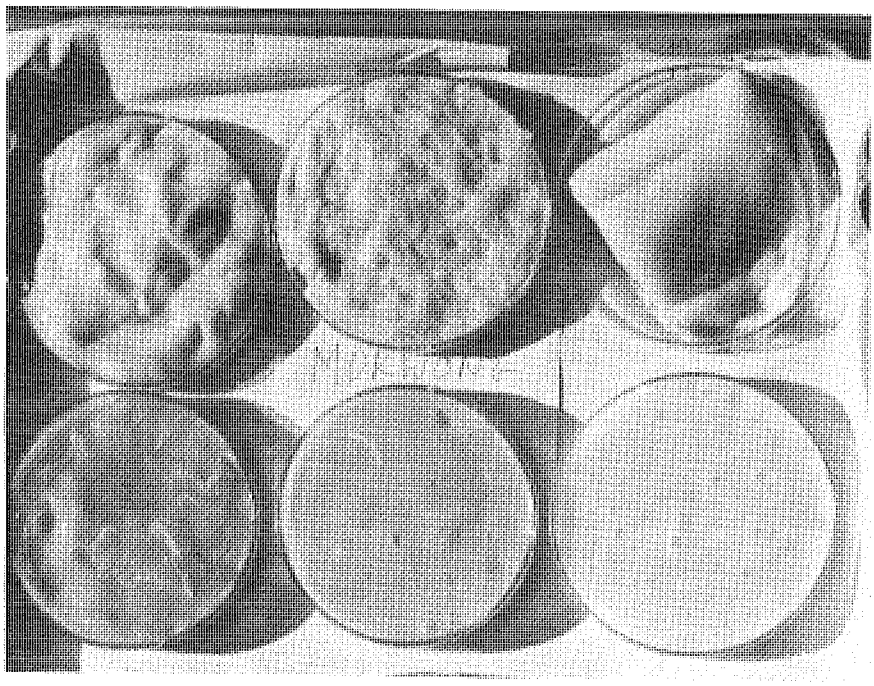


Figure 41 a

Morwong homogenate of varying concentration before gelling

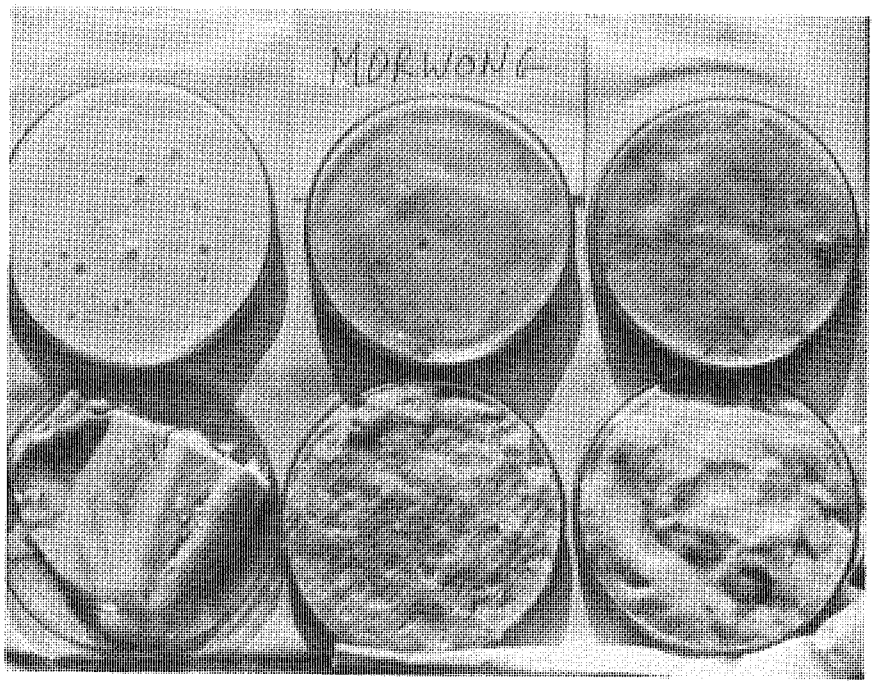


Figure 41 b

Morwong homogenate of varying concentration after gelling

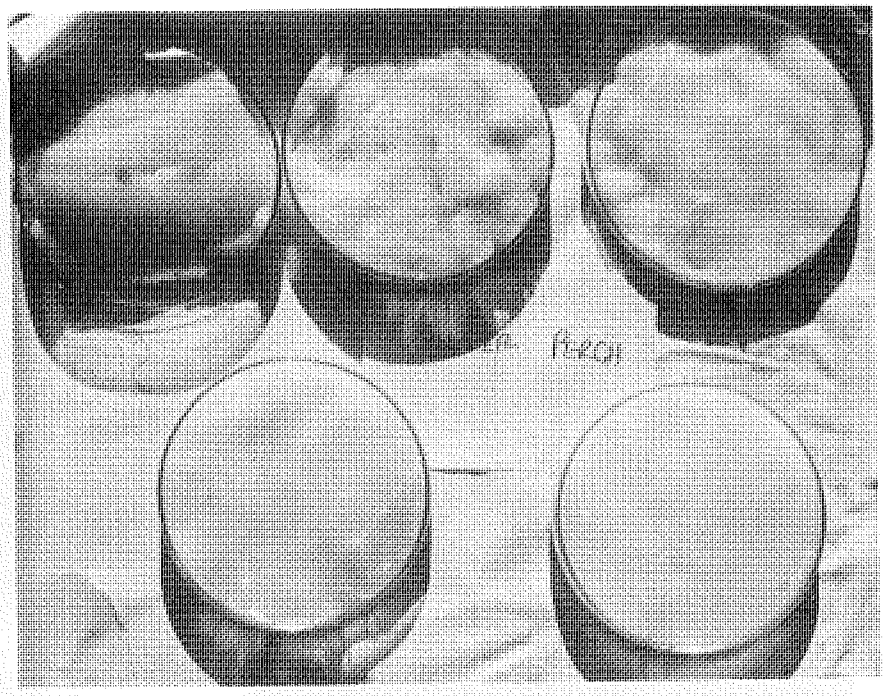


Figure 42 a

Silver Perch homogenate of varying concentration before gelling

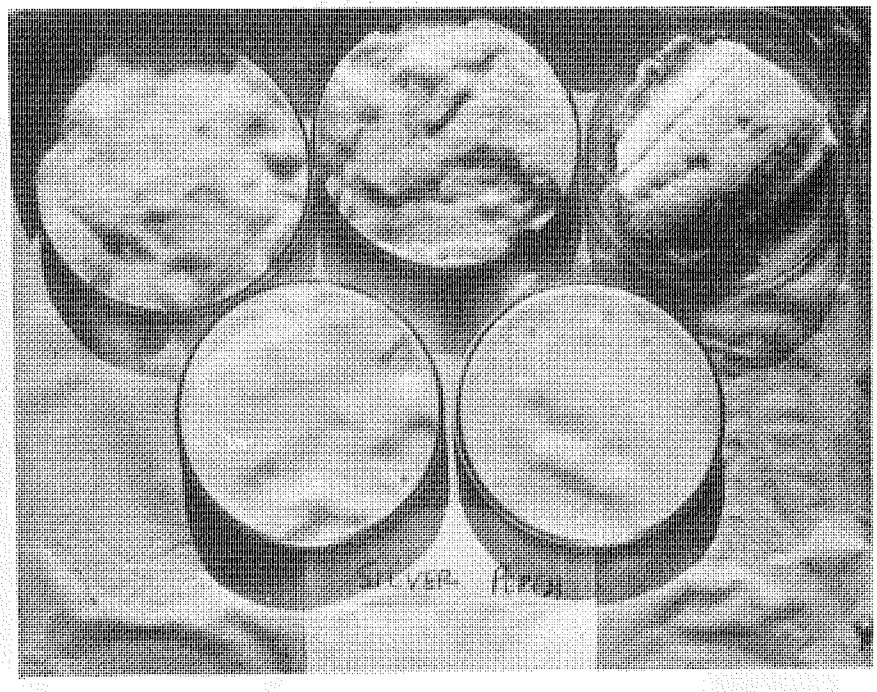


Figure 42 b

Silver Perch homogenate of varying concentration after gelling

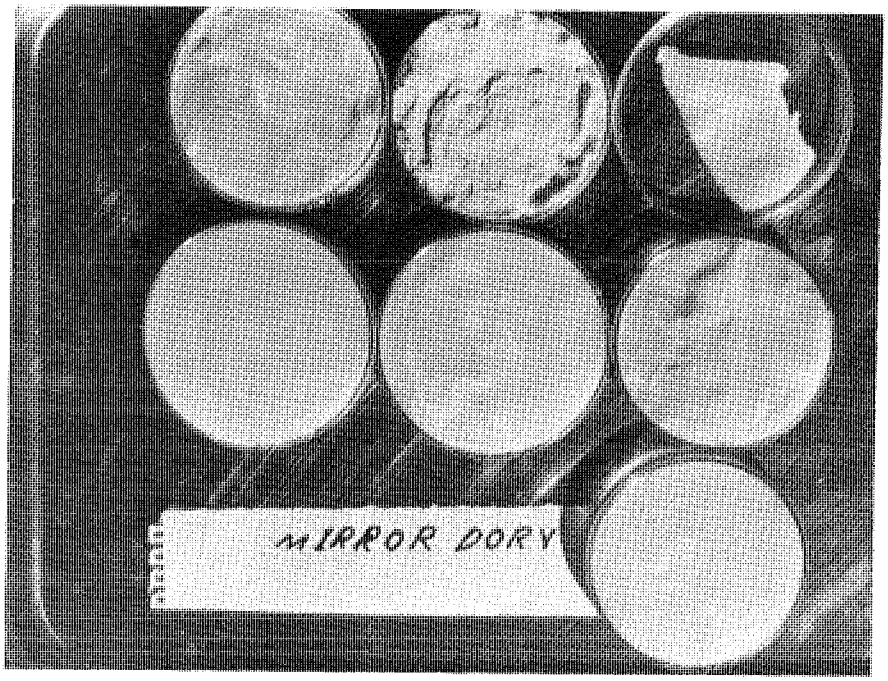


Figure 43 a

Mirror Dory homogenate of varying concentration before gelling

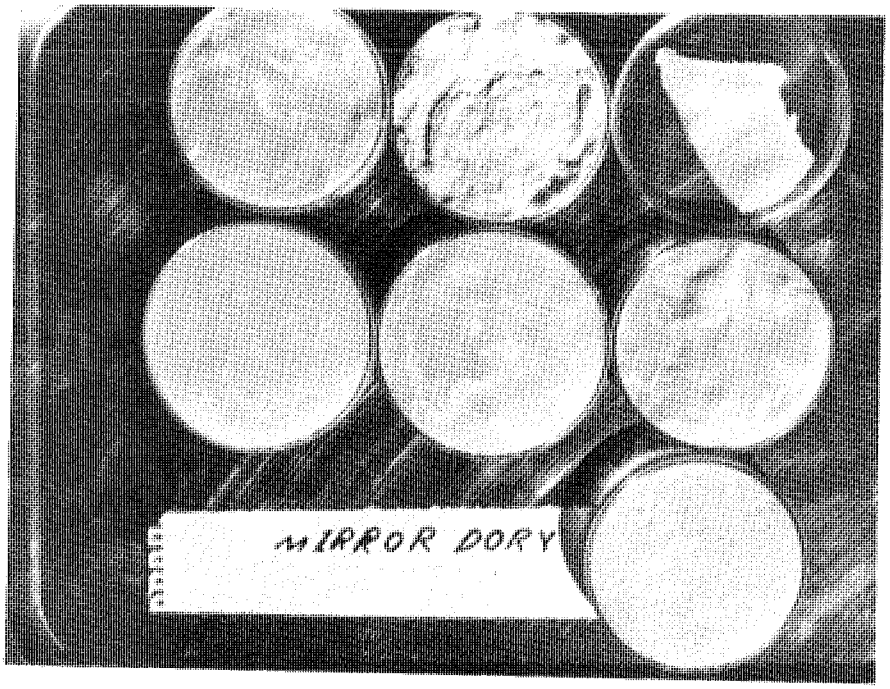


Figure 43 b

Mirror Dory homogenate of varying concentration after gelling

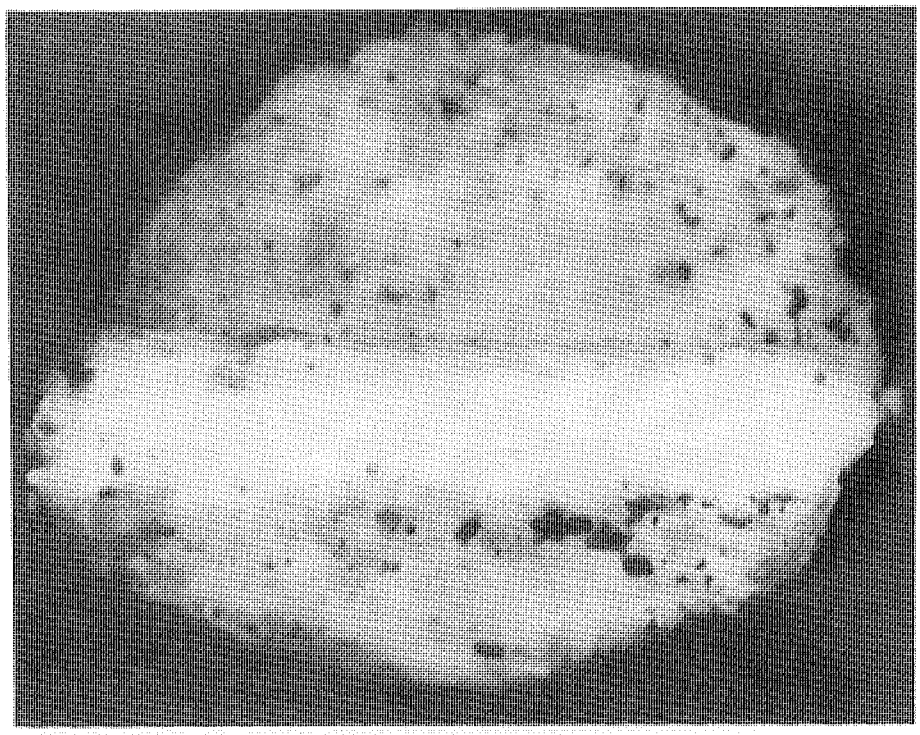


Figure 44

Potato, gelled blended fish sandwich

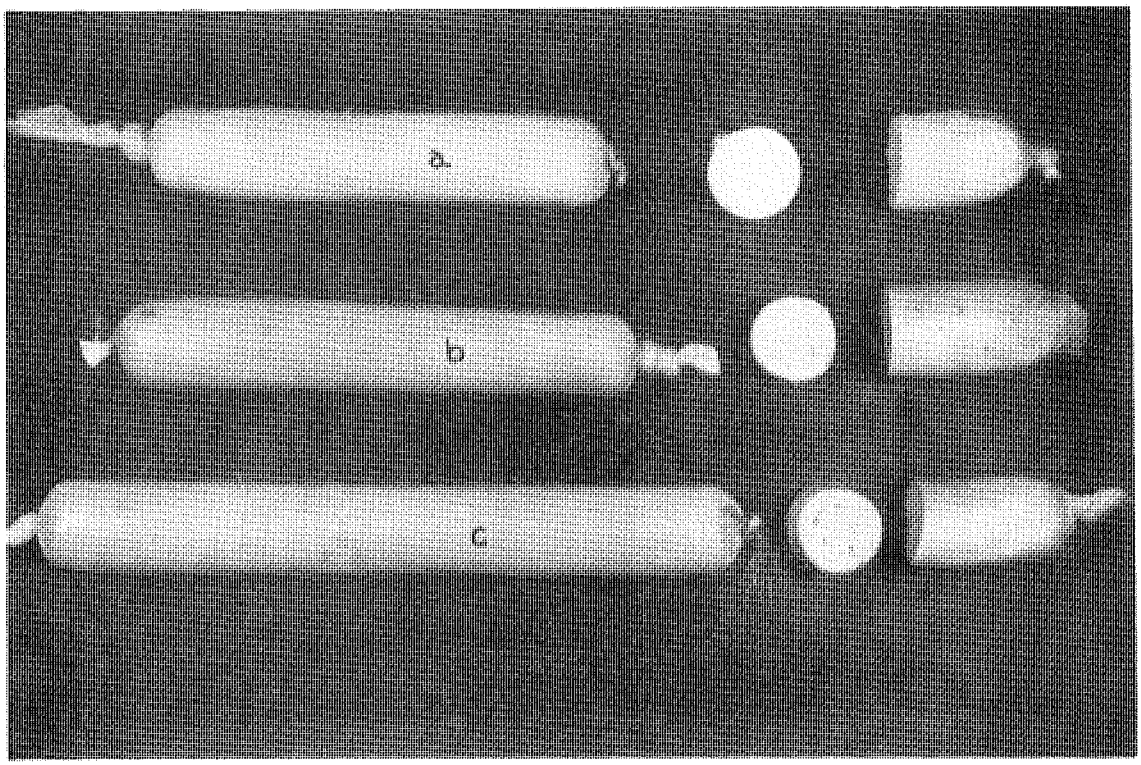


Figure 45

Blended gemfish - Jack Mackerel Sausage
a = gemfish
b = 50% gemfish/50% Jack Mackerel
c = Jack Mackerel

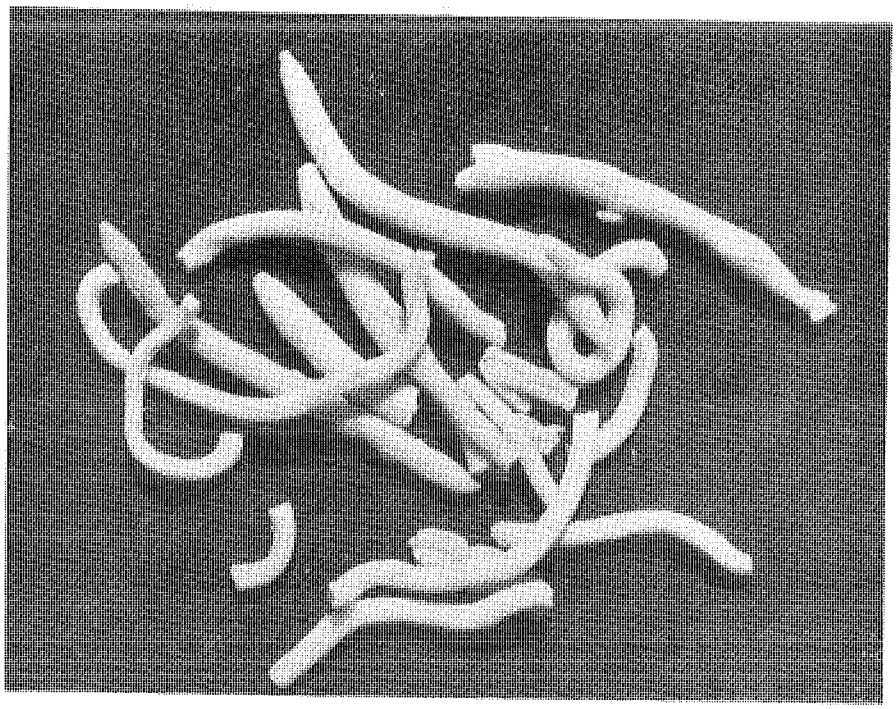


Figure 46

Extruded - baked dried fish straws

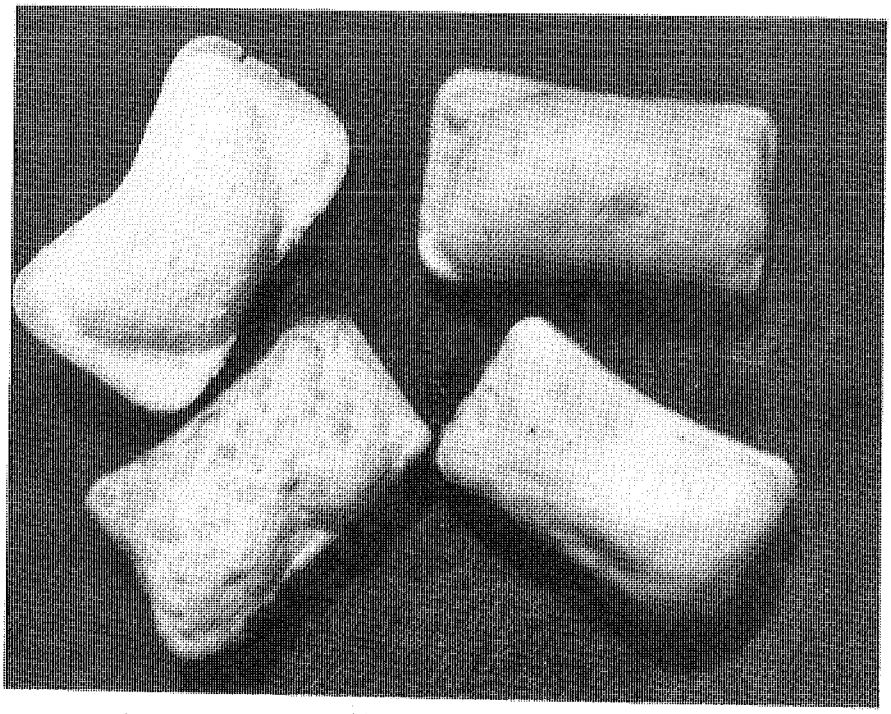


Figure 47

Extruded puffed fish snack

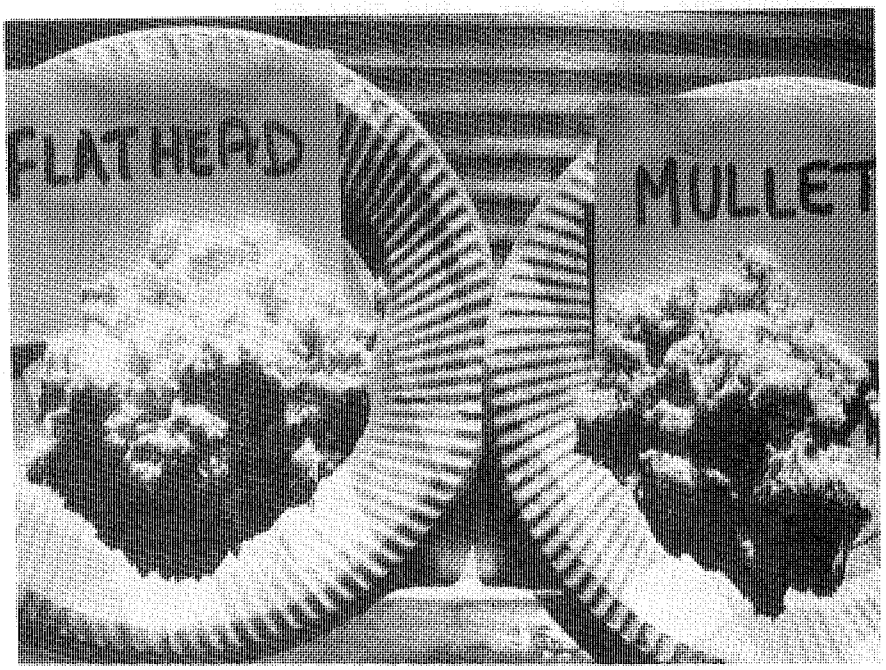


Figure 48

Fish protein isolates from flathead and mullet

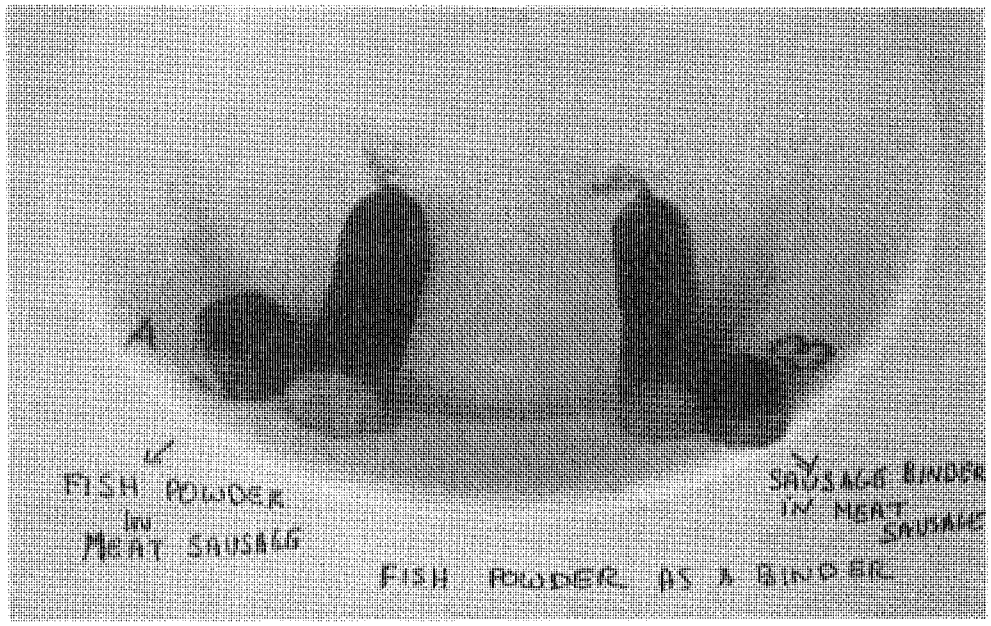


Figure 49

Fish protein isolates used as binder in meat sausages

8. RESEARCH INVESTIGATORS PROFILE

Mr. P. A. Baumgartner - Principal Investigator

Academic qualifications: B.Sc.Agr., Dip.Tert.Ed.

Status: Head, School of Food Sciences, Hawkesbury Agricultural College.

Fellow of the Australian Institute of Food Science and Technology (and past president) of the N.S.W. Branch.

Experience: Prior to joining the College he was the Chief Chemist for a period of nine years with Swift/Mayfair group of companies, producers of processed products, hams, bacon and frozen meats for export.

Mr. Baumgartner was in West Germany working on a research project at the Institute of Technologie, Kulmbach.

Participation in research programmes has resulted in the publication of the following, which are relevant to this proposal:

1. Thomas, M. A., Baumgartner, P. A., Board, P. W., Gipps, P. G. (1973), "Evaluation of some non-meat proteins for use in sausage", J. Fd. Tech., 8, 184-185

2. Thomas, M. A., Baumgartner, P. A., Hyde, K. A., (1974), "A Study of Some of the Functional Properties of Calcium Co-Precipitates in a Model System", Aust. J. Dairy Tech. 29, 59-64.

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4. Baumgartner, P. A. (1975), "Meat and Fat Quality Review", Proc. Australian Pig Production Review Conference, Gatton.

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7. Baumgartner, P. A., (1979), "Different Training Requirements for Food Technologists", Food Tech. in Australia, 31, 41-49

8. Baumgartner, P. A. and Hicks, P. A. (1979), "Pilot Plant as Food Process Engineering Education", Pace 32 No. 9, 13-20

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10. Baumgartner, P. A. and Klettner, P. G., (1980) "The Influence of Temperature on Some Parameters for Dry Sausage During Ripening", Meat Science 4, 191-201.

Mr. A. F. D'Mello - Associate Investigator

Academic qualifications: B.Sc. (USA) A.A.I.F.S.T.
B.Sc.(Hons) Bombay University with honours in Chemistry and Physics
M.Sc. University of Washington (USA) in Food and Fisheries Technology
Chemical Engineering training at the University of Bombay
2 years at Massey University, New Zealand, keeping terms for the Ph.D. degree (Food Tech.)

Status: Lecturer in Food and Fisheries Technology

Mr A. F. D'Mello has been lecturing in Food and Fisheries Technology for the past 11 years, and is responsible for the teaching and application of the course. As part of the normal course of Food Processing Operations, the students, under the guidance of Mr. D'Mello, have produced a large variety of fish products, which include canned fish in different sauces, fish sausage, meat/fish patties and sausages, fishballs, fish frankfurters, smoked fish products and recently, during 1975/76 several projects on 'gel' fish proteins, hydrolysis of fish and shellfish waste were carried out.

During the period February 1975 to June 1975, Mr. D'Mello worked at the Humber Laboratory in Hull, England, which is a sub-station of the Torry Research Station. As a result of his participation, Mr. D'Mello has contributed to the standardisation of a process for the mechanical hot smoking of fish.

Prior to taking up the position at Hawkesbury Agricultural College, Mr. D'Mello was the Assistant Professor of Fisheries Technology at the Government of India Institute of Fisheries Education, under the Ministry of Food and Agriculture, prior to which he held the position of Fisheries Research Officer for a period of ten years.

The School of Food Sciences at Hawkesbury Agricultural College has actively participated in the Australian Fishing Industry and the Schools for Seafood Processors held at the College since 1974 were convened by Mr. D'Mello, who is also the co-editor of the published proceedings. Mr. D'Mello was invited to present a paper at the Seafood Expo '76 in Melbourne.

Mr. D'Mello has published the book "Freeze Fish and Have Fun". This publication gives details on how, when and where to buy fish and how to freeze it, and is based on the work undertaken by Mr. D'Mello at the School of Food Sciences, Hawkesbury Agricultural College. The manual on Sensory Evaluation of Foods is also written by Mr. D'Mello and is used as a text book.

Mr. L. Gullifer - Staff temporarily employed on this investigation

Qualifications - Dip. App. Sci. (Food Tech.) H.A.C.

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