

Studies on the Utilization of Whale Meat by the use of Pancreatic Tryptase of Whales

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The present experiments were carried out in order to obtain enzymes suitable for bacterial culture and production of diphtheria antitoxin from whale meat digested by whale pancreas. The authors prepared 25 kinds of peptone from a definite quantity of meat at a definite pH value with varying amounts of enzyme and at different lengths of time. With each of the peptone obtained, the ration of total nitrogen to free amino radical-N and the yield of peptone were measured. With each peptone obtained under various conditions, test culture of diphtheria bacillus and production of its antitoxin were tested. As a result of these experiments, following observations were made:

1) It was found that better yields of peptone were obtained when reaction time was prolonged. For example, when 3 g of pancreatic powder was used, yield increased proportionately with increase in reaction time.

Table 1

No.	Reaction time (Hours)	Nitrogen of liquefied portion (g)	Percent of digestive (%) (Comment. 1)	Amino nitrogen : Total nitrogen
12	12	4.42	36.0	1 : 3.0
2	24	6.63	51.5	1 : 2.3
6	48	6.93	56.5	1 : 2.7
8	72	9.51	77.3	1 : 2.7
23	120	12.06	98.0	1 : 2.2

2) When the reaction time was made constant, the amount of amino nitrogen increased with the increase in the amount of enzyme but no effect

Table 2

No.	Amount of used pancreas (g)	Amino nitrogen : Total nitrogen	Nitrogen of liquefied portion (g)	Percent of digestive (%)
1	2	1 : 3.4	6.88	56.0
2	3	1 : 2.9	6.33	51.5
3	5	1 : 1.9	7.46	60.7
4	7.5	1 : 1.3	8.00	65.0

1) As is described later, the amount of nitrogen in Frozen whale-meat is 16.4%. This value was obtained by dividing the amount (in gram) of nitrogen found in liquefied portion by $0.164 \times 300 \times 0.25$ and then multiplied by 100.

was observed in the yield of peptone, as can be seen from the following graph where the reaction was limited to 24 hours.

3) Addition of duodenum powder amounting to about 1/3 the amount of pancreas powder results in the increase of 20% in the yield even when the length of reaction time is the same.

Table 3

No.	Amount of used pancreas (g)	Amount of used duodenum (g)	Reaction time (hours)	Yield (g)
13	1	0.3	24	35.5
24	1	0.3	24	43.5
2	2	1.7	24	44.0
25	2	0.7	24	50.5
2	3	1.0	24	44.1
26	3	1.0	24	55.0

4) As a result of experiments in diphtheria culture and production of antitoxin, under various, it was found that some of the peptone obtained by the authors were as good as the market products. It was also found that those suited to the production of antitoxin were those in which the ratio of total nitrogen to amino nitrogen was in the order of 3.9.1.

EXPERIMENTAL

1) Preparation of enzyme powder

Enzyme powder as described in the paragraph on 'Trypsin in a whale (*Balaenoptera borealis* Lesson)' was used. This was prepared by drying whale pancreas with acetone and ether, grinding in mortar and putting through a sieve No. 5. Duodenum was also prepared in the same manner.

2) Preparation of whalemeat peptone

300 g of frozen whale-meat was chopped finely with a knife and put through a mincing machine. 530 cc water was added to this²⁾ and boiled³⁾ for 10 minutes. This was then cooled and placed in a thermostat at 40°C. pH value at this juncture is 5.6 which was corrected to pH 8.6 with suitable addition of caustic alkali. To this solution are added enzyme powder and a small amount of toluene as a preservative. This is then kept in a thermostat but for the first 6 hours, pH value must be corrected every 1 to 2

2) This amount of water was determined from the amount of water (75%) found in frozen whale-meat as described later, Calculation: $300 \times 0.25 \times 10 = 300 \times 0.75 = 530$.

3) pH values were determined with the test papers T. B., B. T. B., and C. R. of Tokyo Roshi Co.

hours because pH values tend to shift to acidic range at first. For instance, if enzyme powder is added to a solution of pH 8.6, it will shift to pH 7 after 90 minutes. After 6 hours from the time of the addition of enzyme powder, pH values do not vary greatly, so that pH values are determined only in 4 to 6 hours. By this correction, pH value is value is maintained at 8.6.⁴⁾ After a lapse of definite time, the solution is taken out of the thermostat, boiled for 10 minutes to break the enzyme, pH corrected to 4.7 with hydrochloric acid and filtered while hot through folded filter paper.⁵⁾ The residue is washed once with 300 to 400 cc of hot water and the washing is added to the filtrate. The amount of filter and the residue are weighed, 1 cc of which is taken to determine total nitrogen by Kjeldahl method and amino nitrogen by Van Slyke method. The filtrate is neutralized with sodium hydroxide and concentrated on a steam bath as much as possible to a paste. This paste is tentatively called whale-meat peptone. The time of reaction was varied at 12, 24, 48, 72 and 120 hours, and the amount of enzyme to 1, 2, 3, 4, 5 and 7.5 g. Tests were also made in which duodenum powder to the amount of 1/3 of pancreatic powder was added at the same time and another in which 5 g dried powder of 4th stomach was used in place of pancreatic powder.

3) Determination of the amount of water and total nitrogen in frozen whale-meat.

5 g Frozen whale-meat forced through the mincing machine was mixed with 5 g sand and held in a thermostat at 110°C until there was no change in weight. The loss in weight was taken as the amount of water contained in frozen meat.

Weight at the end of 22 hours 1.2508 g

$$\text{Water \%} = (5 - 1.2508) / 5 \times 100 = 75.0\% \quad (75.0\%)$$

The frozen whale-meat was dehydrated and degrease with acetone and ether, dried and the amount of total nitrogen determined by Kjeldahl method.

Sample 50 mg. N/10 H₂SO₄ 20 cc (F=0.902), N/10 NaOH 13.87 cc (F=1.000)

$$\text{N\%} = 1.4 (20 \times 0.982 - 13.78) \times 100 / 50 = 1.64\%$$

4) pH values were determined by the test paper T. B., B. T. B., and C. R., of the Tokyo Roshi Co.

5) If filtration is performed while hot, filtration is rapid enough even at this pH value. Filtration cannot be done at alkaline state and even at pH 4.7 it becomes difficult if allowed to cool.

4) Culture of diphtheria bacilli and production of antitoxin

Whale-meat pepton No. 11 and 25 were used for the culture media for the production of diphtheria toxin. Composition of culture medium was as follows:

Horse meat bouillon ⁶⁾	250 cc
Pepton	2.5 g (1%)
CH ₃ COONa	1.25 g (0.5%)
MgSO ₄	0.05 g (0.02%)
NaHPO ₄	0.25 g (0.1%)
CaCl ₂	0.25 g (0.1%)

The pH value of the medium was corrected to 7.6 with NaOH, boiled for 10 minutes, filtered and the pH value of the filtrate again corrected. This solution was then poured into D-type flask of 250 cc capacity, sterilized at 110°C for minutes and 2.5 cc 20% rice syrup⁷⁾ (amount of syrup to medium: 0.2%) added aseptically. To this media was planted diphtheria bacilli, strain No. 8 of park-William's, and held for 7 hours at 37°C.

Table 4

Pepton	pH at inoculation	Growth after 7 days	pH at end time	Lf value/c.c.	Kf value
11	7.6	###	8.8	8	6
15	7.6	###	9.0	7	6
Carno	7.6	###	9.0	7	6

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- 6) Preparation of horse-meat bouillon:
Finely chopped horse-meat was added to 5 times its amount in weight of 5% acetic acid, kept for 30 minutes and the pH corrected to 5.8 with 20% NaOH. This was boiled for 10 minutes and filtered.
- 7) Preparation of rice syrup:
Market product of rice syrup was dissolved in water to obtain 20% solution, poured into test tubes and left for 1 day. They were then disinfected at 100°C for 15 minutes, intermittently.
- 8) Signs used to designate the amount of growth:
- Bacterial film not covering more than half of the surface after 7 days.
 - + Bacterial film covering more than half but not all of the surface after 7 days.
 - ++ Bacterial film covering the whole surface after 7 days.
 - +++ Bacterial film covering the whole surface and constituting a layer.