

Experiment on Digestion of Whale Meat by Koji-mould

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Introduction.

It has already been reported (Akiya, Ishikawa, Tejima) that whale pancreas can be used for manufacturing peptone from whale meat and a product suitable for bacteria culture can be obtained by digesting whale meat with this enzyme.

However, the proportion of pancreas compared with the size of the whale is very small, 3—5 kg in the case of sperm whale of about 45 ft in length and 10 kg in the case of sei whale of about the same size. Also, the total quantity of whale pancreas in the country is very small so that the production of peptone is naturally limited by the quantity of pancreas. Furthermore, pancreas is an important raw material for pharmaceuticals such as protease, insulin which is the special medicine for diabetics, etc, and since there is much use for it in the future, it is necessary to find an enzyme source other than pancreas, for manufacturing peptone.

The authors' attention was turned to koji-mould (*Aspergillus Oryzae*), which is relatively easy to obtain and easy to manufacture, as a substitute for this pancreatin.

The reaction of koji-mould on protein, by which the protein is decomposed into amino acids by the protease, is widely used in various fermentation industry, but when whale meat is used as a source of protein and decomposed, peptone, peptide and amino acids are produced. The fact that peptone is suitable for bacteria culture by this method has already been explained (Tejima).

In carrying on this decomposition, there is a great difference in its enzymic action according to kind of koji-mould, kind of culture substrate, culture conditions, enzyme reaction conditions, and pretreatment conditions of whale meat, etc.

The object of this experiment is to find out these conditions. Protein will first become water soluble peptone by the reaction of enzyme so that the degree of decomposition at the beginning can be determined by the amount of water soluble portion.

Therefore, roughly, the quantity of peptone can be calculated by evaporating and drying the water soluble substance produced by decomposition of whale meat by koji-mould.

In this experiment, sweet potato flour and potato starch were chosen as culture substrate and whale meat broth was used for mixing in this. Ordinarily, rice is used for koji-mould culture, but the present condition in the country does not permit its use. Tejima, used soy bean meal but this is principally and imported goods so it was assumed to be rather undependable as raw material. Also, it is a known fact that the koji-mould will depend on the kind of enzyme produced by different substrate so that whale meat broth was mixed in it in order to produce as much protease. As explained later, the decomposition power was stronger the more whale meat broth used, as anticipated.

Experiment.

Koji-mould Culture.

As substrate, three types were used, one with sweet potato flour kneaded with equal amount of water, formed into pellets the size of soy bean and steamed in an oven, the second with sweet potato flour kneaded with half its quantity of water and half its quantity of whale meat broth, and the third with sweet potato flour kneaded with equal amount of whale meat broth. Whale meat broth referred to here is meat broth produced by decomposing finely chopped whale meat with heat. The object is to supplement the protein in sweet potato flour and also because it was assumed that it will strengthen the protease in the koji-mould.

As conditions for culture, the substrate steamed as above in cooled and koji-mould spores cultured with glutinous rice is shaken on it and experiment on rate of decomposition of whale meat experimented with those left standing for 1 day, 2 days and 3 days at 31—33°C.

In regard to propagation of koji-mould when whale meat broth is not added, only a slight formation of mycelium with the 1 day culture is observed, entirely covered with the 2 days culture and spores were well formed with 3 days culture, but when whale meat broth was added, it became more active, such that 1 day culture was entirely covered with mycelium and spores were entirely formed with 2 days culture.

Digestion of whale meat.

Digestion was carried on by the following method with koji-mould cultured by the above method. For each 5 g of sweet potato flour used for culture, 100 g of finely chopped frozen fin whale meat is boiled, filtered with a cloth, 100 c.c. of sterilized water is added to the insoluble matter, that is, meat protein, boiled to sterilize, koji-mould cultured by the above method and a small amount of toluol is added and preserved at 31–33°C.

Digestion time was divided into three stages, 48 hours, 74 hours and 120 hours. After digestion, this is filtered with suction, using cloth to separate the soluble and insoluble portions and each portion is evaporated, dried (105°C) and weighed.

Peptone was calculated by the following formula :

$$\text{Peptone (g)} = \left\{ \frac{\text{Residue (hydrous) (g)} - \text{dried residue (g)}}{\text{filtrate (g)}} \right\} \times \text{dried filtrate (g) + dried filtrate (g)}$$

Table 1.

Koji-mould culture time	No. of days digestion	Kind of culture	Filtrate (a)	Dried filtrate (b)	Residue (hydrous) (c)	Dried residue (d)	Peptone $\left(\frac{(c)-(d)+1}{(a)} \times (b) \right)$	Remarks	
No. 1 24 hours culture	a (2 days)	A	99 g	4.5g	67 g	26 g	6.35 g	Substrate is 5 g sweet potato flour kneaded with 5 g water	
		B	89	5.2	71	29	7.6	Substrate used is 5 g sweet potato flour kneaded with 2.5 g water and 2.5 g meat extract	
		C	94	5.1	78	25.5	8.0	Substrate used is 5 g sweet potato flour kneaded with 5 g meat extract	
	b (3 days)	A	105	5.3	61	25	7.3	Water added to meat was too much	
		B	97	5.7	61	25	7.85		
		C	125	6.1	67	25	8.15		
	c (5 days)	A	96	5.5	60	21.1	7.7	Filtering somewhat difficult	
		B	115 (113 c.c.)	6.0	62	20.2	8.2		
		C	85 (84 c.c.)	6.0	73	21.8	9.6		
	No. 2 48 hours culture	a (2 days)	A	94	4.2	64	23	6.05	
			B	98	5.3	62	23	7.4	
			C	100	6.0	60	23	8.2	
b (3 days)		A	96	4.9	63	22	7.0		
		B	84	5.0	70	22	7.85		
		C	89	5.7	66	21	8.6		
c (5 days)		A	98	5.8	63	20.6	8.3		
		B	91	6.0	62	19.5	8.8		
		C	81 (79 c.c.)	6.2	69	19.5	10.0		

No. 3 72 hours culture	a (2 days)	A	100	6.1	59	20.7	8.4	Filtering somewhat difficult
		B	93 (90 c.c.)	6.0	65	16.9	9.1	
		C	97	6.6	60	16.9	9.5	
	b (3 days)	A	89 (87 c.c.)	6.1	64	21	9.0	Filtering somewhat difficult
		B	99	6.6	59	21	9.1	
		C	99	7.2	61	19	10.25	
	c (5 days)	A	91	6.0	73	19.1	10.0	Filtering somewhat difficult (c) had con- siderable moisture
		B	84	6.8	66	15.0	10.1	
		C	108	7.8	50	13.2	10.5	
	d (8 days)	D	—	—	—	—	11.2	

Experimental result is as indicated in Table 1.

As can be seen from the above table, digestion is greater when amount of whale meat broth added is greater and when the culture time is longer. Furthermore, digestion will be greater and more soluble matter obtained when digestion time is longer. However, if the digestion time is long, the undecomposed portion will become considerably less and the total of this and the soluble portion will be reduced. It is believed that this is due to the fact that the greater part of the soluble protein is further decomposed and gasified into ammonia gas, etc. Also change in quantity of soluble portion will be retarded when decomposition is continued further. That is, the culture time of koji-mould, in the condition of digestion, had the greatest effect and the effect of time in digestion increased greatly during the first 2 days and becomes very small after that.

In this experiment, 10.5 g of soluble portion was obtained from 100 g of frozen, fresh meat when decomposition was greatest.

The above digestion was carried on at 33°C, but next, digestion was carried on with sperm whale meat under the best conditions of the above experiment, changing the temperature to 37°C and 42°C, the previous experiment was carried on at pH of about 7, but dilute H₂SO₄ was added in this experiment in order to carry it out at pH 4.2.

Furthermore, as substrate potato starch was used instead of sweet potato flour.

The result is as indicated in Table 2.

Table 3 indicates the principal curves of Tables 1 and 2.

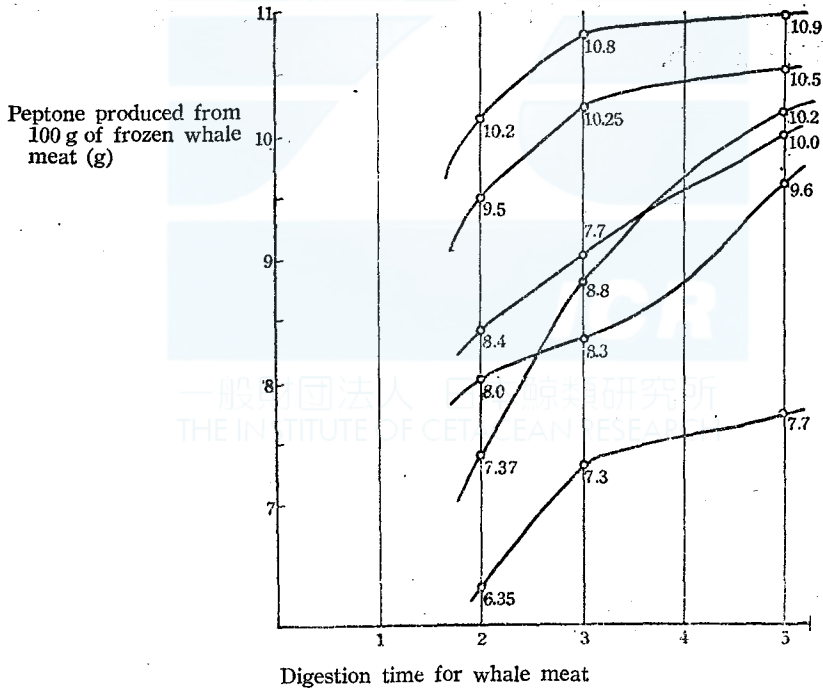
In this experiment, it was difficult to filter after digestion so the unreacted slimy matter was separated with a centrifugal separator.

Table 2.

Decomposition temperature	Substrate for koji-mould culture	Digestion time	Filltrate	Dried filtrate	Slimy matter (hydrous)	Dried Slimy matter	Calculated peptone	Re-remarks
No. 1 37°C	Sweet potato flour + whale meat broth	a (2 days)	110 g	5.3 g	60 g	17.0 g	7.37 g	
		b (3 days)	112.3	6.2	60.8	13.3	8.82	
		c (5 days)	114.8	7.3	55	9.5	10.2	
	Potato starch + whale meat broth	d (5 days)	93.0	5.6	60.7	13.8	8.43	
	Sweet potato flour + whale meat broth	e (5 days)	115.7	6.9	66.2	20.0	9.6	
No. 2 42°C	Sweet potato flour + whale meat broth	a (2 days)	115.0	7.5	57	16.2	10.18	
		b (3 days)	120.9	7.6	57.0	12.7	10.8	
		c (5 days)	127.0	8.3	47.3	9.0	10.9	
	Potato starch + whale meat broth	d (5 days)	119.0	6.8	53.9	10.2	9.3	
	Sweet Potato flour + whale meat broth	e (5 days)	122.5	7.2	66.6	19.4	9.9	

(During digestion, 0.3 g of various oils and fats were obtained)

Table 3.



It can be observed from this table that maximum digestion is at 42°C, digestion is somewhat less at pH 4.2 than at pH 7 and less when potato

starch was used than when sweet potato flour was used. It is assumed that the koji-mould did not propagate fully into the interior because of the formation of gel in case of starch, and also, the culture method was somewhat difficult due to gel formation.

Conclulsion.

The following conclulsion is drawn from the above experiments. The best condition for producing peptone by digestion whale meat with sweet potato koji-mould, within the sphere of this experiment, are as follows:

1. To use a large quantity of whale meat broth with sweet potato flour in producing koji-mould, and to use as much time in culture.
2. Digestion temperature is about 42°C.
3. Digestion time is about 5 days.
4. Care must be taken to prevent abnormal fermentation.

About 10% of peptone can be obtained from frozen whale meat, and since the protein content of frozen whale meat is about 20%, about 50% is decomposed.

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