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Utilization of trash fish solid waste as peptone for potential bacterial growth.

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ABSTRACT

Peptone is a protein hydrolysate prepared by partial proteolytic digestion of protein source. It is an excellent natural source of amino acids, peptides and proteins in growth media and hence, serves as a nitrogen and sometimes carbon source. Also, in medium it acts as a buffer because of its amphoteric nature. The commercial peptone has high market value. Large amount of fish waste is generated daily in fish market which is difficult to dispose. Fish waste can be used as nutrient source for microbial growth as it contains calcium, protein, vitamins, iron and minerals. This new approach can reduce environmental problems associated with the waste disposal. Present study deals with the production of peptone from fish waste which can be used at laboratory level. Fish waste of *Tilapia busumana* (Chilapi), *Rastrelliger kanagurta* (Bangada), *Porthecle dinus* (Dinus) were used for peptone production by alkaline hydrolysis and acid hydrolysis method. The protein content was measured using Folin-Lowry method. It was observed that fish waste of *R. kanagurta* has high protein content. Growth curve studies using commercial peptone and peptone from fish waste were done on *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescence*, *Staphylococcus aureus*, and *Klebsiella* spp.

Keywords: Fish waste, Peptone, Alkaline hydrolysis, Acid hydrolysis, Growth curve.

INTRODUCTION

India is a major producer of fish. In 2013-14, India holds second ranks in the world after China; contributing to 5.68% of global fish production. The country has a long coastline of 8118 km and inland fishery resource include 1.96 lakh km stretch of rivers and canals, 29.07 lakh hector reservoirs 24.40 lakh hector ponds and tanks (Handbook on Fisheries Statistic, 2014). In recent years, total fish production is 9.58 million metric tons with a contribution of 6.14 million metric tons from inland

sector and 3.44 million metric tons from marine sector respectively (Chandasudha Goswami et.al. 2015). The overall growth in fish production in 2013-14 has been 5.9%, which has been mainly due to 7.3% growth in inland fish production and in marine fish production has been 3.7% (Handbook on Fisheries Statistics, 2014).

Approximately 131 (85%) million tonnes of fish were directly utilized as food and the rest (15%) was underutilized as live bait for fishing, ornamental products (pearls and shells), feed for carnivorous farmed species and marine worm. The production of fish in China Indonesia, India and Russia has increased while fish production decreased in other countries over the ten year period. (Ghaly A.E. et.al. 2013). About 75% fish resource was used for human consumption and remaining 25% is consider as waste. (Faouzi B.R. et.al. 2013). According to the Food and Agricultural Organization of the United Nations, in appropriate terms over 100 million metric tons of fish waste or discard in water body is generated worldwide annually, with only a small portion used in the production of fishmeal and fish oil.

During 2006-07, it was estimates that 3, 02,750 tonns of (both processing and pre-processing taken together) waste was generated. In the context of environmental pollution waste generated in fish processing industry is a matter of great concern. Among the maritime state the largest waste generation was observed from Gujarat (30.51%) followed by Maharashtra (23%) and Kerala (17.5%). (A. A. Zynudheen).

Majority of fish processing operations require potable water which results into large amount of waste water. Although, it is bio-waste, but it has effect on surrounding environments, habitats and organisms is highly controversial. It is a natural pollutant so, it has the ability to **affect oxygen levels, salinity, temperature, pH levels** and **the organisms** in sea water. Large amount of fish waste are disposed off in the ocean. The aerobic bacteria present in the water breakdown the organic matter in the presence of oxygen leading to a considerable reduction of oxygen in water. There are also overloads of nitrogen, phosphorus and ammonia which leads to pH variations and turbidity get increases. It leads to decomposition of algae which in turn produce planktons blooms, which may causes mass fish mortality. The reduction in water oxygen content creates an anaerobic condition that release foul gases such as hydrogen sulphide and ammonia, organic acids and greenhouse gases such as carbon dioxide and methane. (Ghaly A.E. et.al. 2013).

The composition of fish varies according to the type of species, sex, age, nutrition and health. Most of the fish contains 15-30% protein, 0-25% fat and 50-80% moisture. Solid fish waste consists of head, tails, skin, gut, fins and frames. These are great sources of proteins and amino acids, collagen and gelatine, oil and enzymes. Fish muscles are made up of 70-80% structural proteins, 20-30% are sarcoplasmic proteins and 2-3% of insoluble connective tissue proteins. Myofibrillar proteins are the primary food proteins and they make up 66-77% of total protein content in fish meat. These Myofibrillar proteins comprise of 50-60% myosin and 15-30% actin. The myosin fibres can be cleaved

by proteases trypsin and chymotrypsin on one end and on the other end with papain. (Kristinsson HG, et.al.2000).

Peptone is protein hydrolysates prepared by partial proteolytic digestion of protein source. It is an excellent natural source of amino acids, peptides and proteins in growth media. Peptones is widely used source of nitrogen in microbial media and also carbon in some extent. Also, in medium it acts as buffer because of its amphoteric nature. The commercial peptone has high market value.

Present study focuses on the production of peptone from fish waste which can be used at laboratory level. This new approach can reduce environmental problems associated with the waste disposal.

MATERIALS AND METHODS

1. Collection of sample:

Mackerel (*Rastrelliger kanagurta*) and Chilapi (*Tilapia busumana*) were used in these experiments. Fish waste which consists of head, bones, fins and viscera were collected from Hadapsar, (Pune) fish market. These fish waste was washed thoroughly and blended. Then stored at 0^0C until used.

2. Hydrolysis:

a. Alkaline hydrolysis

Blended fish waste was subjected to defatting process using petroleum ether (1:2).Then it was mixed with D/W (1:10). 3M NaOH was added to obtain pH 10. Incubated for 92 min. at 50^0C. Centrifuged the solution at 3000 rpm for 15 min. Supernatant containing soluble proteins was collected for further process. (Nurdiyana Husin et.al 2015). Degree of hydrolysis: 20ml of protein hydrolysate was mixed with 10% TCA. Then mixture was left for 30 min. at RT. Then centrifuged the mixture at 3000 rpm for 15 min. Supernatant was collected. (Nurdiyana Husin et.al 2015) and stored for further process.

b. Acid hydrolysis:

Fish waste is mixed with D/W in 1:10. Homogenate was divided into four various portions pH adjusted at 6, 4.5 and 3 by using 4N HCl. Then incubated it for 24hrs and 30hrs with shaking. Then it heated at 85^0C for 20 min. Centrifuged the heated sample at 3000 rpm for 15 min.Supernatant was collected. (Sahar F. Deraz et.al. 2011)

3. Protein concentration by Folin-Lowry method:

Protein concentration of alkaline peptone and acidic peptone was measured by Folin-Lowry method using standard dose response curve of bovine serum albumin (10 -100mg/ml). (Nurdiyana Husin et.al 2015).1ml supernatant was taken in clean test tube. Then add 5ml alkaline copper solution. Well mixed. Allowed it to stand for 10 min. Then 0.5 ml Folin- Ciocalteau reagent was added and incubated

it at room temperature in dark for 30 min. Then absorbance is measured at 660nm.

4. Bacterial Growth curve :

Bacterial strains such as *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescence, Staphylococcus aureus*, and *Klebsiella* spp were grown in nutrient medium using commercial peptone and *R. kanagurta* fish waste peptone produced by alkaline and acid hydrolysis process separately. Analysis of microbial growth with commercial peptone and *R. kanagurta* fish waste peptone by measuring optical density (660nm) and by taking dry weight (gm).

RESULT AND DISCUSSION:

Fish protein was hydrolysed using alkaline and acid methods and further precipitation was done using trichoroacetic acid. The protein content was measured using Folin-Lowry method. Peptone prepared from *R. kanagurta and T. busumana* by alkaline hydrolysis showed protein concentration of 83.92 mg/ml and of 54.46 mg/ml respectively. As compared to *T. busumana, R. kanagurta* waste shows high concentration of protein hence, peptone prepared from *R. kanagurta* fish waste was used for further study.

Table I: Concentration of protein of *R. kanagurta* peptone prepared by acid hydrolysis at different pH level

Incubation period (hrs.)	pH	Absorbance (nm)	Concentration of protein (mg/ml)
24	3	0.44	40
	4.5	0.29	26.36
	6	0.19	17.27
30	3	0.16	14.54
	4.5	0.24	21.81
	6	0.21	19.09

From Table I, peptone prepared from *R. kanagurta* fish waste by acid hydrolysis showed highest protein concentration at pH 3.

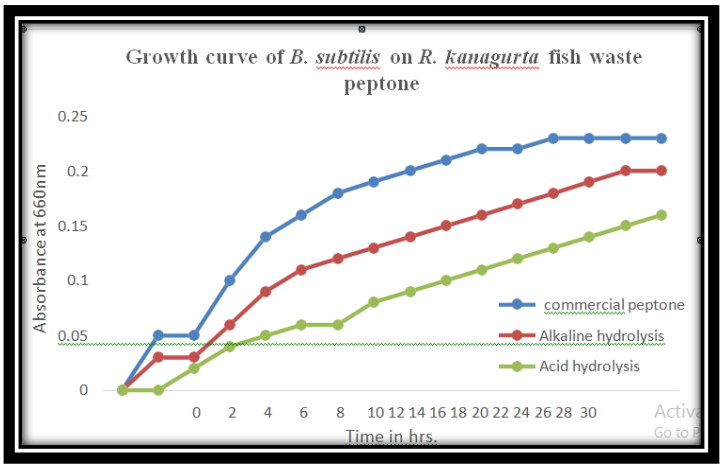

Fig.1. Growth curve of *Bacillus subtilis* at 650 nm

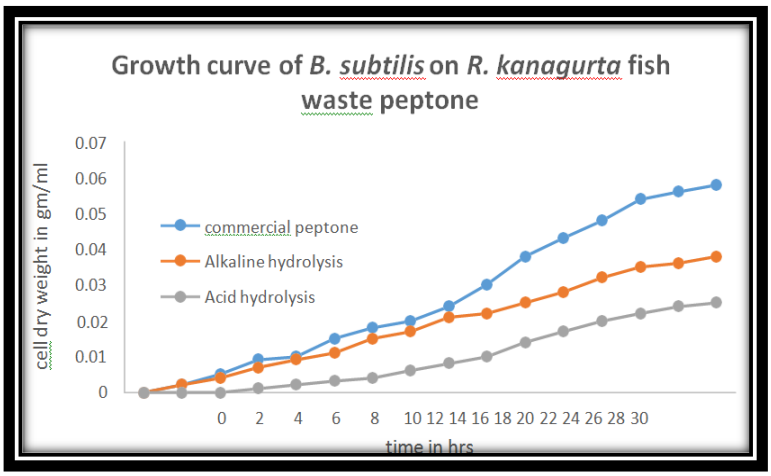

Fig.2. Growth curve of *Bacillus subtilis* using cell dry weight (gm)

Growth curve study was carried out as shown in fig 1 and fig 2 on selected microorganism

According to the experiment carried out, *B. subtilis* growth with alkaline and acidic peptone were significantly near to the commercial peptone. But alkaline peptone showed maximum growth when compared to the peptone obtained from acid hydrolysis. It yielded 0.20 and 0.16 maximum optical density with alkaline and acid peptone respectively. *S. aureus* growth with alkaline and acidic peptone is significantly near to the commercial peptone. Peptone obtained from both alkaline and acid hydrolysis show similar growth. It yielded 0.13 and 0.15 maximum optical density with alkaline and acid peptone respectively.

P. fluorescens growth with alkaline peptone shows almost similar results as that of the commercial peptone. And alkaline peptone shows maximum growth when compared to the peptone obtained from acid hydrolysis. It yielded 0.39 and 0.25 maximum optical density with alkaline and acid peptone respectively. *E. coli* growth with alkaline peptone and acidic peptone shows relatively low growth as compared to the commercial peptone. It yielded 0.13 and 0.14 maximum optical density with alkaline and acid peptone respectively. *Klebsiella* sp. growth with alkaline and acidic peptone is significantly near to the commercial peptone. But alkaline peptone shows maximum growth when compared to the peptone obtained from acid hydrolysis. It yielded 0.18 and 0.13 maximum optical density with alkaline and acid peptone respectively.

From the results obtained it shows that, the growth of microorganisms was significantly higher with peptone produced by alkaline hydrolysis as compared to peptone produced from acid hydrolysis. In conclusion, peptone produced by alkaline hydrolysis method has potential to replace existing commercial peptone for microbial growth. Optimization and estimation of nitrogen content of alkaline and acidic peptone is essential to make it more efficient. From the current studies, this new approach can reduce environmental problems associated with the fish waste disposal and could be converted into a rich cheap source of peptone.

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