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Comparison of Microbial Growth on Fish Waste Peptones from Different Hydrolysis Methods

Nurdiyana Husin [1 +], Siti Mazlina Mustapa Kamal [2], Ling Tau Chuan [3], Nurul Fadzni Muhammad [1], and Norhani Jusoh [1]

[1] Section of Bioengineering Technology, Universiti Kuala Lumpur, Malaysian Institute of Chemical and Bioengineering Technology (UniKL MICET), Lot 1988, Bandar Vendor Taboh Naning, 78000 Alor Gajah, Melaka, Malaysia

[2] Dept. of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

[3] Institute of Biological Sciences, Faculty of Science Building, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract. This study utilized mixture of sardine and mackerel wastes from fish processing industry for peptone recovery. The recovered peptones from different methods of hydrolysis (alkaline and enzymatic) were supplied as nitrogen sources for microbial growth. Peptone performance in microbial growth was found to be affected by hydrolysis method. *Saccharomyces cerevisiae* growth using peptone from enzymatic (Alcalase enzyme) was superior compared to its growth using other types of peptone. The same trend was observed for *Bacillus subtilis* growth. Hydrolysis process played important roles in producing peptone with high protein content and degree of hydrolysis, which contributed to better performance of the fish waste peptone in microbial growth. In conclusion, peptones produced from the mixture of sardine and mackerel waste are potentially viable to replace existing commercial peptone as microbial cultivation media.

Keywords: Alcalase enzyme, fish waste peptone, degree of hydrolysis

1. Introduction

Fish processing industry is one of the producers of insignificant garbage due to abundant generation of waste mainly composed of fish bodies' parts. However, fish waste provides a high supply of protein, which is up to 58% of dry matter and just slightly lower than the protein from commercial fish meals [1]. The idea to utilize wastes or residual materials from the industry is essential to solve the problem of abundant waste generated every year. One of the alternatives is to produce fish protein hydrolysate that can be used as carbon and/or nitrogen source in microbial growth, which is not only an eco-friendly approach but can also produce cheaper raw materials for media formulation. This hydrolysate is also known as peptone.

Most of earlier studies on alternative peptones only focused on peptones performance towards different types of microorganisms rather than methods of hydrolysis to produce peptones. To date, reports on degree of hydrolysis on the performance of peptones (particularly from fish waste) in microbial growth are still scarce. Therefore, the goals of this study were to obtain peptones from protein hydrolysate from a mixture of sardine and mackerel industrial waste recovered from different methods of hydrolysis and to validate the ability of the fish waste peptones as a microbial media component.

2. Materials and Methods

[+] Nurdiyana Husin. Tel.: + 6065512027; fax: +6065512001.
E-mail address: nurdiyana@unikl.edu.my

2.1. Raw Materials

Fish waste was supplied from a canned fish company in Malaysia. The waste (heads and viscera) consists of sardine (*Sardina pilchardus)* and mackerel (*Rastrelliger kanagurta),* both types of fish waste were mixed in equal percentage of weight (ratio 1:1). Then, the fish waste was blended and underwent de-fatting process using petroleum ether.

2.2. Alkaline Hydrolysis

Fish waste was mixed with distilled water with a ratio of 1:10 before the addition of 3M NaOH [2]. Hydrolysis process was done in incubator shaker with 200 rpm agitation rate. Pre-studies and optimization were done using Research Surface Methodology (RSM) (data not shown) and the optimum levels of the three parameters were obtained at pH 10, temperature 50^{o}C, and 92 minutes. Following the hydrolysis process, the solution was centrifuged at 5000 rpm, 4^{o}C for 15 minutes [3]. Supernatant containing soluble proteins was collected for further process.

2.3. Enzymatic Hydrolysis

Fish waste was boiled at 85^{o}C for 20 minutes [4]. Pre-studies and optimization were done using RSM (data not shown) and optimum combined condition for degree of hydrolysis using Alcalase was found at pH 11, temperature 40 ^{o}C, time 240 minute, and ratio of enzyme to sample at 4.83.During the hydrolysis process, pH was checked for every 30 minutes in order to maintain the desired value by using 3M NaOH. Following the hydrolysis process, the solution was boiled at 95^{o}C for 20 minutes [4]. The solution was cooled to room temperature and was centrifuged at 5500 rpm, 4^{o}C for 20 minutes. Supernatant containing soluble proteins was collected for further process.

2.4. Degree of Hydrolysis and Protein Concentration

The degrees of hydrolysis and protein concentration were calculated according to percentage of trichloroacetic acid ratio. After hydrolysis, 20 mL of protein hydrolysate was added into 20 mL of 20 % (w/v) TCA to produce 10 % TCA soluble material. The mixture was left for 30 minutes to allow precipitation, followed by centrifugation (7800 *g* for 15 minutes). Supernatant was measured for protein content using Kjehdahl method. Protein concentration in the supernatant obtained from the TCA treatment was determined through the Bradford method.

2.5. Molecular Weight Analysis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was done on all samples on a discontinuous buffered system according to the method of Laemmli [5].

2.6. Drying of Soluble Peptides

The soluble protein hydrolysate was dried using spray dryer (Model: Buchi Mini Spray Dryer, Switzerland). The powdered peptones were kept in glass container for proper storage before further analysis.

2.7. Microbial Growths

The strains of *S. cerevisiae* and *B. subtilis* were used to test the microbial growth performance. The tested mediums consisted of (wt/wt) 1.5% glucose, 0.4% peptones, 0.2% KH_2PO_4, 0.013% $CaCl_2$ H_2O, 0.001% $FeSO_4$ – 7 H_2O and $MgSO_4$ – $7H_2O$, pH 7.2 [6]. Inoculum size was 5% (v/v). Both strains were grown at 37^{o}C in liquid media. Cultivation was performed for 30 hours on a rotary shaker. The growths of both microbes were monitored every 3 hours by measuring optical density (650_{nm}) and dry weight of cell.

3. Results and Discussion

3.1. Growth of Bacillus Subtilis and Saccharomyces Cerevisiae

B. subtilis growth (Figure 1) was significantly higher when the peptone produced from enzymatic hydrolysis by Alcalase was used as compared with other peptones; the growth when this peptone was used yielded 3.96 maximum optical density (OD). This research showed promising results when compared to previous study by Aspmo [7], which tested the viability of peptone from viscera of the Atlantic cod, the growth curve of *B. subtilis* was monitored for 35 hours, and the maximum OD was observed at 1.75 (650

nm). As for the cell dry weight observation, it demonstrates a similar trend in comparison with optical density. The peptones from enzymatic hydrolysis by Alcalase showed the highest value of dry cell weight, and the peptone from alkaline hydrolysis gave the lowest value.

The growth curves of *S. cerevisiae* are shown in Figures 2. Cell density for the peptones from fish waste showed superior performance in *S. cerevisiae* growth as compared to the commercial peptone. Among the three types of produced peptones, the peptone from enzymatic hydrolysis by Alcalase gave the highest absorbance reading of around 4.18 at 650 nm. A study by Aspmo [7], showed a maximum OD of *S. cerevisiae* growth at 0.5, demonstrating that the current research of peptone from enzymatic hydrolysis by Alcalase showed good results in promoting the growth of *S. cerevisiae*. The trend for cell dry weight was similar to the cell density. The highest cell dry weight was observed to be 0.13 g after 30 hours of cultivation.

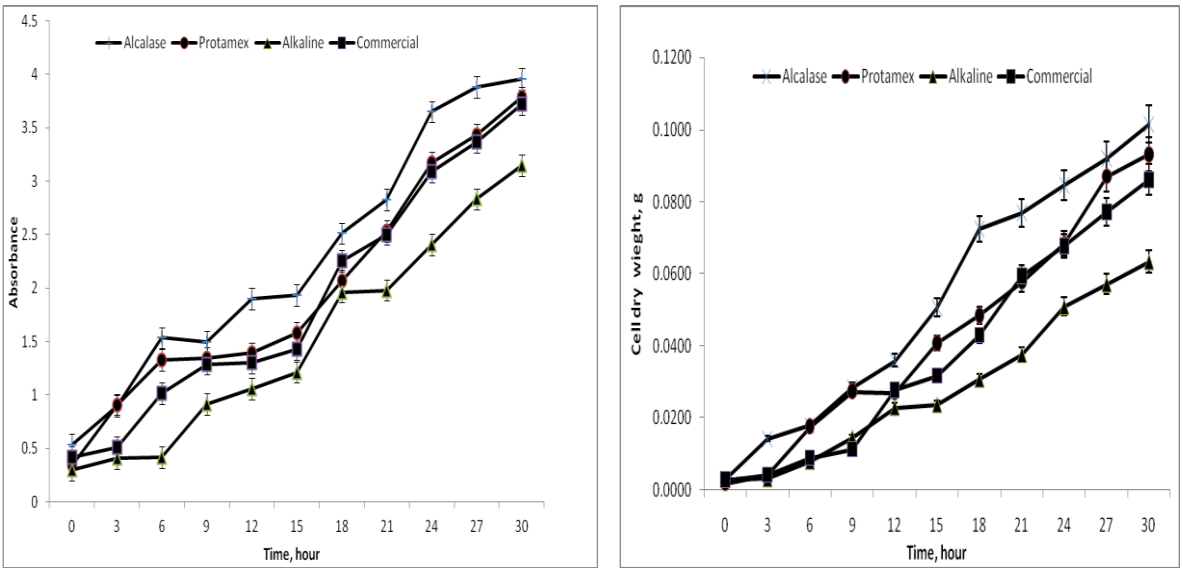

Fig. 1: Growth curve of *Bacillus subtilis* observed at optical density of 650 nm (left) and its growth monitored using cell dry weight (right).

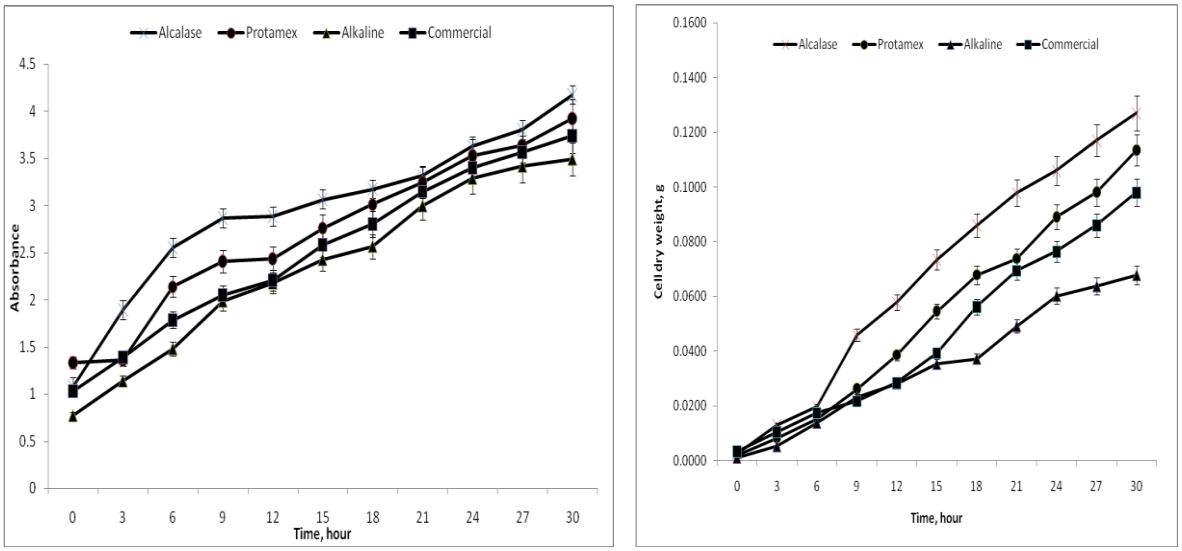

Fig. 2: Growth curves of *Saccharomyces cerevisiae* monitored using optical density at 650 nm (left) and observed throught dry weight (right).

3.2. Characteristics of Peptones Affect Microbial Growth

The peptone from enzymatic hydrolysis by Alcalase showed superior performance in both microorganism growth profile due to high degree of hydrolysis. High degree of hydrolysis reduces molecular weight and helps the peptone to be effectively consumed by microorganisms [8]. Table 1 depicts molecular

weight and optical density of each peptone. It was observed that when the molecular weight was low, the optical density of microorganism was high. High optical density reflects good ability of microorganism to grow in the medium. The results also showed that high protein concentration provided more nitrogen source to the medium. This was proven by a proportional trend between dry weight and optical density towards protein concentration.

Table 1: Protein concentration, degree of hydrolysis, molecular weight, and optical density of peptones from different type of hydrolysis processes

Type of peptone	Concentrati-on of Protein (mg/mL)	Degree of Hydrolysis (%)	Molecular Weight (kDa)	Absorbance		*Dry Weight (g)	
				B. subtilis	***S. cerevisiae***	***B. subtilis***	***S. cerevisiae***
Alcalase	7.72	58.37	< 6.5	3.96	4.18	0.10	0.13
Alkaline	1.03	18.80	< 21.5	3.15	3.49	0.006	0.07
Commercial	-**	-**	< 6.5	3.72	3.74	0.008	0.1

*Highest value. **The reading can only be done for liquid proteins, while the commercial peptone is already in powder form.

4. Conclusion

This research revealed that the method of hydrolysis of peptone influenced the function of peptone as a vital component in the media for microbial growth. Conclusively, the peptones produced from fish waste, especially from enzymatic hydrolysis using Alcalase, have potentials to replace existing commercial peptone for microbial growth.

5. References

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