Extraction of crude peptone from fish wastes for use as a nitrogen source in microbiological media

Najim, S. M. [*1]; Al-Noor, J. M. [1] and Al-Waely, W. A. [2]
[1]Department of fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq
[2]Department of Food Sciences and Biotechnology, College of Agriculture, University of Basrah, Basrah, Iraq

*Corresponding author E-mail: salahm63@yahoo.com

ABSTRACT

This study was carried out to exploit the feasibility of production of crude peptones from fish wastes. Autolysis under acidic and alkaline conditions was used to hydrolyze fish wastes. The proximate analysis of the two produced crude peptones and the commercial peptone showed that crude peptones contained fair levels of nitrogen (up to 10.8%) although commercial product was superior with 15.1 % nitrogen. Amino acid composition of the three examined peptones demonstrated that crude peptones have a well-balanced amino acid profile with no acute deficiencies or absence of any amino acid existed in the commercial product. Three microorganisms i.e. *Pseudomonas aeruginosa*, *Lactobacillus acidophilus* and *Saccharomyces cerevisiae* were used to test the produced crude peptones as nitrogen sources in culture media. Results of OD_{600} indicated that the three species show good growth efficiency into different media supplemented with crude or commercial peptones in spite of the relative superiority of the commercial product.It could be concluded that crude peptones produced by acid and alkaline autolysis of fish wastes could be exploited as a suitable nitrogen sources for different microbiological media. The fair nitrogen content and balanced amino acid composition were obviously reflected in the good growth of different tested microorganisms. Keywords: Fish waste, crude peptone, acid, alkaline autolysis, culture media

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1- INTRUDUCTION

Total production of world fisheries reached 158 million tons in 2012 and it is expected to increase in the future mainly from the expansion of aquaculture activities. However, it is estimated that 30-60% of total fish catch is discarded as by-catch and processing wastes **(FAO, 2014).** It was found that fish wastes are profitable materials to produce valuable products like different bioactive proteins, amino acids, peptone, oil, enzymes and pigments. Recycling of fish wastes could be economically viable as well as it could participate in solving disposal problem of fish wastes **(Ghaly *et al*., 2013).**

The utilization of fish viscera has been limited to animal feed, but the abundance of proteolytic enzymes and protein in viscera opens the possibility of further utilization. Fish viscera contain high levels of digestive enzymes and this makes autolytic digestion of fish protein more economically viable for marine waste utilization as enzyme costs are nil. During autolysis, proteins of the incubated wastes are hydrolyzed into soluble low molecular weight products in a complex mixture of peptides and amino acids and may be used as a nitrogen source in microbiological media **(Bhaskar and Mahendrakar, 2008; Jayathilakan *et al.*, 2012).**

Nitrogen sources are the most expensive component of microbiological media, and are supplied primarily from animal products in the form of low molecular weight proteins. Many attempts were carried out to use fish materials as sources of nutrients for microorganisms **(Dufosse *et al*. 2001; Poernomo and Buckle, 2002).** Fish and shrimp wastes have also been investigated as peptone sources for use as nitrogen sources in microbiological media **(Ovissipour *et al*., 2009; Kandra *et al*., 2012).**

This study was carried out to explore the possibility of extracting crude peptone from fish waste and evaluate its suitability against the standard material for use as a nitrogen source in microbiological media used for culturing different microorganisms.

2- MATERIALS AND METHODS

Fish wastes were obtained from local fish markets at Basrah province center during November 2014. It included mainly fish viscera with negligible quantities of heads, fins and scales. Eight kilograms of wastes were minced using meat mincer and 1 kg subsamples were put into 2 kg plastic containers. In order to compare autolysis and peptone production under different pH conditions, three containers were treated with acid (4N HCl until pH 4.2) to apply acid autolysis **(Sahar *et al*., 2011)** and another three containers were treated with alkali (3M NaOH until pH 10) to apply alkaline autolysis **(Husin *et al*., 2015).** Containers were put into dark plastic bags an incubated for 72 h at 45ºC. After incubation, containers were set to laboratory temperature (25ºC) and the upper oily phase was skimmed off. The aqueous phase was centrifuged at 3000 rpm for 3 minutes and the supernant was collected as a crude peptone (Crude peptones A and B for both acid and alkaline autolysis, respectively). The liquid products were spray dried and preserved into tightly capped glass container under refrigeration at 5ºC until further utilization and analysis. The Proximate composition of raw material and produced peptones were assessed according to the methods mentioned in **AOAC (2003).** Amino acid composition was determined using high performance liquid chromatography (Shimadzu Spd-6AV UV-visible detector) on samples previously hydrolyzed with 6N HCl for 22 h at 110^{0}C **(Poernomo and Buckle, 2002).**

Three microorganisms of health and economic importance i.e. *Pseudomonas aeruginosa, Lactobacillus acidophilus* and *Saccharomyces cerevisiae,* were used to test the suitability of produced crude peptones as nitrogen sources in different culture media. Commercial pepton (Oxoid, U.K) was used as a standard for comparison purpose. *P. aeruginosa* was cultured on CAJ medium which was supplemented with peptone (5 g/l) and nutritive broth, *L. acidophilus* was cultured into MRS broth supplemented with peptone (10 g/l) while *S. cerevisiae* was cultured into YPD broth containing 20 g/l of peptone **(Corry et al., 2003).** Microbial growth was observed for 30 hours at 3 hour intervals using OD_{600} (optical density spectrophotometrically measured at 600 nm wavelength, **Poernomo and Buckle, 2002).** Data were statistically analyzed using SPSS statistical package version 19.

3- RESULTS

Table (1) show the proximate composition of raw fish wastes, the two produced crude peptones and the standard peptone. The most important component is nitrogen content which was higher in commercial peptone in comparison with both crude peptones A and B (15.1, 10.8 and 9.7 %, respectively). However, nitrogen content in crude peptones was higher than waste material (9.3 %). In spite of these variations, the tow produced crude peptones were not significantly different while commercial peptone was significantly different ($P < 0.05$) from both crude materials. Another variation of less significance could be observed in ash, lipid and moisture contents.

Table (1) Proximate composition of fish waste, produced and commercial peptones

Component %	Fish waste	Crude peptone A	Crude peptone B	Oxoid® peptone
Moisture	5.5	3.1	2.9	1.5
Total nitrogen	9.3	10.8	9.7	15.1
Lipid	23.3	0.8	0.9	0.4
Ash	12.9	8.8	8.3	5.0

The amino acid composition of the two produced crude peptones and the commercial peptone show slight variations (Table 2). The three peptones were not significantly different. However, the three materials demonstrate some richness and deficiency trends for specific amino acids. Crude peptone A was very rich in glutamic acid while it was deficient in phenylalanine and valine (143, 18 and 19 mg/g, respectively). On the other hand, crude peptone B was rich in serine and deficient in lysine (38 and 48 mg/g, respectively). It is noteworthy that no single amino acid was absent in both produced peptones in comparison with commercial peptone.

Table (2) Amino acid composition of produced and commercial peptones

Amino acid (mg/g protein)	Crude peptone A	Crude peptone B	Oxoid® peptone
Alanine	60	61	69
Arginine	48	55	60
Aspartic acid	85	69	74
Cystine	12	11	9
Glutamic acid	143	95	115
Glycine	48	46	57
Histidine	33	21	18
Isoleucine	21	25	26
Leucine	48	23	49
Lysine	66	48	60
Methionine	22	24	15
Phenylalanine	18	25	24
Serine	29	38	31
Threonine	23	21	29
Tyrosine	20	19	22
Valine	19	30	31

Figures (1) illustrate the growth curve of *P. aeruginosa* which cultured into CAJ broth supplemented with the three studied types of peptone. It could be observed that the commercial peptone was superior in supporting bacterial growth in comparison with the two produced crude peptones. However, crude peptone A show closer trend to the commercial material in supporting bacterial growth than crude peptone B. At the end of incubation period, the three examined peptones showed no significant differences in bacterial growth.

An approximately similar trend of peptone source influence on bacterial growth was indicated by *L. acidophilus* cultured into MRS broth supplemented with peptone (Figure 2). However, the variations were smaller than that observed previously with *P. aeruginosa*. The three peptone types reflected good ability in supporting growth of these important lactic acid bacteria in spite of some differences especially at the middle of incubation period.

From figure (3) it could be observed that yeast Saccharomyces cerevisae have been grown at the same efficiency into YPD broth supplemented with the three examined peptone sources. The two crude peptones A and B show similar trends most of incubation period. However, some divergence was observed at the end of incubation period.

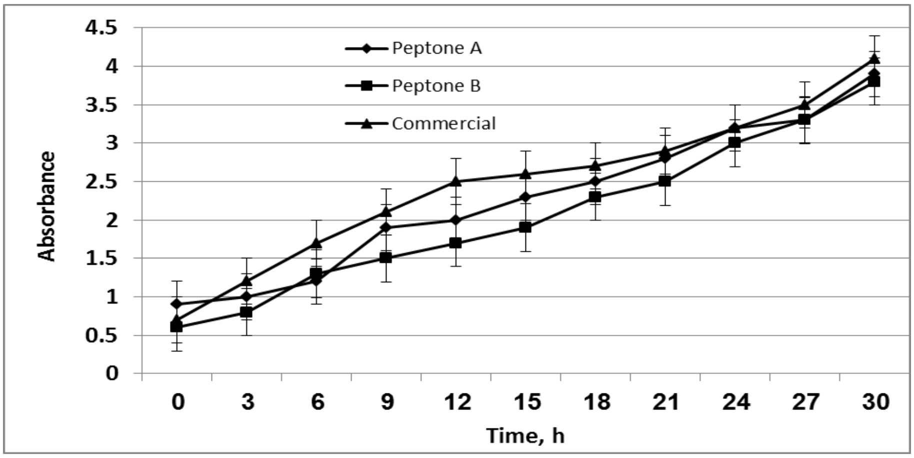

Figure (1) Growth curves of *Pseudomonas aeruginosa* using different peptone sources (observed at optical density 600 nm, OD_{600}, ± S.E.).

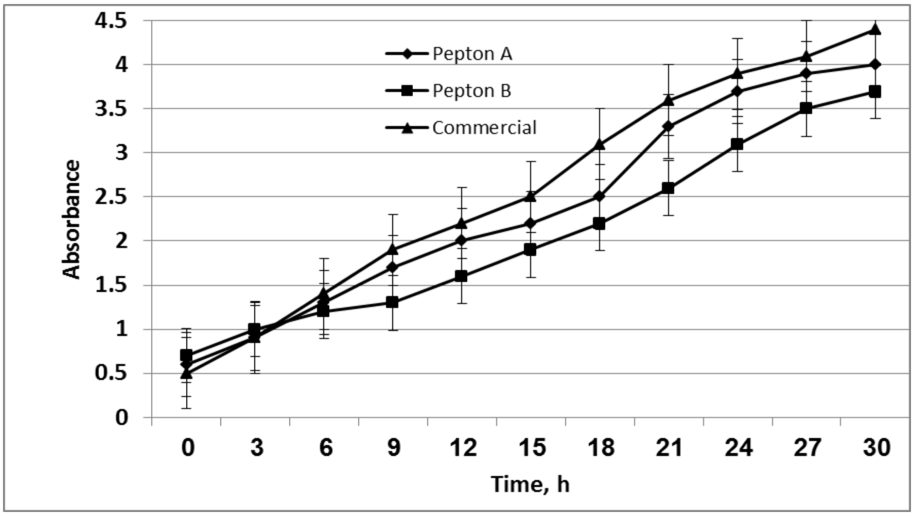

Figure (2) Growth curves of *Lactobacillus acidophilus* using different peptone sources (observed at optical density 600 nm, OD_{600}, ± S.E.).

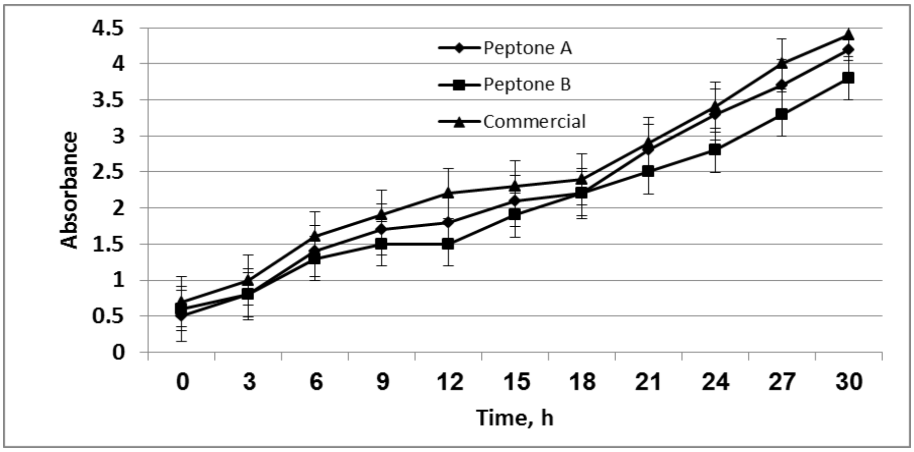

Figure (3) Growth curves of *Saccharomyces cerevisiae* using different peptone sources (observed at optical density 600 nm , OD_{600}, ± S.E.).

4- DISCUSSION

Crude peptone from fish wastes could be a good nitrogen source for microorganisms in culture media in spite of preparation method i.e. acid, alkali or enzymatic as shown by previous researches **(Horn *et al.*, 2005).** Although nitrogen content in crude protein preparations are usually lower than that in commercial peptones, the low cost of production makes it a feasible technology for pulverizing fish waste materials.

It was observed in the current study that crude peptone produced with acid autolysis was slightly better than that produced with alkaline autolysis. This could be explained by the sensitivity of different endogenous enzymes in the crude material to pH level. The high rate of enzymatic activity hydrolyzes more protein into the aqueous phase and consequently yields more peptone **(Jayathilakan *et al.*, 2012; Duarte *et al.*, 2015)**. Freshness of the raw material could have an effect on the final peptone yield. So, it is crucial to maintain good transport, handling and storage conditions to preserve raw material freshness **(Haddar *et al***., **2010).**

Amino acid composition is very important aspect in preparation of culture media for different microorganisms. This is related with the different needs of microorganisms for specific amino acids which could be growth limiting factors if it was deficient in culture media. Some specific culture media may be supplemented with special crystalline amino acids **(Corry *et al.*, 2003).** The variation in amino acid composition in the two types of studied crude peptone was not unexpected. Amino acid have different sensitivities for pH levels in hydrolysis media. This can be reflect directly on the ratios of some amino acids in the produced peptones as reported previously **(Poernomo and Buckle, 2002).** However, both crude peptones show nearly balance amino acid composition in comparison with the commercial peptone.

The three tested microorganisms i.e. *P. aeruginosa, L. acidophilus* and *S. cerevisiae* showed good growth potency into the different culture media which supplemented with crude and commercial peptones. This indicates that microorganisms were able to use peptones efficiently as nitrogen sources. Nitrogen sources in microbiological media mainly peptone play vital roles in supporting microbial growth. The efficiency of crude peptones in supporting the growth of the selected microorganisms illustrates the versatility of these nitrogen sources for use with different microbial culture media. *P. aeruginosa* is one of the well-known pathogenic bacteria, *L. acidophilus* is one of the important species in lactic acid bacteria group of large economic importance so as the yeast *S. cerevisisea* which is widely used in industrial fermentation as well as bakery products **(Horn *et al*., 2005; El-Fouly *et al*., 2015)**

It could be concluded that crude peptones produced by acid and alkaline autolysis of fish wastes could be exploited as a suitable nitrogen sources for different microbiological media. The fair nitrogen content and balanced amino acid composition were obviously reflected in the good growth of different tested microorganisms. Further studies are indeed needed to improve the composition of crude peptones using modern technologies especially when intended for production on a commercial scale.

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استخلاص الببتون الخام من مخلفات الاسماك للاستخدام كمصدر نتروجين في الاوساط الزرعية الميكروبية

صلاح مهدي نجم[١] ، جلال محمد النور[١] ووائل علي الوائلي[٢]
[١] قسم الأسماك والثروة البحرية، كلية الزراعة، جامعة البصرة ، البصرة، العراق
[٢] قسم علوم الأغذية والتقانات الأحيائية، كلية الزراعة، جامعة البصرة، البصرة ، العراق

أجريت الدراسة الحالية لاستكشاف امكانية انتاج الببتون الخام من مخلفات الاسماك. استخدم التحلل الذاتي تحت الظروف الحامضية والقاعدية للتحليل المائي لمخلفات الاسماك. اظهر التحليل التقريبي للببتون الخام والتجاري ان الببتون الخام احتوى مستويات مقبولة من النتروجين (وصلت الى ١٠,٨ %) رغم ان المنتج التجاري كان متفوقا باحتوائه نسبة ١٥,١ % نتروجين. واوضح تركيب الاحماض الامينية لأنواع الببتون الثلاثة المدروسة ان الببتون الخام يمتلك تركيبا جيد التوازن من الاحماض الامينية دون نقص حاد او فقدان لأي من الاحماض الامينية الموجودة في المنتج التجاري. استخدمت ثلاثة أنواع من الأحياء الدقيقة هي *Pseudomonas aeruginosa* و *Lactobacillus acidophilus* و *Saccharomyces cerevisiae* لاختبار الببتون الخام المنتج كمصادر نتروجين في الاوساط الزرعية. واوضحت نتائج الكثافة البصرية OD_{600} أن الأنواع الثلاثة تظهر كفاءة نمو جيدة في الأوساط المختلفة المدعمة بالببتون الخام أو التجاري بغض النظر عن التفوق النسبي للمنتج التجاري. وقد استنتج أن الببتون الخام المنتج يحتاج للتحسين من أجل تعزيز خواصه المختلفة.