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Tracing Growth of *Staphylococcus Aureus* in a Medium Containing Peptone Derived from Rainbow Trout (*Oncorhynchusmykiss*) Viscera

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ABSTRACT

One of the most important applications of protein hydrolysate is in bacteria media.Peptones are extensively used in microbial media and consequently, producing cheap peptones with even higher performance than commercial peptones is very important. The present study aimed at determining growth of *Staphylococcus aureus* in a medium containing peptone derived by acid hydrolysis of rainbow trout (*Oncorhynchusmykiss)* viscera. Hydrolysis was performed using HCl at 85°C and degree of hydrolysis was determined through the method proposed by kristinsson and Rasco (2000a).Growth rates of the bacteria were measured by reading absorption at 600 nm at seven times (0, 3, 6, 9, 12, 15, and 18 hours after the introduction of the bacteria). Statistical analysis of data was performed via one-way ANOVA and Duncan's test ($p<0.05$).The results of the present study showed that 15 gr/l protein hydrolysate obtained from rainbow trout viscera resulted in improvement in growth of *S. aureus* although it was less efficient compared with commercial medium. In conclusion, rainbow trout viscera can be used as a rich source of protein for improving growth of *Staphylococcus aureus*in growth media.
KEYWORDS: growth medium, peptone, protein hydrolysate, rainbow troutviscera, *Staphylococcus aureus*.

1- INTRODUCTION

Fishery products are rich in protein and unsaturated fatty acids resulting in susceptibility to rapid rancidity. They are also considered suitable substrates for lactic acid fermentation and sources of protease-producing bacteria (Bhaskar et al., 2007). Since almost 50 percent of fish is edible and around half of fish meat is consumed by human, a considerable amount of fish meat is left unused. During the past 50 years, several attempts have been devoted to elevate usability of marine products. One of such efforts is extraction of protein from whole fish, gutted fish, and fish viscera by solvent producing fish protein concentrate (FPC) with high protein percentage. Several efforts have been also devoted for hydrolyzing fish protein via several methods (Aspmo et al., 2005).

Protein hydrolysis consists of hydrolytic cleavage of peptide bonds either chemically or enzymatically. Chemical hydrolysis of proteins is performed through peptide bonds cleavage by use of acid and/or base. This method has been a method of favor in food science and technology due to its low cost and ease; however, it suffers from some limitations such as difficulty in controlling chemical hydrolysis, products with weak functional properties, etc. (Kristinsson and Rasco, 2000a). Duringalkaline hydrolysis, such amino acids as cysteine, serine, and threonine are damaged and unfavorable compounds such as lysinoalaninemay form. Furthermore, racemization of amino acid residues might occur during alkaline hydrolysis (Onodenaloreand Shahidi, 1996). Protein hydrolysis with chemicals and solvents is done in extreme temperatures and pH values leading to the products with low nutritional and functional properties limiting its applications as flavoring compounds (Kristinsson and Rasco, 2000b). Nowadays, acid hydrolysis of proteins is limited because of formation of chloropropanol; moreover, acid hydrolysis decompose tryptophan, an essential amino acid for human body (Kristinsson and Rasco, 2000a).

One of the most important applications of protein hydrolysate is in bacteria media. Standard media used for various bacteria contain protein hydrolysate from casein, meat, soy bean, and yeast (as nitrogen source) and growth factors (vitamins, fatty acids, purine, pyrimidine, and amino acids). Some compounds in microbial media such yeast concentrate are different according to the producer companies and different ratios are adopted (Horn et al., 2007). Bacteria are microorganisms that need minerals, carbon, and nitrogen for growth in artificial media. Nitrogen is one of the most expensive components of media which are sold as meat peptones, casein peptones, and yeast concentrate. For instance, proteins from casein, meat, and yeast concentrate are used to grow lactic bacteria. It should be noted that such peptones are not economical for mass culture of lactic bacteria. Peptones are extensively

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used in microbial media and consequently, producing cheap peptones with even higher performance than commercial peptones is very important. The present study was formulated in order to determine growth of *Staphylococcus aureus* in a medium containing peptone derived by acid hydrolysis of rainbow trout (*Oncorhynchusmykiss)* viscera.

2- MATERIALS AND METHODS

2-1- Materials

Hydrochloric acid and sodium hydroxide were purchased from Merck Co., Germany. Trichloroacetic acid was prepared from Panreac Co., Italy. Protein standard was purchased from Zist-Shimi Co., Iran. The bacteria were prepared from Scientific and Industrial Research Organization, Iran. The tools used in the present study were as follows: laboratory scale (GF-300, A&D Co., Japan), pH-meter (827, Metrohm, Switzerland), spectrophotometer (6305, Jenway, England), centrifuge (GmbH Z206A, Hermleabortechnic, Germany), micro-centrifuge (Ependorf, Germany), oven (Shimas, Iran), incubator (Aria Teb, Iran), water bath (W614-B, Fatrerizpardaz, Iran), and grinder (Sania, Iran).

2-2- Preparation of viscera protein hydrolysate

Rainbow trout viscera was provided from a local fish market in Mazandaran Province, Iran and stored at -20°C until use, the viscera was thawed at 4°C in refrigerator and 100 gr viscera was ground. The heated viscera was then mixed with distilled water at a ratio of 1:2 and homogenized and heated at 85°C for 20 min in order to inactivate internal enzymes. Reaction was performed at 85°C and pH=3.3. Acid hydrolysis was performed by using hydrochloric acid and terminated by use of sodium hydrochloride. The reaction was performed in 250 ml flasks in shaking water bath for 18 hours. Then, the sample was centrifuged at 5000 rpm and 4°C for 15 min. the supernatant was collected and filtered and put in oven at 85°C for 18-24 hours and free-dried (Ovissipour, et al. 2009).

2-3- Chemical composition

Total protein in raw material was derived through the Kjeldahl method (AOAC, 2005). Supernatant protein was obtained via biuret method and bovine albumin serum was used as standard protein and absorption was read at 540 nm (Layne, 1957).

2-4- Degree of hydrolysis

The degree of hydrolysis was evaluated on the basis of kristinsson and Rasco (2000a) and expressed as the proportion (%) nitrogen soluble in 10% TCA with respect to the total N in the sample (Ovissipour, et al. 2009).

2-5- Application of protein hydrolysate in *S. aureus* media

In order to compare growth of *S. aureus*in a commercial medium (Tryptic Soy broth) and the medium containing viscera protein hydrolysate, primary compounds of TSB is prepared and the prepared peptone was used instead of commercial peptone and sterilized at 121°C for 15 min. growth rates of the bacteria were measured by reading absorption at 600 nm at seven times (0, 3, 6, 9, 12, 15, and 18 hours after the introduction of the bacteria) (Safari et al., 2009).

2-6- Data analyses

In order to perform statistical analysis of data, one-way ANOVA and Duncan's test were used ($p<0.05$). All the analyseswere performed using SPSS (version 18).

3- RESULTS

Total protein in rainbow trout raw viscera and viscera hydrolysate were found to be 16.25±0.65 and 43.25±0.55 mg/ml, respectively. Degree of hydrolysis was detected to be 23.42±0.37%.

In order to use rainbow trout viscera protein hydrolysate, hydrolyzed peptone after 18 hours with the highest level of protein as the final product of hydrolysis was used. Table 1 and Figs. 1 and 2 show variations of absorption of *S.aureus*in media containing protein hydrolysate prepared by acid and commercial medium (Tryptic Soy broth).

Table 1: variations of absorption of *S.aureus*in media containing protein hydrolysate prepared by acid and commercial medium (Tryptic Soy broth)

Time (h)	Hydrolysis by acid	TSB (control sample)
0	0.51±0.01f	0.41±0.02g
3	0.67±0.03f	0.82±0.03f
6	1.18±0.07e	1.48±0.07e
9	1.64±0.03d	2.21±0.06d
12	2.11±0.06c	2.68±0.06c
15	2.45±0.04b	3.06±0.08b
18	3.22±0.06a	3.83±0.05a

Different letters indicate significant differences ($p<0.05$).

There are increasing trends of absorption of *S.aureus*from the time o to 18 h in both treatments (acid and control); however, this trend was better in the commercial medium compared to the one containing protein hydrolysis. In control sample, absorption reached from 0.41 nm at the time 0 to 3.81 nm at the time 18 h while in the sample with protein hydrolysis, it reached from 0.51 nm at the time 0 to 3.22 nm at the time 18 h. significant differences were seen in increasing trends of bacteria in both samples ($p<0.05$). In addition, increasing trend of absorption by *S.aureus*in different times were found to be significant ($p<0.05$).

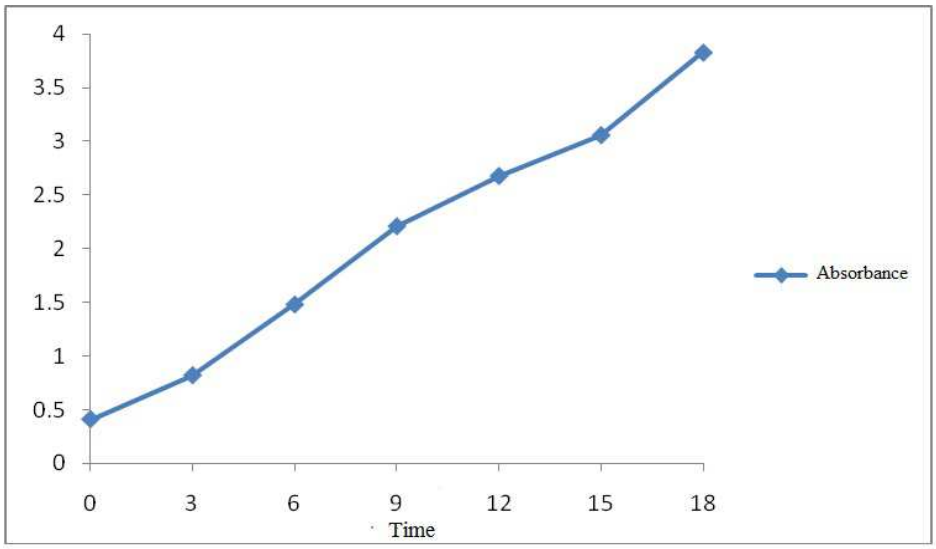

Figure 1: Variations of absorption of *S.aureus*in media containing a commercial medium (Tryptic Soy broth)

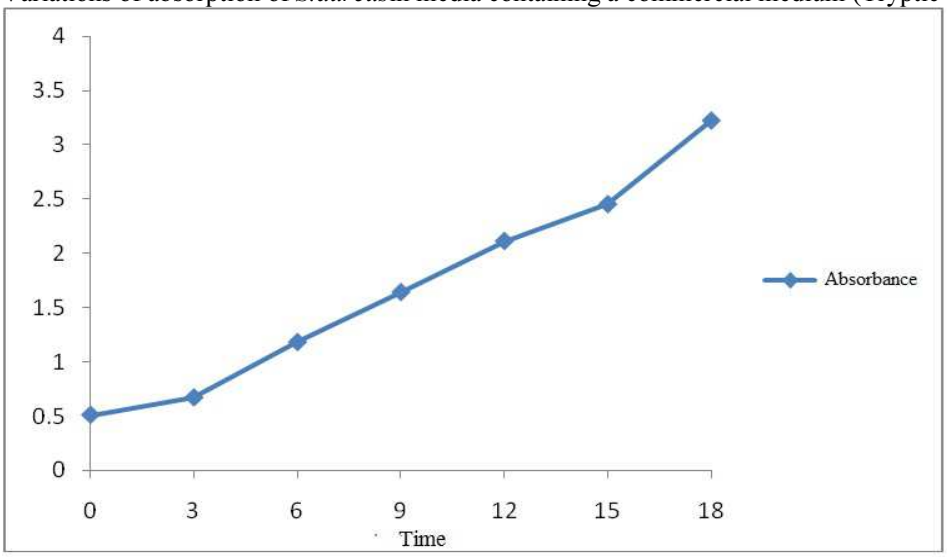

Figure 2: Variations of absorption of *S.aureus*in media containing protein hydrolysate prepared by acid

4- DISCUSSION

In recent years, several investigations have been performed on hydrolysis of different parts of fish by acids, bases, and commercial enzymes. Changing pH to increase protein extraction is a suitable method to use fish wastes. This method can also reduce secondary contamination by these wastes. The results obtained from the present study showed that hydrolyzed protein in rainbow trout viscera was 43.25±0.55 mg/ml by acid hydrolysis at 85°C. On a study on white muscles of *Clupeaharengus* through acid hydrolysis, protein content was found to be 74% (Hoyle and Merritt, 1994). Also, protein content of *Oreochromisniloticus* hydrolysis by acid was found to be 56-61% (kristinsson and Rasco, 2000a). Furthermore, protein content of *Salmosalar* hydrolysis by use of acid was found to be 71.5% (kristinsson and Rasco, 2000b).

Peptone used in the present study was 15 gr/l of commercial medium. The commercial medium in the present study was TSB with 20 gr/l peptone. The results of the present study showed that 15 gr/l protein hydrolysate obtained from rainbow trout viscera resulted in improvement in growth of *S. aureus*although it was less efficient compared with commercial medium. It seems that peptones produced by acid hydrolysis couldn't be used very well in the medium and therefore, the bacteria couldn't use peptones properly to reach desirable level of growth because the peptones in the hydrolysate were not suitable in terms of molecular weight and peptide chain length (Ovissipour et al., 2009).

Of course, it should be noted that different species might have varying effects on properties of hydrolysate. For example, Gildberg et al. (1989) reported that lactic bacteria had better growth rates in substrates containing blue whiting protein hydrolysate compared to capelin protein hydrolysate.

Although growth of the bacteria in the medium containing rainbow trout viscera hydrolysate wasn't as much as that in commercial medium, it is still recommended to make use of such peptone sources because they are cheaper and can be considered as value-added products. Vazquez et al. (2004a) indicated that fish and seafood protein hydrolysate not only improves bacteria growth but also is suitable to be used as media in order to produce various bacteriocins and single cell proteins. The results acquired from the present study showed that rainbow trout viscera protein hydrolysate can be used as a suitable substitute for commercial peptides in media used for *Staphylococcus aureus*.

Taken together, it can be concluded that rainbow trout viscera be used as a rich source of protein for improving growth of *Staphylococcus aureus*and protein hydrolysate provided from the viscera can be utilized as a value-added substitute for commercial peptones in microbial media such as casein, beef, and soybean.

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