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# Utilization of Hydrolysis of Protein Concentrates Prepared From the Waste of Common Carp Fish using Trypsin Enzyme for the Growth of Lactic Acid Bacteria

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**Abstract**

This study aimed to use trypsin enzyme hydrolysates of protein concentrates prepared from the waste of common carp fish (Cyprinus carpio) for the growth of lactic acid bacteria. Trypsin enzyme has been used in the hydrolysis of acid and base protein concentrates for various times, including 60, 120, and 180 minutes. The solubility and emulsification property of the produced hydrolysates was studied. The EAI and ESI at pH 2 and 12 gave the highest values for solubility, , while the lowest values at pH 4. Hydrolysates were used to prepare culture media as an alternative to the MRS medium. Peptospecial and Beef extract were replaced in the commercial media with hydrolysates, and two types of culture media were prepared, one containing yeast extract and the other without it. The enzymatic reaction time had a significant effect on the number of Lactobacillus acidophilus and Bifidobacterium animalis colonies, and yeast extract had a significant effect on the number of colonies of Lactobacillus rhamnosus GG. In contrast, the enzymatic reaction time had not affected the number of colonies of Lactobacillus plantarum and Bifidobacterium infantis. This study is recommended to study the response of other types of lactic acid bacteria and the suitability of the culture media prepared in this study to their growth requirements.

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## Introduction

Fish waste is an important source of protein for the production of protein hydrolases and biologically active peptides (Shahidi *et al.*, 2019). The most common chemical and enzymatic methods for producing protein hydrolases use enzymes derived from plants, animals, and microbes sources (Rodríguez-Vázquez *et al.*, 2020). The type of enzyme determines the enzymatic modification endopeptidases hydrolyze the internal peptide bonds, at the same time, exopeptidases catalyze the cleavage of the terminal peptide bonds in a sequential manner of the substrate (Senadheera *et al.*, 2021). Proteolytics are the product of the enzymatic hydrolysis of proteins. They contain peptides with low molecular weights and free amino acids. Therefore, they are an important source of amino acids and have many applications in different fields (Nurdiani *et al.*, 2022). Commercial enzymes are used to modify physical, chemical, and sensory properties while increasing the digestibility coefficient and improving nutritional value under moderate conditions without isomers reactions, as in chemical decomposition (Prihanto *et al.*, 2019). The physical and chemical properties of the resulting proteolytic are affected by the type of enzyme used, the type of substrate, and the degree of hydrolysis. Enzymes with an acidic pH generally show less catalytic activity than neutral or basic enzymes. Thus,

both neutral and basic enzymes are used to obtain protein hydrolysates with a high degree of degradation (Priatni *et al.,* 2020). Fish waste contains unstable fats that are susceptible to oxidation during the enzymatic hydrolysis process, and the oxidation of heme proteins enhances the appearance of rancidity in the final product (Yarnpakdee *et al.*, 2012; Al-Shamary *et al.*, 2023). When fats are more than 1% in the final product, it is necessary to use organic solvents or add antioxidants to them, such as butylated hydroxytoluene, butylated hydroxyanisol, and propylgallate. Hydrolyzed fish proteins also become dark in color when they contain a high percentage of fat, and the formation of brown pigments may result, due to Aldol condensation of carbonyls from lipid oxidation (Ahmed and Al-Shamary, 2019). The common carp is a freshwater fish (Al-Khafaji and Al-amary, 2016; Al-Hassani and Mustafa, 2022). It is an omnivorous fish that feeds on various plant and animal sources (Alrudainy and Jumaa, 2016; Mohammad, 2023), it grows quickly and reaches maturity after two years (Yousef and Al-Khshali, 2023). Therefore, it is of great economic importance in some countries and a riverine resource in others (Tessema *et al.,* 2020). Carp fish waste contains varying percentages of protein, as it constitutes 22.1% of scales, 13.9% of fins, and 25.9% of bones (Maktoof et al., 2020). Complex commercial media are used to grow lactic acid bacteria such as De Man Rogosa and Sharpe (MRS), Sodium Lactate (NaLa), Brain Heart Infusion (BHI), Trypticase Soy Broth Yeast Extract (TSBYE) and M17, these media are designed for specific strains, such as MRS for lactobacilli and M17 for lactococci (Abbasiliasi *et al.,* 2017; Awda and Fayyadh, 2018; Hasan et al., 2019). MRS used to grow lactic acid bacteria in the laboratory, are considered to be very expensive, because they contain expensive nitrogen sources, such as yeast and meat extract, and they do not support the growth of all types of lactic acid bacteria (Muhsin and Hassan, 2019; Hayek *et al.,* 2019). Many studies indicate the possibility of replacing expensive commercial growing media with alternative media that contain agricultural or industrial waste at low or medium cost (Al-Lami *et al.,* 2015; Anggraini *et al.,* 2019). The study aimed to exploit the waste of common carp fish polluting the environment in the preparation of an inexpensive peptone for the growth of some types of bacteria.

## Materials and Methods

### Microorganisms

*Lactobacillus acidophilus*, Natures bounty, *Lactobacillus rhamnosus GG*, Lacto 66, Lactobacillus *plantarum*, Swanson, *Bifidobacterium anmals Bb12*, Sandoz, *Bifidobacterium infantis*, Biocodex.

### Cultural medium

DeMan, Rogosa and Sharpe broth, Diagnostici Liofilchem, Italy

### Preparation of protein concentrates

The method mentioned in Tian *et al*. (2016) was followed in preparing protein concentrate using the pH shifting method, by mixing defatted fish waste powder with deionized water in a ratio of 1:9, using a magnetic stirrer (Stuart SB162, China), the pH was adjusted to 2 and 12 using the hydrochloric acid solution and sodium hydroxide 2N. The mixing process was completed using a magnetic stirrer for 60 min at 40°C, followed by centrifugation (Hermile GmbH, Germany) at 10,000 x g, for 30 min, at 4°C. The precipitate was removed, acid supernatant and base supernatant fraction were collected, the pH was adjusted to 4 using sodium hydroxide and hydrochloric acid solutions 2N, and the solutions were mixed using a magnetic stirrer for 60 min, at 40°C, followed by centrifugation 10,000 x g, for 30 min at 4°C. The supernatant was removed and the precipitate was collected to obtain the acidic protein concentrates (ACO) and base protein concentrates (BCO), washed with deionized

water, and the centrifugation process was repeated. The precipitate was collected, pH was adjusted to 7 and lyophilized (ALPHA 1-2 LDplus, Germany).

**Preparation of protein hydrolysates**

Protein hydrolysate was prepared according to the method explained (Ramezani *et al.*, 2020). The protein concentrates prepared from defatted fish waste powder were mixed with phosphate buffer pH (8) in a ratio of 1:4 and placed in a water bath water at 85°C for 15 minutes, then cooled to 37°C, the trypsin, ≥ 10,000 BAEE. Units/mg protein, from bovine pancreas, Sigma Aldrich (USA), was added at a rate of 1.5 grams/100 grams of protein concentrate, in a shaking incubator at 37°C. The samples were withdrawn after 60, 120, 180, and 240 minutes, and placed in a water bath at 85°C for 15 minutes.

**Degree of Hydrolysis (DH)**

The degree of Hydrolysis was estimated according to the method (Nurdiani *et al.*, 2022) by mixing 2 ml of protein Hydrolysis with 2 ml of TCA solution 20% (w/v), leaving the mixture for 30 minutes, then centrifuging (Hermile GmbH, Germany) at 5000 x g for 30 minutes, the protein in the mixture and the supernatant fraction was estimated using the Kjeldahl method, and the degree of Hydrolysis was calculated according to the following equation:

$$\textbf{DH \% } = \frac{\textbf{10\% } \textit{TCA soluble nitrogen in the sample}}{\textit{Total nitrogen in the sample}} * 100$$

**Solubility**

The solubility was estimated according to the method mentioned in (Thuy *et al.*, 2015) The solution of protein hydrolysates was prepared by dissolving 200 milligrams of hydrolysate powder with an amount of distilled water, completing the volume to 20 milliliters, and adjusting the pH using hydrochloric acid 0.1 N and sodium hydroxide solution 0.1 N to 2, 4, 6, 8, and 10, using pH meter (Crison basic 20, CHINA). The solutions were mixed for 30 minutes at 150 rpm, and centrifuged (Hermile GmbH, Germany), at 8000 x g for 20 minutes, the supernatant was collected, the protein was estimated using the Kjeldahl method, and the solubility was calculated from the following equation:

$$\textbf{Solubility \% } = \frac{\textit{protein content in the supernatant}}{\textit{total protein content in the sample}} * \textbf{100}$$

**Emulsification**

The method mentioned by (Jamdar *et al.*, 2010) was used to estimate both the Emulsifying Activity Index (EAI) and the Emulsifying Stability Index (ESI), by dissolving 450 milligrams of hydrolysate powder in an amount of distilled water, bringing the volume to 45 ml. The pH was adjusted using hydrochloric acid solution 0.1 N and the sodium hydroxide solution 0.1 N was to 2, 4, 6, and 8. The hydrolysate solutions were mixed with 15 milliliters of sunflower oil (Zer, Iraq) separately, and homogenized at a speed of 19,000 rpm for one minute. Then 50 microliters of solution were mixed with 5 milliliters of SDS 0.1 %. The optical absorbance of the homogenized solutions was measured immediately after homogenization, and 10 minutes after homogenization, at a wavelength of 500 nm using a spectrophotometer (SCO-TECH SPUV-26, Germany), and the EAI and ESI were calculated by:

$$\text{EAI } (m^2 / g) = \frac{A^0 \; x \; 2 \; x \; T \; x \; dilution \; factor}{C \; x \; \Phi \; x \; 1000}$$

$$\text{ESI (min)} = \frac{A^0 \; x \; \Delta t}{A^0 - A^{10}}$$

Where $A^0$ = absorption value at 500 nm, $A^{10}$ = absorption after 10 minutes, Φ = oil volume fraction (0.25), DF = dilution factor (100), C = protein concentration, Δt = 10, T = 2.303.

**Activation of bacteria**

The MRS broth medium prepared by Diagnostici Liofilchem (Italy) was prepared by dissolving 54.3 grams of the medium in

1000 milliliters of distilled water according to the supplied company's instructions (Schöpping *et al*., 2021). The MRS medium was distributed in tubes and sterilized with an autoclave (Tomy ss-325, Japan). After cooling to 37°C, the medium was inoculated with the contents of the lyophilized capsule of *Lactobacillus acidophilus, Lactobacillus rhamnosus GG*, Lactobacillus *plantarum*, *Bifidobacterium infantis*, and *Bifidobacterium animalis* under sterile conditions, and incubated at 37°C for 72 hours, repeat the process three times, and store at 4 °C until use.

**Preparation of MRS agar medium**

The method mentioned by (Schöpping *et al*., 2021), was used to prepare De Man, Rogosa and Sharpe (MRS) agar medium supplied by Diagnostici Liofilchem by dissolving 69.3 grams of the medium in 1000 ml of distilled water according to the supplied company's instructions and mixed using a hot plate magnetic stirrer to completely dissolve the medium.

**Preparation of alternative media**

Alternative media were prepared according to the method mentioned in (Chen *et al*., 2015) replacing Peptospecial and Beef extract in the MRS commercial medium with the acidic and basic protein hydrolysate, in the presence and absence of yeast extract, without changing the rest of the components of the commercial medium as in Table 1. All Media were sterilized using autoclaves at 121 °C for 15 minutes, and 15 lb/in².

**Table 1. Formulation of culture media**

| Ingredients (g / L) | MRS | Without yeast extract | With yeast extract |
|---|---|---|---|
| Protein hydrolysates | Null | 25 | 20 |
| Peptospecial | 10 | Null | Null |
| Beef extract | 10 | Null | Null |
| Yeast extract | 5 | Null | 5 |
| Glucose | 20 | 20 | 20 |
| Triammonium citrate | 2 | 2 | 2 |
| Sodium acetate | 5 | 5 | 5 |
| Magnesium sulphate | 0.2 | 0.2 | 0.2 |
| Manganese sulphate | 0.05 | 0.05 | 0.05 |
| Tween 80 | 1 | 1 | 1 |
| Di – potassium phosphate | 2 | 2 | 2 |
| agar | 15 | 15 | 15 |
| PH 6.2 | | | |

**Bacterial growth in commercial and alternative media**

The method mentioned in (Ahmed and Al-Shamary, 2019) was followed in preparing the decimal dilutions. The pouring plate method was used in growing the bacterial species, as 1 milliliter of each dilution was transferred to each type of bacteria into the Petri dishes, the commercial MRS agar medium, and the alternative media, the plates were left to solidify, they were transferred to the anaerobic incubation vessel, upside down, the air was removed and replaced with carbon dioxide gas, and incubated at 37°C for 48 hours.

**Result and Discussion**

**Degree of Hydrolysis (DH)**

Figure 1 shows the degree of hydrolysis of the acidic and the basic protein concentrate by trypsin enzyme after 60, 120, 180, and 240 minutes of enzymatic reaction time. The degree of hydrolysis of the acidic protein concentrate was 10.9, 19.3, 29.2, and

30.1%, respectively, while the degree of hydrolysis of basic protein concentrate was 11.2, 17.5, 31.4, and 31.8 %, in the same order. The degree of hydrolysis in both protein concentrates increased with increasing enzymatic reaction time, and there were no significant differences in the degree of Hydrolysis after 120 and 180 minutes of enzymatic reaction time. Wisuthiphaet and Kongruang (2015) prepared protein hydrolysates by Papain at a concentration of 2, 4, and 6 % from a mixture of Ponyfish (*Eubleekeria splendens*), Mackerel (*Decapterus maruadsi*), and Yellow stripe travally (*Selaroides leptolipis*), the degree of hydrolysis was 10, 14, and 10 % after 5 hours, respectively, 19, 15, and 14.5 % after 10 hours, and 20, 24, and 23% after 15 hours, in same order. Prihanto *et al.,* (2019) found that when using parrotfish heads (*Chlorurus sordidus*) to prepare autolytic protein hydrolysates, the degree of hydrolysis ranged between 0.26 - 30.65 %, the highest degree of hydrolysis was observed at pH 9 after 24 hours, while The lowest degree of hydrolysis was at pH 7 after two hours. Ali (2019) found when preparing protein hydrolysates from the skin of *Silurus glanis* fish, by Papain, that the degree of hydrolysis after 4, 7, 10, and 13 hours was 21.1, 33.3, 51.1, and 54.28 % respectively. The variation in the degrees of hydrolysis is due to the type of enzyme and the Substrate.

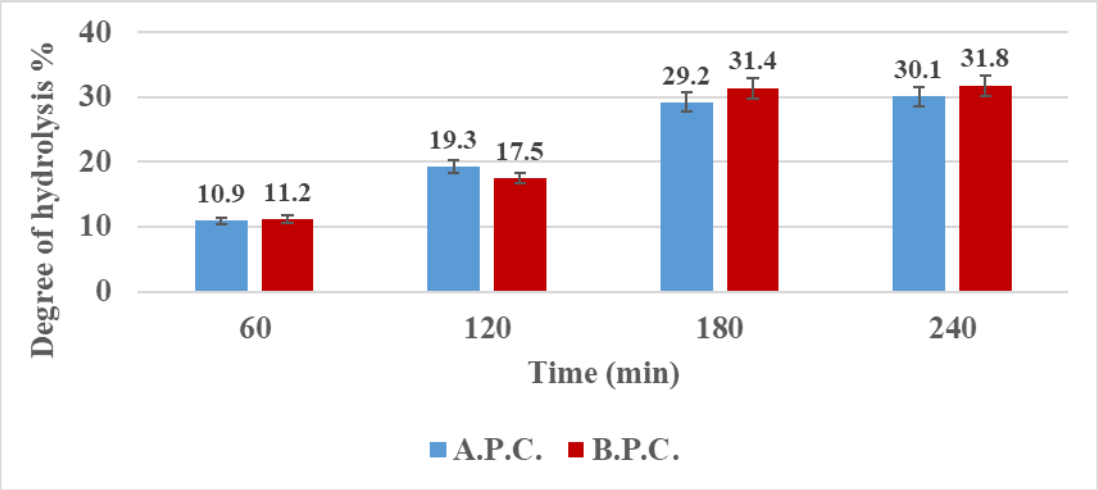


**Figure 1. Degree of hydrolysis of the acidic protein concentrate (A.P.C.) and the basic protein concentrate (B.P.C.) with the enzyme trypsin at a concentration of 1.5 grams/100 grams of protein concentrate**

## Solubility

Figures 2 and 3 show the solubility of hydrolysates prepared from the acidic protein concentrate and the basic protein concentrate by trypsin enzyme after (60, 120, and 180) minutes of enzymatic reaction time at various pHs. The solubility increased with increasing enzymatic hydrolysis time, while it decreased at pH 4. Proteins generally have a positive charge at pHs lower than their isoelectric point, and a negative charge at pHs higher than their isoelectric point, in both cases proteins spread in solutions and remain suspended due to repulsion between similar charges, and the resultant charge is zero at the isoelectric point, meaning that total negative charge is equal to total positive charge, as the different charges attract and proteins precipitate, and the number of negative charges increases whenever the pH is higher than the pH isoelectric point, due to the ionization of the alpha carboxylic groups belonging to the side chains of amino acids, while the number of positive charges increases as the pH decreases from the isoelectric point due to the ionization of the

amine groups. Molecular weight, pH, and hydrophilic groups affect solubility, Klomklao and Benjaku (2018) prepared protein hydrolysates from the guts of tuna fish (*Katsuwonus pelmamis*) by Alcalase enzyme, and solubility was 91.89, 96.39, 97.65, and 100 % at pH 3, 5, 7, and 9 respectively. Ramezani *et al.*, (2020) showed the effect of both pH and hydrolysis time when prepared hydrolysates from ponyfish (*Photopectoralis bindus*), the solubility of protein hydrolysates after one

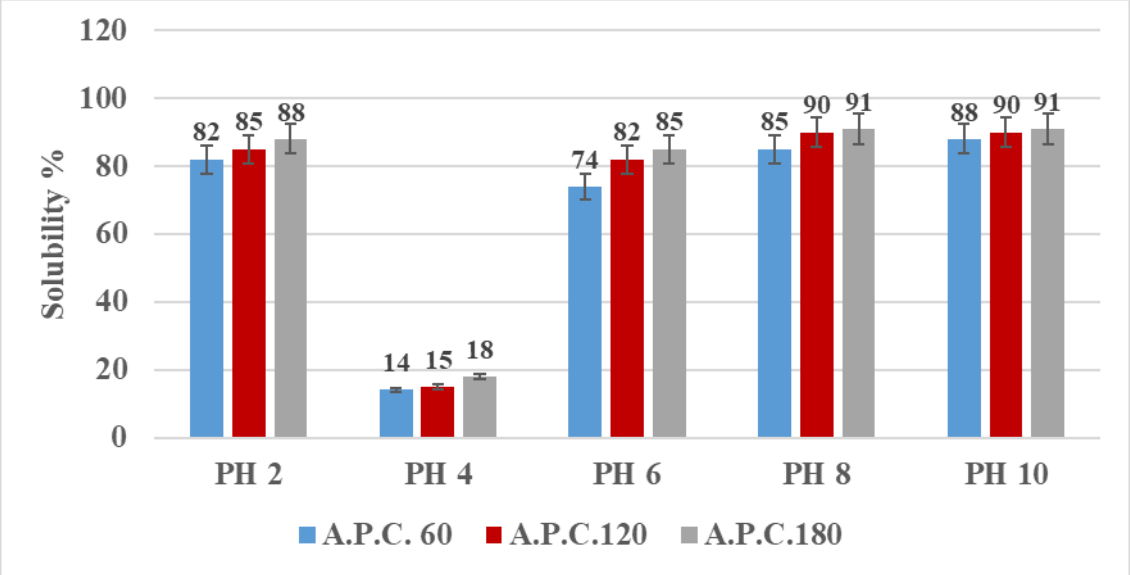


**Figure 2. Solubility (%) of hydrolysates from acidic protein concentrate (A.P.C.) by trypsin enzyme after (60, 120, and 180) minutes of enzymatic reaction time at various pH (2, 4, 6, 8, and 10)**

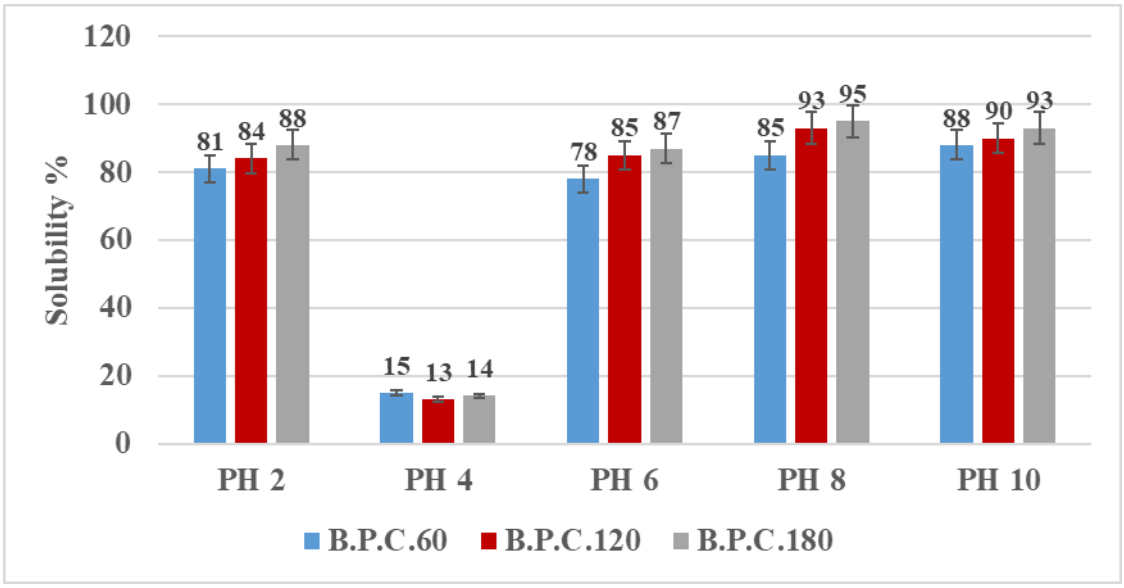


**Figure 3. Solubility (%) of hydrolysates from basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120, and 180) minutes of enzymatic reaction time at various pH (2, 4, 6, 8, and 10)**

hour were 94, 92, 94, and 96 % and after four hours, 98, 95, 98, and 99 %, at pH 3, 5, 7, and 9, respectively. Alahmad *et al.*, (2022) found that solubility increases with increasing degree of hydrolysis when prepared protein hydrolysates from Bighead Carp (*Hypophthalmichthys nobilis*) by Ficin enzyme (610 MCU/mg). Yathisha *et al.*,

(2022) reported that the solubility of protein hydrolysates prepared from the Ribbon fish (*Lepturacanthus savala*) by Alcalase EC.3.4.21.14 (*Bacillus licheniformis* ≥2.4 U/g) after one hour of hydrolysis time was 33.91, 19.23, 33.58, 32.74, 25.57, and 26.51 at pH 2, 4, 6, 8, 10, and 12, respectively, after five hours, they were 85.05, 58.74, 82.35, 81.55, 79.21, and 74.67 % in the same order.

**Emulsification**

Figures 4 and 5 show the Emulsifying Activity Index (EAI) of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after 60, 120, and 180 minutes of enzymatic reaction time at various pH, EAI increased after 120 minutes of enzymatic reaction time, then decreased after 180 minutes, in both protein concentrates. The lowest values were at pH 4.

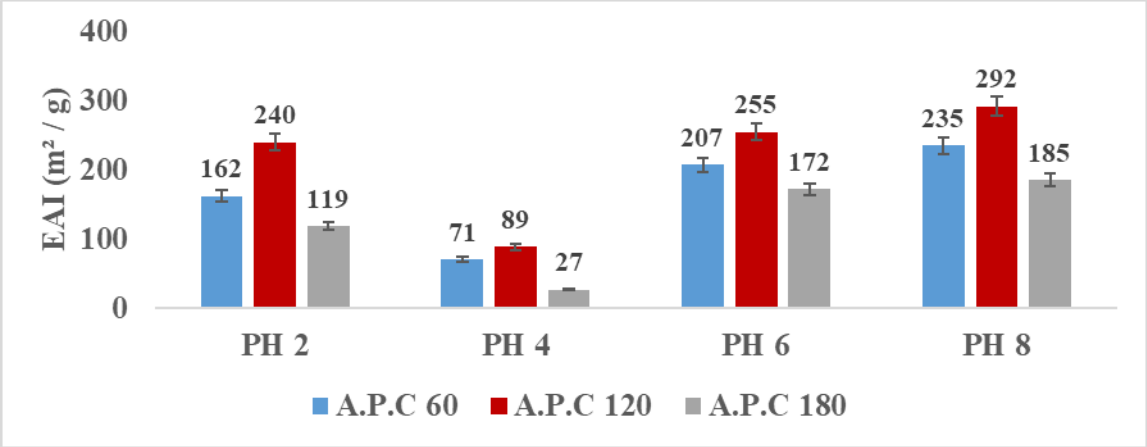


**Figure 4. Emulsifying Activity Index (EAI) of hydrolysates from acidic protein concentrate (A.P.C.) by trypsin enzyme after (60, 120 and 180) minutes of enzymatic reaction time at various pH (2, 4, 6 and 8)**

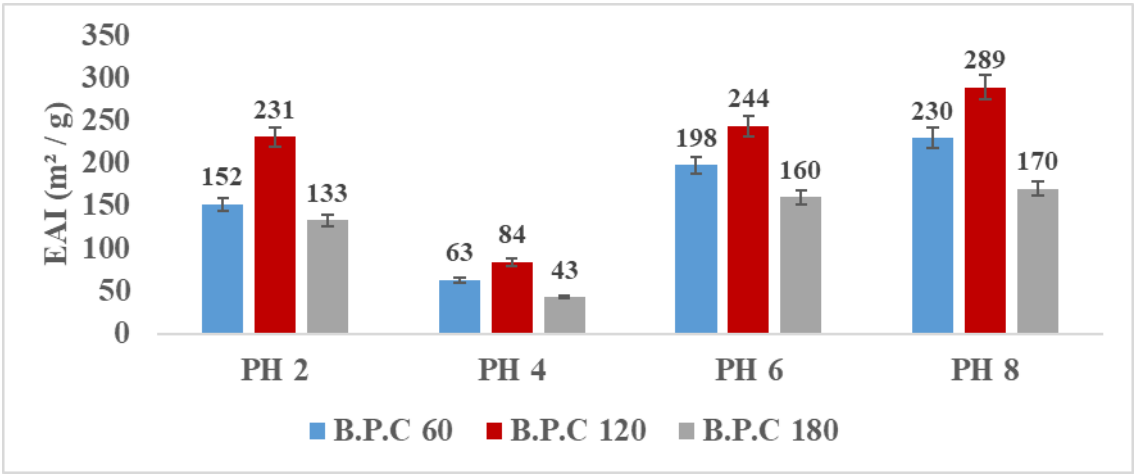


**Figure 5. Emulsifying Activity Index (EAI) of hydrolysates from basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120, and 180) minutes of enzymatic reaction time at various pH (2, 4, 6, and 8)**

Figures 6 and 7 show the Emulsifying Stability Index (ESI) of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after 60, 120, and 180 minutes of enzymatic reaction time at various pH.

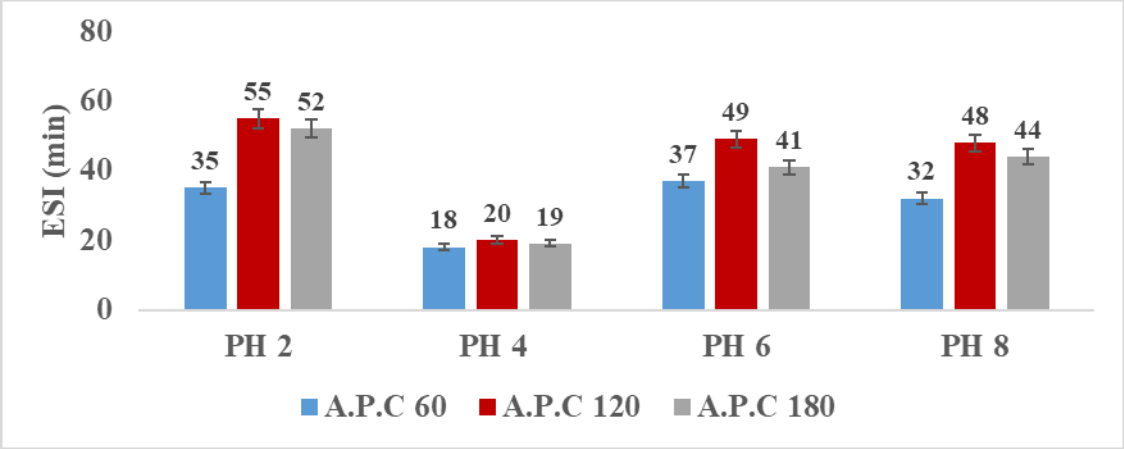


**Figure 6. Emulsifying Stability Index (ESI) of hydrolysates from acidic protein concentrate (A.P.C.) by trypsin enzyme after (60, 120 and 180) minutes of enzymatic reaction time at various pH (2, 4, 6 and 8)**

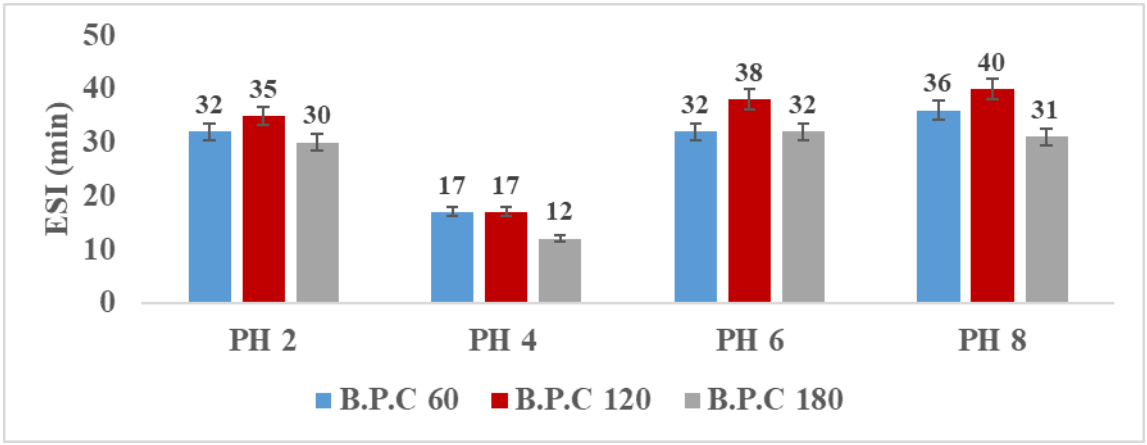


**Figure 7. Emulsifying Stability Index (ESI) of hydrolysates from basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120, and 180) minutes of enzymatic reaction time at various pH (2, 4, 6, and 8)**

The ESI values of the acidic protein concentrate hydrolysates at pH 2 were 35, 55, and 52 minutes after 60, 120, and 180 minutes of enzymatic reaction time, respectively, at pH 4 (18, 20, and 19) minutes, at pH 6 (37, 49, and 41) minutes, and at pH 8 (32, 48, and 44) minutes, in same order, while the ESI values for basic protein concentrate hydrolysates at pH 2 were 32, 35, and 30 minutes after 60, 120, and 180 minutes of enzymatic reaction time, respectively, at pH 4 (17, 17, and 12), minutes, at pH 6 (32, 38, and 32), and at pH 8 (36, 40, and 31), in same order. Thuy *et al.*, (2015) used the Alcalase 2.4L (declared activity 0.5 IU/g subtract) in prepared hydrolysates from the heads and skeletons of *Pangasius hypophthalmus* fish, The emulsification capacity increased from 10.58 mL oil/g FPI after 30 minutes of enzymatic reaction time to 20.99 mL oil/g FPI after 75 minutes, then decreased to 10.02 mL oil/g FPI after 150 minutes of enzymatic reaction time. Alahmad *et al.*, (2022) found when studying the emulsifying property of hydrolysates prepared from Bighead Carp (*Hypophthalmichthys nobilis*) by the Ficin enzyme (610 MCU/mg), after mixing different concentrations of hydrolysates (0.1, 0.5 and 1)% with 10 ml from soybean oil, EAI at a degree of Hydrolysis of 13.36% was 92.75, 27.36 and 10.68 $m^2/g$, respectively, while the ESI was 51.39, 29.94 and 23.46 minutes in the same order, at 17.09% the EAI were 76.82, 14.98 and 8.22 $m^2/g$, ESI was 45.87, 20.16 and 18.40 minutes, and at 20.15% the EAI was 72.04, 16.11 and 8.17 $m^2/g$, ESI was 36.06, 19.78 and 11.83 minutes.

**Growth of lactic acid bacteria on MRS medium and alternative media**

Figures (8, 9, 10, 11 and 12) show effect of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after 60, 120, and 180 minutes of enzymatic reaction time as a source of nitrogen instead of the nitrogen source in the MRS medium on growth of bacteria *Lactobacillus acidophilus, Lactobacillus rhamnosus GG*, *Lactobacillus plantarum*, *Bifidobacterium infantis*, and *Bifidobacterium animalis* respectively. The number of *Lactobacillus acidophilus* colonies in culture media containing basic protein concentrate hydrolysates was higher than in culture media containing acidic protein concentrate hydrolysates, the number of colonies increased with increasing enzymatic reaction time in media containing both protein concentrates and adding yeast extract had a significant effect on the number of colonies. The enzymatic reaction time did not have a significant effect on the number of *Lactobacillus rhamnosus GG* colonies, in contrast to the addition of yeast extract to hydrolysates of both acidic and basic concentrations hydrolysates. The yeast extract had no significant effect on the number of colonies of *Lactobacillus plantarum* and *Bifidobacterium infantis*, while the enzymatic reaction time and the addition of yeast extract had a significant effect on the number of *Bifidobacterium animalis* colonies in culture media containing hydrolysates of both concentrates. LAB needs amino acids to meet its need for amino nitrogen, which can be obtained from proteolysis. Proteolysis systems consist of proteinases associated with the cell wall, which are responsible for digesting proteins outside the cell and converting them into oligopeptides, and peptide transporters, which transport peptides into the cell. To reduce the amount of energy needed to absorb amino acids, intracellular peptidases are responsible for converting peptides into short peptides and free amino acids. Plasmids encode for these systems; Therefore, there is variation between different strains depending on the presence or absence of the plasmids that encode them (Abiola *et al.*, 2022). Studies show that the size of the genome has an impact on the amino acid needs of microorganisms. *L. plantarum*, which has a large genome size, needs only three amino acids and therefore requires a simpler environment from a nutritional standpoint, compared to *L. acidophilus* bacteria, which needs 14 amino acids. The size of the genome gives a clear picture of the microorganism's possession of different enzyme systems, which makes it more adapted to simple culture media (Barrangou *et al.*, 2012). Schöpping *et al.* (2021) found that *Bifidobacterium animalis* can synthesize all protein amino acids except cysteine, because it is unable to absorb inorganic sulfur, and methionine cannot compensate for its absence, it can synthesize methionine from cysteine through direct conversion to homocysteine and then to methionine, but the opposite is not true. *Lactobacillus acidophilus* bacteria do not only need amino acids, they have many requirements, and they need many growth factors that were not sufficient for their growth, as Chen *et al.* (2015) showed that different sources of nitrogen have an impact on the growth of *Lactobacillus acidophilus* bacteria. It was mentioned that organic nitrogen sources had higher growth than inorganic nitrogen sources because it contains free amino acids, peptides, glycosides, lipids, and growth factors. It was found that complex nitrogen sources prepared from more than one type of peptone gave higher growth rates than yeast extract alone, as studied by Meng *et al.* (2022) Growth requirements of *Lactobacillus acidophilus* LA-5, found that aspartic and asparagine are the most consumed amino acids with 0.94 mmol/L, glutamic and glutamine with 0.69 mmol/L, alanine with 0.55 mmol/L, leucine with 0.48 mmol/L, and the least consumed amino acid was phenylalanine at 0.03 mmol/L. In

contrast, the rest of the amino acids ranged between 0.06 - 0.44 mmol/L. *L. acidophilus* bacteria lack cytochromes, porphyrins and respiratory enzymes, and as a result they are unable to produce energy by oxidative phosphorylation. Since they use sugars such as glucose, esculin, cellobiose, galactose, lactose, maltose, salicin and sucrose as substrates for fermentation, the energy production is two ATP. As a result, these bacteria metabolize large amounts of the substrate to generate sufficient energy, so their generation time is longer than the rest of the species of lactic acid bacteria, and the absence of one of their nutritional requirements will significantly affect the number of growing cells (Hasan *et al*., 2019)

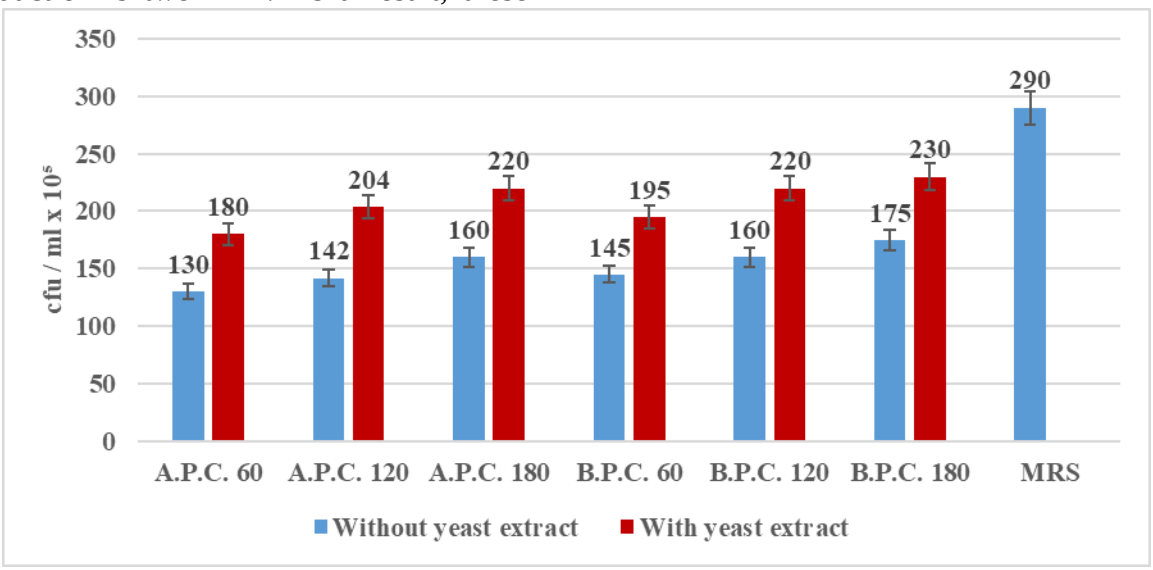


**Figure 8. Effect of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120 and 180) minutes of enzymatic reaction time as a source of nitrogen instead of the nitrogen source in the MRS medium on the growth of bacteria *Lactobacillus acidophilus***

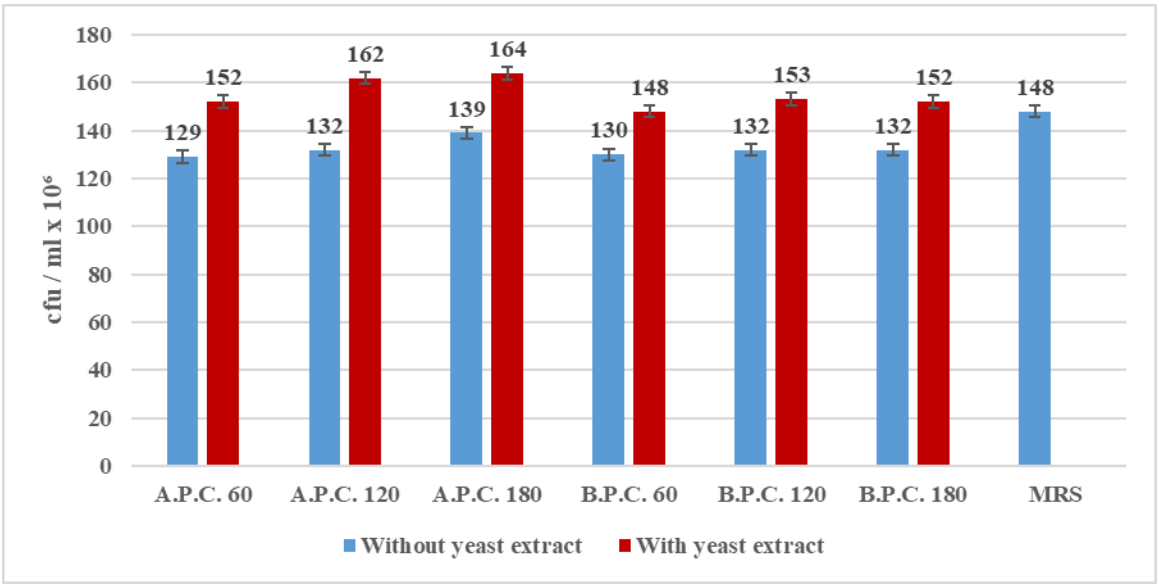


**Figure 9. Effect of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120 and 180) minutes of enzymatic reaction time as a source of nitrogen instead of the nitrogen source in the MRS medium on the growth of bacteria *Lactobacillus rhamnosus GG***

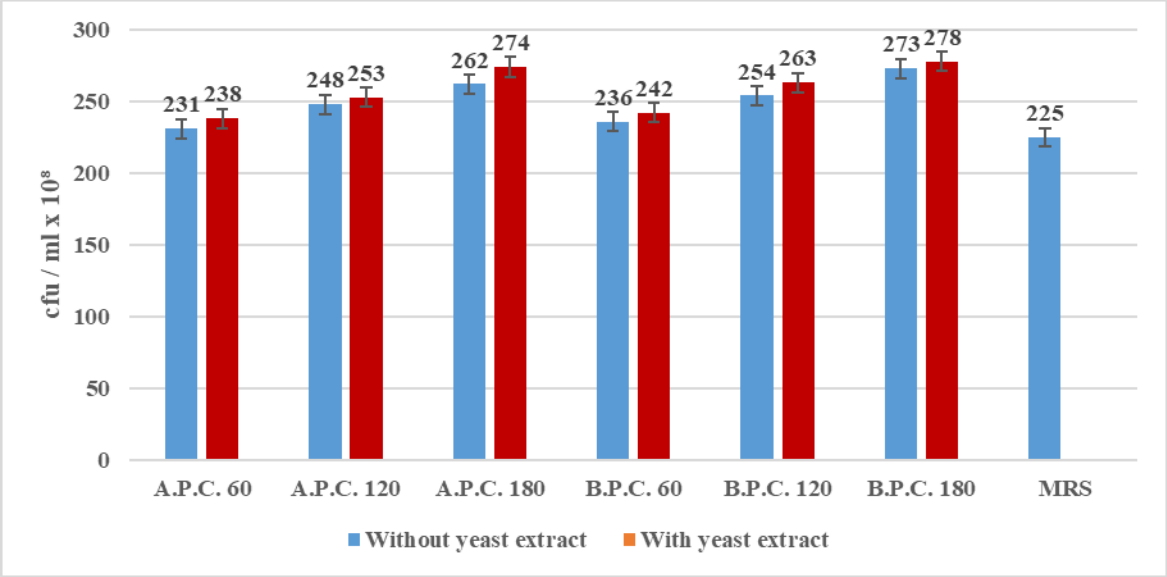


**Figure 10. Effect of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120 and 180) minutes of enzymatic reaction time as a source of nitrogen instead of the nitrogen source in the MRS medium on the growth of bacteria *Lactobacillus plantarum.***

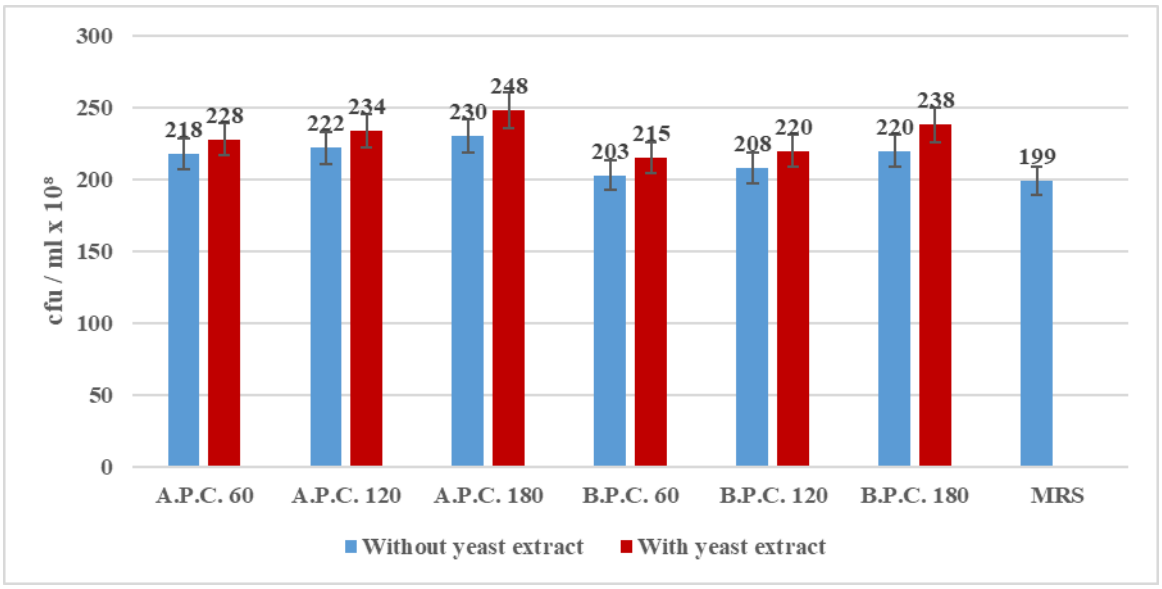


**Figure 11. Effect of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120 and 180) minutes of enzymatic reaction time as a source of nitrogen instead of the nitrogen source in the MRS medium on the growth of bacteria *Bifidobacterium infantis***

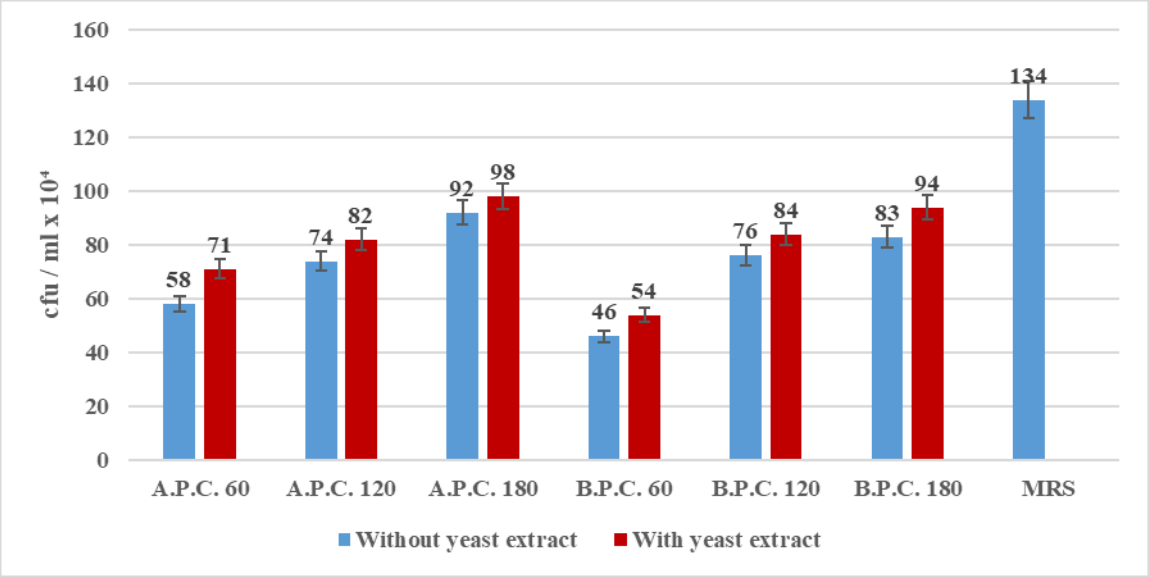


**Figure 12. Effect of hydrolysates from acidic protein concentrate (A.P.C.) and basic protein concentrate (B.P.C.) by trypsin enzyme after (60, 120, and 180) minutes of enzymatic reaction time as a source of nitrogen instead of the nitrogen source in the MRS medium on the growth of bacteria *Bifidobacterium animalis.***

## Conclusions

The degree of hydrolysis by trypsin enzyme at a concentration of 1.5 grams/100 grams of protein concentrate increased with increasing hydrolysis time. Protein hydrolysates with the enzyme trypsin after 60, 120, and 180 minutes of enzymatic reaction time gave the highest solubility at pH 10 and 2, respectively, while it had the lowest solubility at pH 4. The value of EAI and ESI increased after 120 minutes of enzymatic reaction time but decreased after 180 minutes. The time of enzymatic hydrolysis and the addition of yeast extract affected the growth of lactic acid bacteria.

## Conflict of interest

The authors confirm that there are no conflicts of interest to declare regarding the publication of this paper.

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