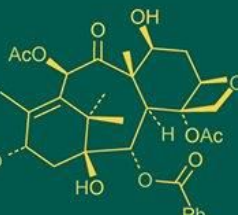
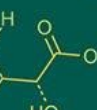
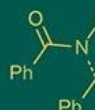


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Optimization of chicken heart protein hydrolysates production using lactobacilli fermentation

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Abstract

Objective of this study was to optimize the processing conditions for preparation of chicken heart protein hydrolysates. Chicken heart protein hydrolysates were prepared by fermentation using *L. helveticus* and *L. plantarum*, and their concentrations and processing conditions were optimized according to pH, soluble protein content and degree of hydrolysis%. It was observed that there was significant ($p < 0.05$) reduction in pH of the samples with increase in fermentation time and microbial concentration. Two hydrolysates namely, 2Hh16 (2% *L. helveticus* fermentation for 16 h) and 2Hp16 (2% *L. helveticus* fermentation for 16 h) achieved better soluble protein content (14.52 and 12.84 mg/mL respectively) and degree of hydrolysis % (41.62% and 38.78% respectively). These findings indicate Lactobacilli fermentation can be utilized effectively for the production of chicken heart hydrolysates having good techno-functional and antioxidant properties and may find its way in food industry.

Keywords: Protein hydrolysates, chicken heart, degree of hydrolysis, lactobacillus

Introduction

Indian poultry industry has undergone substantial growth over the past few decades and has effectively evolved from traditional backyard farming into a thriving agri-based industry. Amongst all poultry meat, chicken meat is the most preferred meat consumed throughout the country. The increase in production and consumption of meat has also led to an increase in production of slaughter house by-products. Slaughterhouses as well as meat processing industries generate a sizable amount of usable by-products. Each year, the meat industry produces over 100 million tonnes of by-products worldwide (Mora *et al.*, 2019) [10]. Visceral organs, bones, blood, head, feet, and feathers constitute the major portion of by-products of the poultry processing sector, making up approximately 28–30% of the total live weight. Poultry by-products are typically discarded through methods like landfilling, incineration, burial or rendering. Each of these methods comes with certain drawbacks, including cost implications, labor intensiveness, the generation of environmental pollutants and odors, and so forth. Improper handling and disposal of poultry slaughter waste can pose a potential risk of disease outbreaks on poultry farms, resulting in direct losses such as mortality and decreased in productivity.

Traditionally, poultry by-products are commonly utilized as animal feed and organic fertilizers. However, recent findings have revealed that these by-products contain abundant proteins, fats, carbohydrates, minerals, vitamins, and various other nutrients. Heart usually remains underused because of limited storage life. The amino acids threonine, leucine, lysine, tryptophan, and some other amino acids have been found in abundant concentrations in the heart muscles. Heart meat is often regarded as relatively tough meat, reflecting the nature of the cardiac muscle (Jayatilakan *et al.*, 2012) [8]. This makes them a promising option for discovering novel compounds and functional ingredients, and also presents an opportunity to maximize the utilization of existing protein resources in our diets. Proper utilization of these slaughter wastes not only enhances economic returns but also safeguards against undesirable side effects. In a world where resources are limited, the reduction, recycling, and utilization of by-products become increasingly crucial. Valorization of wastes is an effective strategy to lower disposal expenses (Mora *et al.*, 2019) [10].

Adding value to poultry slaughter by-products entails a level of innovation that transforms a by-product otherwise regarded waste into raw material for further processing into appealing edible food items for consumers (Toldrá *et al.*, 2021) [12]. By-products could be used effectively to generate 7–12% of the slaughterhouse's revenue (Abdulla *et al.*, 2020) [1].

Protein hydrolysates refer to a complex blend of oligopeptides, peptides, and free amino acids resulting from the partial or extensive hydrolysis of proteins. Protein hydrolysates derived from animals have gained significant attention as promising candidates for the development of a variety of health-enhancing functional and designer meat and other food products. One of the methods for production of protein hydrolysates is through microbial fermentation. During microbial fermentation, proteolytic enzymes, as well as lactic and propionic acids that build up during the metabolism of these bacterial species, are crucial in altering the structure of by-products. Producing a large quantity of protein hydrolysates relies on the careful selection of microbial strains and proteolytic activity and specificity, as microorganisms and their secreted proteases play a significant role in breaking down proteins during fermentation. The specific characteristics of peptides, such as their size, sequence, and properties, are determined by the activity of microbial proteolytic enzymes that cleave proteins. While research has explored the use of microbial fermentation to derive protein hydrolysates from foods like fish and milk, there has been limited investigation into using chicken heart for producing these hydrolysates, particularly with lactic acid bacteria. Therefore, the current study was envisaged to optimize the fermentation technique for extraction of protein hydrolysates from chicken heart on the basis of pH, soluble protein content and degree of hydrolysis%.

Materials and Methods

Source of heart samples

Chicken hearts were obtained from the Experimental poultry dressing plant of Post-Harvest Technology (PHT) Division, ICAR-Central Avian Research Institute, Izatnagar. Heart samples were washed, drained off excess liquid and finally, packed into LDPE (Low density polyethylene, 50 µm thickness) packages. The samples were then stored under frozen temperature of -20 ± 2 °C in a deep freezer for further use.

Standard bacterial cultures

Freeze dried *Lactobacillus helveticus* (NCDC-292) and *Latiplantibacillus plantarum* (NCDC-025) were obtained from National Collection of Dairy Culture, NDRI, Karnal for preparation of protein hydrolysates.

Chemicals and Media

High purity analytical grade chemicals were obtained from reputed firms such as Loba Chemie Pvt. Ltd. Mumbai, India and Sigma Aldrich (Merck) Pvt. Ltd. USA. Chemicals used for analyzing antioxidant activity of hydrolysates such as 2,2-azinobis 3-ethylbenzathiazoline-6-sulfonic acid (ABTS) was obtained from Central Drug House (P) Ltd. New Delhi, India. BLUelf Prestained Protein Ladder (245-5 kDa) was procured from Simply, India for SDS-PAGE.

Preparation of chicken heart hydrolysates

Freeze dried *Lactobacillus helveticus*-NCDC-292 and *Latiplantibacillus plantarum*-NCDC-025 cultures were revived using 12.5% sterilized skimmed milk. Activated culture was maintained in litmus milk by repeated sub-culturing at every 10 days. Working culture was prepared by 1 mL litmus milk containing activated culture to 12.5% sterilized skimmed milk to make volume to 100 mL, which was then incubated at 37 °C overnight. Working culture with 10^7 cfu/mL bacterial counts was used for fermentation. Fermentation time and concentration of microbial culture were standardized based on preliminary trials and available literature. The fermentation times taken were 4h, 8h, 12h and 16h. Control sample was considered at 0 h (without fermentation). Microbial concentrations used were 1% (10^7 cfu/mL bacterial counts) and 2% (2×10^7 cfu/mL bacterial counts) levels, while 5% lactose was added into each group of sample based on reported literature. Fermentation process was controlled at 37 °C.

For processing, first frozen heart samples were defrosted at chilling temperature (4 ± 1 °C), and then they were suspended with DDW in 1:1 ratio separately and homogenized using ULTRA-TURRAX T25 (Janke & Kunkel IKA Labortechnik, USA) in 13,500 rpm until a uniform homogenate was obtained. pH was checked and then the homogenates were divided into 40 batches in 50 mL test tubes. After adding lactose and culture aseptically in a laminar flow (except in the control groups), the samples were kept in a shaking incubator (Orbitek® LEBT-BH, Scigenics Biotech Pvt. Ltd. Chennai, India) at optimum temperature of 37 °C and speed 140 rpm for undergoing fermentation. Samples were taken out of the incubator at regular intervals of time to check the efficiency of fermentation and protein hydrolysis for periods of 4 h, 8 h, 12 h and 16 h. The fermented products were immediately heat processed at 85 °C for 15 min to inactivate the cultures, pH was recorded and then they were centrifuged (HermLe, Z326 K, USA) at speed of 10,370 rcf for 25 min at 4 °C. The protein hydrolysates recovered were evaluated and compared on the basis of pH, soluble protein content and degree of hydrolysis (DH%).

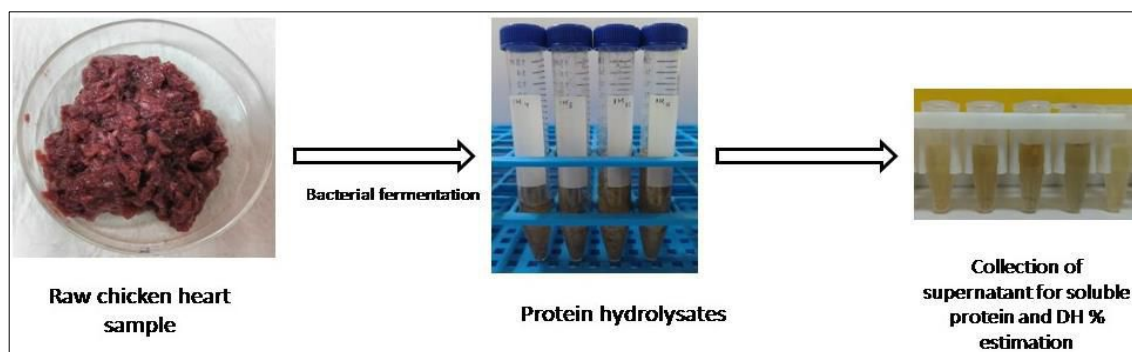


Fig 1: Production of chicken heart protein hydrolysate by Lactobacilli fermentation

Determination of pH

pH of the chicken heart protein hydrolysates were determined with the help of a digital pH meter (Electronic Corporation of India Limited, India) according to the procedure of Troutt *et al.* (1992) [13]. The electrode of the pH meter was dipped into the samples to record the readings.

Determination of Soluble Protein Content

The protein content of the chicken heart protein hydrolysates were estimated following the method as described by Lowry *et al.* (1951) [9]. About 500µL alkaline reagent was mixed properly with 500µL of protein hydrolysate and left undisturbed for around 10 min. Freshly prepared Folin-Ciocalteu phenol reagent was added and then the mixture was heated at water bath for 5 min at a temperature of 55 °C. After cooling, OD at 650nm was taken against the blank in Spectrophotometer (Eppendorf Biospectrometer® Basic, USA). Bovine serum albumin (BSA) was taken as standard.

$$\text{Protein (mg/mL)} = \frac{\text{OD (test)}}{\text{OD (Standard)}} \times \text{Concentration of standard}$$

Determination of Degree of Hydrolysis%

The approximate degree of hydrolysis (%) of chicken heart protein hydrolysate were calculated according to the method described by Hoyle and Meritt (1994) [6]. Briefly, 500µL aliquot of the protein hydrolysates was mixed with 500µL of 20% (w/v) trichloroacetic acid solution to solubilize the protein. The mixture was kept at room temperature (25±2 °C) for approximately 30 min and then centrifuged (HERMLE Z 446 K, High-speed centrifuge, Labortechnik, Germany) at 1,990 rcf speed for 15 minutes at 4 °C. The protein content of the solubilized protein was determined by the method described above (Lowry *et al.*, 1951) [9] by taking bovine serum albumin (BSA) as standard. The degree of hydrolysis was determined by using the following formula:

$$\text{DH (\%)} = \frac{\text{Solubilized protein content in 20\% TCA (9 mg)}}{\text{Total protein content}} \times 100$$

Results and discussion

pH of chicken heart protein hydrolysates

Results indicated that at the end of 16 fermentation, the pH values of 1Hh, 2Hh, 1Hp and 2Hp samples were 4.42±0.02, 4.41±0.01, 4.44±0.03 and 4.38±0.01, respectively. No significant ($p > 0.05$) differences in pH were observed amongst the hydrolysates at this stage. In a related study, Ali *et al.* (2021) [2] observed a similar pH reduction from an initial 6.52 to 4.38 in chicken gizzard hydrolysates processed by *Pediococcus acidilactici* ATTC 8042 fermentation over 18 h.

Organic acids, primarily lactic acid produced by LABs, play a crucial role in lowering the pH during fermentation process (Gan *et al.*, 2017) [5]. A low pH can inhibit spoilage bacteria (Yu *et al.*, 2020) [14].

Soluble protein content of chicken heart protein hydrolysates

Table 1 details the soluble protein content of chicken heart protein hydrolysates fermented with *L. helveticus* and *L. plantarum*. Significant ($p < 0.05$) increases in soluble protein content were noted after a 12h fermentation period. Hydrolysates fermented with 2% microbial concentration exhibited significantly ($p < 0.05$) higher soluble protein content compared to those fermented with 1% microbial concentration by the 16h mark. Notably, 2Hh demonstrated the highest soluble protein content (14.52±0.19 mg/mL) at 16 h, significantly ($p < 0.05$) surpassing other hydrolysates. These results align with the findings of Jain and Anal (2017) [7], who reported a gradual increase of protein concentration in fermented eggshell membrane hydrolysates during extended period of fermentation. Additionally, Pan and Guo (2010) [11] noted that *L. helveticus* synthesizes large amounts of proteolytic enzymes during milk fermentation at an initial pH of 7.5, enhancing the protein hydrolysate production. One of the possible explanations for the increasing protein level was that some insoluble proteins converted into soluble ones by fermentation process (Fang *et al.*, 2017) [4].

Degree of hydrolysis% of chicken heart protein hydrolysates

The degree of hydrolysis (DH%) of chicken HPH were increased significantly ($p < 0.05$) across all samples by the end of 16 h, with 2Hh exhibiting the highest DH% value of 41.62±0.51%. This indicates that 2Hh underwent more extensive peptide bond cleavage, resulting in the formation of smaller peptides and free amino acids. The higher DH% in 2Hh suggests that *L. helveticus* at 2% bacterial concentration had superior hydrolytic efficiency for chicken heart samples compared to *L. plantarum* at the same concentration. The increase in DH% was primarily attributed to the protease activity produced by *L. helveticus* and *L. plantarum*. In a similar study, Anggraeni *et al.* (2022) [3] reported a DH% of 44.32% in edamame (a legume) protein hydrolysates after 24 h of fermentation with *Lactococcus lactis*.

Overall, these findings demonstrate significant improvements in soluble protein content and DH% during fermentation, highlighting the impact of bacterial concentration and fermentation time on the hydrolysis process in chicken HPH. It was observed that in case of hydrolysates fermented by both *L. helveticus* and *L. plantarum*, 16 h fermentation and 2% bacterial concentration produced hydrolysates with superior soluble protein content and degree of hydrolysis%.

Table 1: Effect of fermentation on pH, soluble protein content and degree of hydrolysis% of chicken heart protein hydrolysates

Treatments	Fermentation time (h)				
	0	4	8	12	16
pH					
1Hh	6.13±0.01 ^{Aa}	5.43±0.02 ^{Bb}	4.90±0.03 ^{Bc}	4.62±0.01 ^{Bd}	4.42±0.02 ^{Ac}
2Hh	6.11±0.01 ^{Aa}	5.20±0.02 ^{Cb}	4.72±0.02 ^{Bc}	4.45±0.02 ^{Cd}	4.41±0.01 ^{Ad}
1Hp	6.10±0.03 ^{Aa}	6.30±0.08 ^{Aa}	5.58±0.11 ^{Ab}	4.84±0.05 ^{Ac}	4.44±0.03 ^{Ad}
2Hp	6.09±0.02 ^{Aa}	6.22±0.03 ^{Ab}	5.48±0.07 ^{Ac}	4.67±0.06 ^{Bd}	4.38±0.01 ^{Ac}
Soluble protein content (mg/mL)					
1Hh	8.60±0.02 ^{Ad}	8.68±0.04 ^{Bd}	10.13±0.02 ^{Cc}	10.73±0.05 ^{Bb}	12.71±0.04 ^{Ba}
2Hh	8.61±0.02 ^{Ad}	8.98±0.05 ^{Ac}	11.39±0.02 ^{Ab}	11.60±0.03 ^{Ab}	14.52±0.19 ^{Aa}
1Hp	8.61±0.04 ^{Ad}	8.64±0.07 ^{Bd}	9.34±0.09 ^{Dc}	10.33±0.07 ^{Cb}	11.65±0.05 ^{Ca}
2Hp	8.61±0.05 ^{Ad}	8.70±0.05 ^{Bd}	10.39±0.13 ^{Bc}	10.60±0.05 ^{Bb}	12.84±0.04 ^{Ba}
Degree of Hydrolysis (%)					
1Hh	12.25±0.58 ^{Ac}	13.44±0.97 ^{Bc}	27.51±0.87 ^{Bb}	34.34±1.07 ^{Ca}	35.46±1.43 ^{BCa}
2Hh	12.39±0.59 ^{Ac}	19.52±0.28 ^{Ad}	34.62±0.96 ^{Ac}	37.83±0.67 ^{Ab}	41.62±0.51 ^{Aa}
1Hp	12.46±0.08 ^{Ac}	15.58±1.11 ^{Bd}	21.10±0.23 ^{Cc}	29.30±1.04 ^{Db}	33.20±0.63 ^{Ca}
2Hp	12.47±0.06 ^{Ad}	18.61±0.34 ^{Ac}	28.04±1.67 ^{Bb}	35.90±1.17 ^{Aa}	38.78±1.04 ^{Ba}

n=6, Mean±SE bearing different superscripts column wise (capital alphabet) and row wise (small alphabet) differ significantly ($p<0.05$).

1Hh- 1% *L. helveticus*, 2Hh- 2% *L. helveticus*, 1Hp- 1% *L. plantarum*, 2Hp- 2% *L. plantarum*

Conclusion

This study aimed to optimize the fermentation process for the production of chicken heart hydrolysates using *L. helveticus* and *L. plantarum*. In particular, hydrolysates produced by fermentation with *L. helveticus* exhibited higher soluble protein content and degree of hydrolysis% compared to those produced by fermentation with *L. plantarum*. Thus, the fermentation process with *Lactobacillus* bacteria presents a promising method for generating protein hydrolysates from chicken heart.

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Declaration of Competing Interest

The authors declare that they have no conflict of interest, financial or otherwise.

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