ADVANCES IN ENVIRONMENTAL BIOLOGY

ISSN:1995-0756
EISSN:1998-1066

2021 October; 15(10): Pages 1-7
https://doi.org/10.22587/aeb.2021.15.10.1

ORIGINAL ARTICLE AENSI PUBLICATIONS

Effect of Fermentation Time on The Dynamics of *Lactobacillus plantarum* Bacteria using Collagen Extract from Broiler Claw Skin as a Substrate

Muhammad Irfan Said[1], Effendi Abustam[1], Hasma[2], Muhammad Fatahillah Arifin[1], Sitti Nurani Sirajuddin[1], Abdel Rahman Al-Tawaha[3], Abdel Razzaq Al-Tawaha[4]

[1]Faculty of Animal Science, Hasanuddin University, Jl.Perintis Kemerdekaan Km.10 Makassar, South Sulawesi, Indonesia 90245
[2]STKIP YAPTI, Jeneponto Regency, Jl. M. Ali Dg Gassing No I, Binamu Kabupaten Jeneponto 92311
[3]Department of Biological Sciences, Al-Hussein bin Talal University, Maan, Jordan
[4]Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia 43400 UPM Serdang, Selangor, Malaysia

Correspondence Authors: Muhammad Irfan Said, Faculty of Animal Science, Hasanuddin University, Jl.Perintis Kemerdekaan Km.10 Makassar, South Sulawesi, Indonesia 90245.
E-mail: Irfanunhas@gmail.com; irfan.said@unhas.ac.id

Received date: 15 August 2021, Accepted date: 20 October 2021

Cite as: M. I. Said., E. Abustam., Hasma., M. F. Arifin., S. N. Sirajuddin., A. R. M. Said Al-Tawaha., A. R. Al-Tawaha., 2021. Effect of Fermentation Time on The Dynamics *of Lactobacillus plantarum* Bacteria using Collagen Extract from Broiler Claw Skin as a Substrate. Advances in Environmental Biology, 15(10): 1-7. DOI:10.22587/aeb.2021.15.10.1.

Abstract

Research has been carried out to evaluate the growth dynamics of lactic acid bacteria (LAB) *Lactobacillus plantarum* (*L.plantarum*) using collagen extract (CE) from the broiler claw skin (BCS) as a substrate. The bacterial dynamics studied were related to total lactic acid (TLA) production, pH, dissolved protein (DP) and bacterial growth (BG) during the fermentation process. A total of 50 g of BCS was used with fermentation time (24; 48 and 72 hours). The results was showed that fermentation time using L.plantarum as a culture with CE from BCS as a substrate did not affect on bacterial dynamics (TLA, pH, dissolved protein and BG) during the fermentation process. Using L.plantarum with EC from BCS as substrate can be applied with 24 hours fermentation time. The bacterial dynamics produced by the fermentation time were 2.35±0.50%; 5.88±0.10; 45.60±2.21 mg/mL and 15.591±0.92 Log10 CFU/mL respectively.

Keywords: Fermentation, L.plantarum, Collagen, Claw skin, Broiler

INTRODUCTION

The use of chemicals in the production process of collagen extract is a growing concern. This is certainly very important, especially in the food industry [1]. Lately, non-mammal collagen has been widely used in the food and cosmetics industry, medical research, and tissue engineering [2]. Collagen is the main fibrillar protein commonly distributed in vertebrates and contributes to the skin, tendons, bones, cartilage, and other tissues [3,4]. This is because collagen shows low antigenic activity, high cell adhesion properties, and biocompatibility [5]. Exploration of sources of collagen originating from livestock other than cattle has been developed by many researchers. Other collagen sources include fish, poultry, or other livestock [6,7,8,9,10,11]. This is an effort to prevent the transmission of madcow disease outbreaks [12,13]. However, the use of chemicals is feared to have a negative impact on humans. The application of bacterial microorganisms in food processing rapidly grows *Bacillus subtilis* FNCC 0059 bacteria to break down keratin [14].

At present, the application of bacteria in the food fermentation process is increasing and needed by the community. This is closely related to food security. Currently, collagen in the food sector has been widely applied. One of them is as a food supplement for anti-osteoporosis. In addition, collagen has been widely applied as gelling, stabilizing, foarming and emulsifying agent (emulsifying agent) [15,16]. Collagen extract has also been used as a source of natural antioxidants and texturing agents. The use of collagen and other natural ingredients (liquid smoke) can reduce the use of chemical preservatives. The use of natural ingredients in animal food products has also been developed as a source of antioxidants [17,18].

Claw is one of the by-products of broiler chickens rich in collagen compounds. Collagen is the main protein in livestock and accounts for around 30% of the total. Approximately 29 types of collagen have different roles in the body's tissues. Each collagen has a different amino acid sequence and molecular structure [19]. In addition, collagen can be used as a functional food agent [20]. The results of the collagen fermentation process can be developed as a portion of available food to improve the process of absorption of food components and extend product shelf life [21].

Lactobacillus plantarum (*L.plantarum*) as lactic acid bacteria (LAB) in the food industry has been carried out. These bacteria are used to ferment food products as a functional food. The fermentation process increases the benefits of industrial products by increasing production volume, reducing energy use, and reducing the waste production. In addition, this process produces products were environmentally friendly [22]. Some have been used as fermentation agents for meat, milk, eggs and fish [23,24].

The application of lactic acid bacteria (LAB) to the type of *L.plantarum* in the collagen fermentation process in broiler claw skin (BCS) has not been widely known. Therefore, to maximize the potential of *L.plantarum* in hydrolyzing collagen proteins, this research is significant to be carried out. Furthermore, the effect of fermentation time on *L.plantarum* bacteria will affect the performance of these bacteria. Therefore, the study aimed to evaluate the effect of fermentation time on the dynamics of bacteria using CE from BCS as a substrate.

MATERIALS AND METHODS

Research Materials

The BCS was used as the main ingredient in the study. The BCS was a by-product of livestock obtained from poultry slaughterhouses (PSh), Daya, Makassar City, South Sulawesi, Indonesia. The material comes from 45-day-old broiler chickens that are slaughtered in a halal condition. The isolates of *L.plantarum* were obtained from isolation using milkfish extract media in the Lab. Microbiology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar. The materials used for the propagation of bacteria were MRS-Broth (*Oxoid M0359*); alcohol 70% (*One-Med*). In addition, some equipment for the propagation and fermentation process were used, such as electric ovens (*Memmert 100-800*); incubator (*Memmert BE 400*); autoclave (*YXQ.SG41.280*); laminar flow (*AIRTECH*), shaker (*IKA KS 260 basic*); hot plate (*Stuart SD162*); pipette (*HUAWEI 14060806*); vortex (*Vortex IKA 3*); pH meter (*Hanna HI 8424*); analytical scales (*Jc3-B scale/KERN ALS 220-4N*); petri dish (*ANUMBRA*); Erlenmeyer (*SCHOTT DURAN*).

Propagation of L.plantarum Bacterial Culture

Bacterial isolates were propagated using MRS-broth media. MRS-broth media was made by dissolving 5.2 g of MRS-broth media into 100 mL aquadest. The solution was then homogenized. A total of 50 mL of bacterial growing media have been made. Based on the solution, a total of 47.5 mL was put into the Erlenmeyer tube in preparation for the fermentation medium. The liquid membrane was closed using aluminum foil. The media solution was then sterilized using an autoclave at 120-125 ºC for 45 minutes and then cooled. A total of 2.5 mL of the suspension of *L.plantarum* bacteria were grown for 24 hours (concentration of 5%). The bacterial suspension (5%) was then inserted into the Erlenmeyer tube containing growth media.

Preparation of BCS

A total of 5 kg of BCS were washed with running water. The skin part of the claw was separated from the bone using a sterile knife. The skin from the claw was washed with running water. The BCS samples were weighed and dried in an oven at 50ºC for 24 hours. The BCS was then sterilized using 70% alcohol.

Fermentation Process

The tube containing 47.5 mL of MRS-broth media was prepared. A total of 50 g of BCS sample was inserted into the tube. A Total of 2.5 mL of the suspension of *L.plantarum* bacteria were inoculated on the media. The tube was covered with gauze and plastic wrap. The tube was placed on a shaker sway and placed at room temperature. The next fermentation process was carried out at room temperature for 24 hours, 48 hours and 72 hours.

Research Design and Data Analysis

Statistical analysis was used to process research data. The data results of the study were analyzed by ANOVA based on a Complete Random Design (CRD) Pattern. The analysis results showed a significant effect, then tested using Duncan’s Multiple Range Test (DMRT) at the level of 5% [25].

Parameter of Analysis

Total lactic acid (TLA)(%). A total of 10 mL of solution was added with three drops of PP indicator. The solution was titrated using 0.1N NaOH solution. Determination of test results was carried out 3 times. The determination of the value of TLA was done through the titration method which is characterized by a change in pink. TLA (%) = $(V_1)(N)(B)/(V_2)(1{,}000)$, where; V_1 = volume of NaOH (mL); V_2 = volume of suspension solution *L.plantarum* (mL); N = normality of NaOH (0,1); molecular weight of lactic acid (90). pH, the determination of pH was carried out by conducting a calibration process using pH 4 and pH 7. The fermented solution was heated at 70ºC and homogenized. The pH was set at room temperature.

Dissolved protein (DP) (mg/mL). The lowry method was used to determine the amount of DP during the fermentation process. First of all, a sample of 1.5 g was inserted into a scale tube. Furthermore, as much as 7.5 mL of aquadest were added. The solution was homogenized using vortex for 15 minutes. The formed supernatant was boiling using a hotplate. A total of 2 mL of supernatant was added with 1 mL of 10% TCA. The mixture was centrifuged for 15 minutes. The TCA extract (0.1 mL) + aquadest (1.9 mL) + lowry reagent (2.5 mL), homogenized, stored at room temperature for 10 minutes. Folin reagent (0.5 mL) was added to the mixture and incubated at room temperature for 30 minutes. The result will be blue. The absorbance value was measured using a spectrophotometer with a wavelength (λ) = 660 ηm. The next measurement results were compared with the standard bovine serum albumin (BSA) solution. DP(mg/mL), y = ax + b, so that (x) = (y-b)/a, where; a = 3,520; b = 0.058; y = absorbance; R^2 = 0.992.

Bacterial growth (BG) (Log_{10} CFU/mL). The growth of the population of *L.plantarum* bacteria was known using the total plate count (TPC) method. The *L.plantarum* bacteria was a class of lactic acid bacteria (LAB). The calculation process was done by dissolving 1 mL of *L.plantarum* solution in 9 ml of sterile aquadest. The solution was homogenized using vortex. Dilution was done from 10^{-1} to 10^{-12}. Samples were incubated at 37ºC for 24 hours. Calculation of colonies was carried out using standard 25-250 of colonies. Data collection was done 3 times (triploid).

RESULTS AND DISCUSSIONS

Total Lactic Acid

Lactic acid bacteria (LAB) are gram-positive bacteria that do not form spores and can ferment carbohydrates to produce lactic acid. The *L.plantarum* bacteria is one example of LAB which has been widely applied in the food processing industry. LAB can protect against contamination of pathogenic bacteria, improve nutrition, and potentially positively impact human health. These bacteria can maintain stability in the digestive tract of pathogen bacteria [26,27]. The difference in TLA by the activity of *L.plantarum* bacteria on the substrate of CE from BCS was shown in Fig.1.

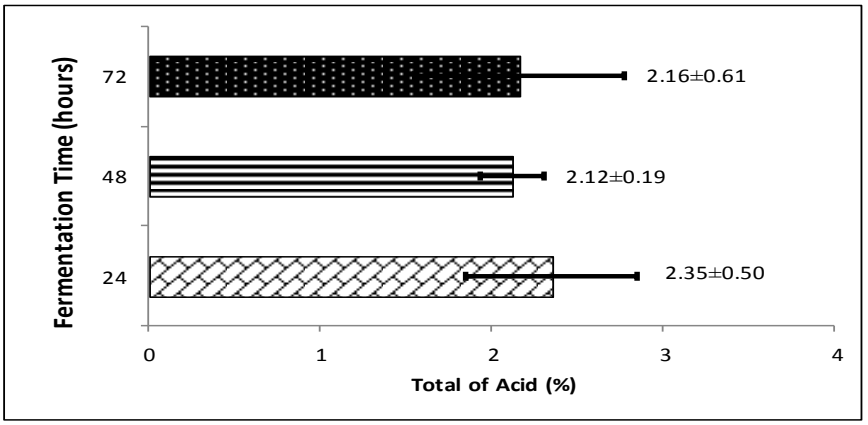

Fig. 1: Total Lactic Acid (TLA) (%) of Bacterial Activity of *L.Plantarum* on The Substrate of CE From BCS Using Different Fermentation Times

Determination of the TLA as a result of the fermentation process can be known from the total acid measured through the titration process. Based on Figure 1, the TLA formed during the fermentation process by the activity of *L.plantarum* bacteria was quite varied. The value of TLA tends to decrease slightly with increasing fermentation time from 24 hours to 72 hours. However, statistically did not show a significant difference (P> 0.05). At 24 hours fermentation, 48 hours and 72 hours each showed TLA of 2.35± 0.50%; 2.12±0.19% and 2.16±0.61%. The results obtained were related to pH values with values that also vary. Therefore, the formation of lactic acid will affect the pH value. Lactic acid was a product formed from fermentation involving carbohydrates. This decrease process occurs because the amount of carbohydrates in the collagen substrate also decreases due to the increasing growth of bacteria. Therefore, the amount of lactic acid formed was influenced by carbohydrates. The substrate of collagen consists mainly of protein molecules.

Bacteria belonging to the LAB have specific characteristics: not having porphyrins and cytochrome, negative catalase, not carrying out electron transport phosphorylation, and only getting energy from the phosphorylation of the substrate. Almost all LABs only get energy from sugar metabolism, so that their growth habitat was only limited to environments that provide enough sugar or can be called nutrient-rich environments. Their ability to produce compounds (biosynthesis) was also limited and the complex

nutritional requirements of LAB include amino acids, vitamins, purines and pyrimidine [28,29]. The bacteria *L.plantarum* has probiotic properties and improved environmental conditions in the digestive tract. *L.plantarum* bacteria can metabolize and synthesize bacteriocin. This bacterium can inhibit the growth of gram-positive and gram-negative bacteria. The *L.plantarum* bacteria has been widely used in food products such as milk and processed products [30]. The *L.plantarum* bacteria produce bacteriocins so that they are resistant to acids and high fermentation temperatures.

pH

The time of fermentation affects the pH value of the solution. Therefore, changes in pH of the solution showed a difference in fermentation activity. The dynamics of the pH of the solution by the activity of *L.plantarum* bacteria during the fermentation process on the CE from BCS as a substrate was shown in Fig. 2.

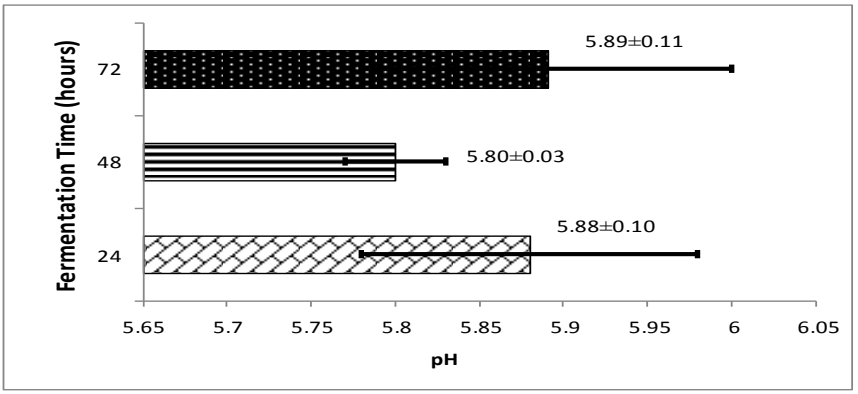

Fig. 2: pH Value of Bacterial Activity of *L.Plantarum* on The Substrate of CE from BCS Using Different Fermentation Times

Fig. 2 shows the change in pH of the solution during the fermentation process. Based on the statistical analysis results, there was no significant difference (P>0.05) in the pH of the solution containing CE from BCS as a substrate during fermentation. The pH value of the solution with a 24-hour fermentation time showed a value of 5.88±0.10. However, 48-hour fermentation time decreased (5.80±0.03). The pH value shows the effect of the growth of *L. plantarum* bacteria. Changes in pH values occur because bacteria have entered the logarithmic phase. In the logarithmic phase, accumulation of lactic acid and organic acids results from the hydrolysis of fat and metabolic results. One factor that influences acid production is environmental factors, namely pH and temperature. In applying the 72-hour fermentation time, it turned out to have increased (5.89±0.11). Changes in varied pH values are influenced by bacterial activity in fermenting carbohydrate molecules on the CE as BCS substrate. At 72 hours of fermentation, carbohydrate levels decrease. This causes the amount of lactic acid formed to decrease so that pH increases. The food sources from bacteria may be obtained from protein molecules on the CE as BCS substrate. The *L. plantarum* bacteria can metabolize these organic acids. Bacteria used the results of the process as a food source in the form of carbon [31]. In fermentation in processed meat (sausage), *L.plantarum* bacteria can inhibit the occurrence of protein oxidation [32]. Production of organic acid affects the pH value. This also affects antioxidant activity [33]. *L.plantarum* bacteria can free phenolic compounds during fermentation [34].

Dissolved Protein

Proteins are the main components of living things that play an essential role in cell activity. Proteins are composed of amino acids bound in straight chains forming peptide bonds. The peptide bond is forming a complex substance. Many factors can cause changes in the physical properties of proteins such as heat, acids, bases, heavy metals, salts and radioactive ray radiation. These changes are the occurrence of compaction. These factors cause the protein not to dissolve quickly. Dissolved protein (DP) shows the amount of oligopeptides that are easily absorbed by the digestive tract. Changes in the amount of DP in the solution of *L.plantarum* bacteria containing CE substrate of BCS were presented in Fig. 3.

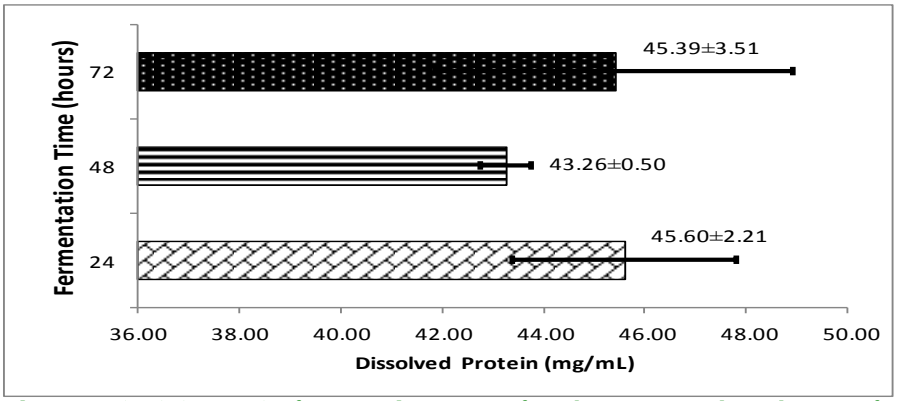

Fig.3: Dissolved Protein (DP) (mg/mL) of Bacterial Activity of *L.Plantarum* onThe Substrate of CE From BCS Using Different Fermentation Times

Based on Fig. 3, it can be seen that the increase in fermentation time by *L.plantarum* bacteria on the CE substrate of BCS produced a variety of DP. Statistically, the difference in fermentation time did not show a significant difference (P>0.05) in the dissolved protein. This is caused by the presence of different bacterial activity at each fermentation time. The fermentation time is 24 hours, the total DP is 45.60±2.21 mg/mL. The 48 hours fermentation time decreased by 43.26±0.50 mg/mL. Furthermore, at 72 hours of fermentation there was a re-increase of 45.39±3.51 mg/mL. The condition of the substrate used affects the difference in the amount of DP. The maximum speed of an enzyme that breaks down the substrate was affected by the condition of the substrate.
The total DP gives an indication of the amount of protein undergoing the degradation process. This occurs due to the activity of proteolytic enzymes from the *L.plantarum* bacteria. The intense denaturation process of sarcoplasmic and actin proteins, transformation of α-helix into β-sheets was a major change in protein [35]. Hydrogen bonds, hydrophobic forces and electrostatic interactions help the process of combining two proteins into a particle plane [36]. The solubility of a protein in water plays an important role in their functions such as foaming and emulsifying. This is very important for those with high surface hydrophobicity [37].

Changes in primary structure affect immunoreactivity of a protein molecule. Proteins can undergo a process of degradation, aggregation, folds, and cross bonds. This affects changes in protein immunoreactivity [38]. Antimicrobial peptides play an important role in improving the performance of LAB and its metabolic processes. Such performance affects microbial safety, total population and the ecology of fermented products [39]. The component of mannan-oligosaccharide (MOS) influences the proliferative process of *L.plantarum* ATCC14917 bacteria in vitro [40].

Bacterial Growth

The level of bacterial growth can be determined by knowing the number of bacteria from each fermentation process. The total bacteria of a medium was influenced by fermentation time, type and availability of substrate and fermentation temperature. A comparison of the growth of *L.plantarum* bacteria on the CE as substrate of BCS was presented in Fig. 4.

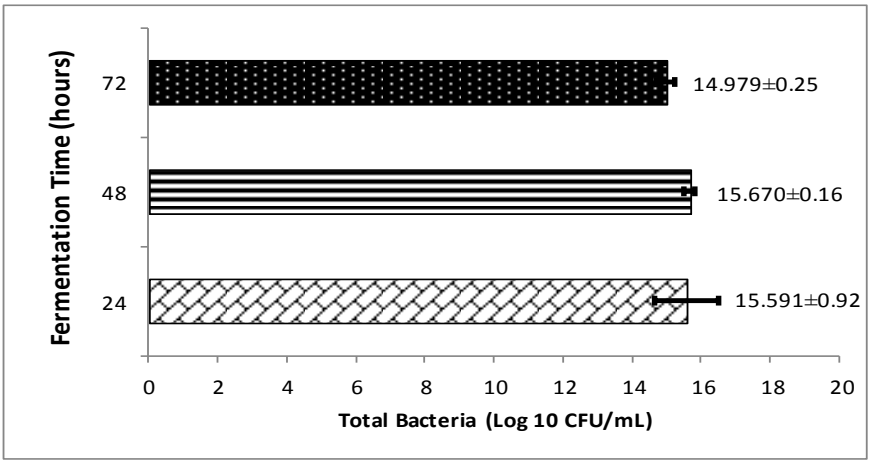

Fig. 4: Bacterial Growth (BG) (Log_{10} CFU/mL) of Bacterial Activity of *L.plantarum* on the Substrate of CE from BCS Using Different Fermentation Times

The results of the study (Fig.4) showed that at 24 hours fermentation, there was a total decrease in *L.plantarum* bacteria (15,591±0,92 Log_{10} CFU/mL) as the fermentation process increased to 72 hours (14,979±0,25 Log_{10} CFU /mL). However, statistically, there was no significant difference (P>0.05) between these treatments. The number of microbes was influenced by fermentation time. The availability of adequate nutrition, among others, protein causes an increase in the microbial population of *L.plantarum* bacteria. Mostly, The BCS is composed of collagen protein which is a nutrient needed by microorganisms (bacteria). The availability of sufficient nutritional compounds influenced bacterial productivity. For the growth process, LAB (*L.plantarum*) requires a source of amino acids or peptides to grow. Animal skin is rich in collagen protein. Collagen proteins contain a number of amino acids glycine, proline and hydroxyproline. Nutrients in the form of proteins needed by bacteria are available in very large quantities on the substrate. Collagen contained in livestock skin was rich in protein compounds, especially amino acids. The *L.plantarum* bacteria can reduce cholesterol by growing cells (84%). The findings of this study indicate that *L. plantarum* can be a probiotic candidate for application in the low cholesterol food industry that has hypocholesterolemic activity in its host [41].

CONCLUSION

The difference in fermentation time by LAB *L.plantarum* bacteria using CE as the substrate from BCS did not affect TAL, pH, DP and BG in fermentation solutions. The application of 24-hour fermentation time can be used to ferment *L.plantarum* by using CE as a substrate from BCS with TAL, pH, DP and BG of 2.35±0.50%; 5.88±0.10; 45.60± 2.21 mg/mL and 15.591±0.92 Log_{10} CFU/mL.

ACKNOWLEDGEMENT

The researcher expressed his special thanks to the Ministry of Research, Technology and Higher Education, Republic of Indonesia, Rector of Hasanuddin University and Head of Institut for Research and Community Service (IRCS) for research funding support through the "Penelitian Terapan" scheme. Thank you also to the student researcher (Alvina) as a technical team in the field laboratory.

REFERENCES

[1] Said, M. I., Abustam, E., Wahab, A. W., Sartini and Hifizah, A. 2015. Chemical characteristics of collagen extract from scapula of Bali cattle (*os scapula*) produced using different extractant. Pakistan Journal of Nutrition. 14(3): 174-179.

[2] Meng, D., Tanaka, H., Kobayashi, T., Hatayama H., Zhang, X., Ura K., Yunoki, S., Takagi Y. 2019. The effect of alkaline pretreatment on the biochemical characteristics and fibril-forming abilities of types I and II collagen extracted from bester sturgeon by-products. Int. J. Biol. Macromol. 131: 572-580.

[3] Gordon, M. K. and Hahn, R. A. 2010. Collagen. Cell Tissue Res. 339:247–257.

[4] Ikoma, T., Kobayashi, H., Tanaka, J., Walsh, D. and Mann, S. 2003. Physical properties of type I collagen extracted from fish scales of Pagrus major and Oreochromis niloticas. Int. J. Biol. Macromol. 32: 199–204.

[5] Zhang, M., Liu, W. and Li, G. 2009. Isolation and characterisation of collagens from the skin of largefin longbarbel catfish (*Mystus macropterus*). Food Chem. 115(3): 826–831.

[6] Parenteau-Barei, R., Gauvin, R. and Berthod, F. 2010. Collagen-based biomaterials for tissue engineering applications. Materials. 3: 1863–1887.

[7] Zhang, X., Azuma, N., Hagihara, S., Adachi, S., Ura, K., Takagi, Y. 2016. Characterization of type I and II procollagen α1chain in Amur sturgeon (*Acipenser schrenckii*) and comparison of their gene expression. Gene. 579(1): 8-16.

[8] Liang, Q., Wang, L., Sun, W., Wang, Z., Xu, J. and Ma, H. 2014. Isolation and characterization of collagen from the cartilage of Amur sturgeon (*Acipenser schrenckii*). Process Biochem. 49: 318–323.

[9] Wang, T., Xu, P., Chen, Z. and Wang R. 2018. Mechanism of structural interplay between rice proteins and soy protein isolates to design novel protein hydrocolloids. Food Hydrocolloids. 84, 361-367,

[10] Wang, R., Xu, P., Chen, Z., Zhou, X. and Wang, T. 2019. Complexation of rice proteins and whey protein isolates by structural interactions to prepare soluble protein composites. LWT. 101: 207-213.

[11] Rosmawati., Abustam, E., Tawali, A. B., Said, M. I. and Sari, D. K. 2018. Effect of body weight on the chemical composition and collagen content of snake head *Channa striata* skin. Fisheries Science. 84(6): 1081-1089.

[12] Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai. and Tanaka T. M. 2005. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). Food Chem. 89(3): 363–372.

[13] Zhang, X., Adachi, S., Ura, K. and Takagi, Y. 2019. Properties of collagen extracted from Amur sturgeon Acipenser schrenckii and assessment of collagen fibrils in vitro. Int. J. Biol. Macromol. 137: 809-820.

[14] Said, M. I., Abustam, E., Yuliati, F. N. and Mide, M. Z. 2018. Characteristics of feather protein concentrates hydrolyzed using Bacillus subtilis FNCC 0059. OnLine J on Biol. Sci. 18(2): 138-146.

[15] Bhuimbar, M. V., Bhagwat, P. K. and Dandge, P. B. 2019. Extraction and characterization of acid soluble collagen from fish waste: Development of collagen-chitosan blend as food packaging film. J of Env. Chem. Eng. 7(2). doi.org/10.1016/j.jece.2019.102983

[16] Sato, K. 2019. Food for Skin Health: Collagen Peptides, Editor(s): Laurence Melton, Fereidoon Shahidi, Peter Varelis, Encyclopedia of Food Chemistry, Academic Press: 344-348.

[17] Abustam, E., Said, M. I. and Yusuf, M. 2018[a]. The effect of antioxidant activity of liquid smoke in feed supplement block on meat functional of muscle longissimus dorsi. Proceeding. IOP Conf. Ser: Earth Environ. Sci. 119 012046.

[18] Abustam, E., Said, M. I. and Yusuf, M. 2018[b]. Effect of aging time on changes in smoke flour compounds on meatballs and fresh meat of Bali beef. Proceeding. IOP Conf. Ser: Earth Environ. Sci. 247: 012026.

[19] Mc-Cormick, R. J. 2009. Collagen, in: M. Du, R.J. McCormick (Eds.), Applied Muscle Biology and Meat Science, CRC Press, London: 129–148.

[20] Pal, G. K and Suresh, P.V. 2016. Sustainable valorisation of seafood by-products: Recovery of collagen and development of collagen-based novel functional food ingredients. Innovative Food Sci & Emerging Technol. 37 (Part B): 201-215.

[21] Abustam, E., Said, M. I. and Yusuf, M. 2019. Organoleptic characteristics of Bali beef meatballs based on collagen concentration in UKKMB and time of maturation. Proceeding. IOP Conf. Ser: Earth Environ. Sci. 255: 012005.

[22] Haq, K. H., Mukhtar, H., Daudi, S., Ali, S. and Qadeer, M. A. 2003. Production of protease by a locally isolated molds culture under lab condition. Biotechnology. 2(1): 30-36.

[23] Ummadi, M. and Curic-Bawden, M. 2010. Use of protein hydrolysates in industrial starter culture fermentations. In V. K. Pasupuleti and Demain A. L. (Eds.), Protein hydrolysates in biotechnology. Dordrecht: Springer Netherlands: 91-114.

[24] Fioramonti, J., Theodorou, V. and Bueno, L. 2003. Probiotics: what are they? What are their effects on gut physiology? Best Practice & Research Clinical Gastroenterology. 17(5): 711-724.

[25] Steel, R. G. D and Torrie, J. H. 1991. Principle and Procedure of Statistics. 2[nd] .ed. International Book Company, Tokyo.

[26] Hooper, L. V. and Gordon, J. I. 2001. Commensal host-bacterial relationship. Science 292: 1115–1118.

[27] Bäckhed, F., Ley, R. E., Sonnenburg, J. L., Peterson, D. A. and Gordon, J. I. 2005. Host-bacterial mutualism in the human intestine. Science. 307: 1915–1920.

[28] Lahtinen, S., Ouwehand, A. C., Salminen, S. and Wright, A. V. 2011. Lactic Acid Bacteria: Microbiological and Functional Aspects, Fourth Edition. CRC Press Published. ISBN 9781439836774 - C
[29] Madigan, M. T., Bender, K. S., Buckley, D. H., Sattley, W. M. and Stahl, D. A. 2006. Brock Biology of Microorganisms (15th Edition). Pearson Education International. ISBN 0-13-196893-9: 375-377.
[30] Spano, G. and Massa, S. 2006. Environmental stress response in wine lactic acid bacteria: beyond *Bacillus subtilis*. Crit. Rev. Microbiol 32: 77.
[31] Lerena, M., Rojo, M., Sari, S., Mercado, L., Krieger-Weber, S. and Combina, M. 2016. Malolactic fermentation induced by *Lactobacillus plantarum* in Malbec wines from Argentina. South African J of Enology and Viticulture, 37(2):115-123.
[32] Ge, Q., Chen, S., Liu, R., Chen, L., Yang, B., Yu, H., Wu, M., Zhang, W. and Zhou, G. 2019. Effects of *Lactobacillus plantarum* NJAU-01 on the protein oxidation of fermented sausage. Food Chemistry. 295: 361-367.
[33] Mousavi, Z.E. and Mousavi, M. 2019. The effect of fermentation by Lactobacillus plantarum on the physicochemical and functional properties of liquorice root extract, LWT - Food Sci and Tech. 105: 164-168.
[34] Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I. and Kim, G. B. 2014. Effect of fermentation on the 363 antioxidant activity in plant-based foods. Food chemistry. 160: 346-356.
[35] Zhou, H. Y., Wang, C., Cai, J. H., Bai, Y., Yu, X. B., Li C. B., , Xu, X. L., Zhou, G. H. and Cao, J. X. 2019. Evaluating the effect of protein modifications and water distribution on bitterness and adhesiveness of Jinhua ham. Food Chem: 293.
[36] Wang, L., Liang, Q., Chen, T., Wang, Z., Xu, J. and Ma, H., 2014[a]. Characterization of collagen from the skin of Amur sturgeon (*Acipenser schrenckii*). Food Hydrocolloid. 38: 104–109.
[37] Wang, L., Liang, Q., Wang, Z., Xu, J., Liu, Y. and Ma, H. 2014[b]. Preparation and characterisation of type I and V collagens from the skin of Amur sturgeon (*Acipenser schrenckii*). Food Chem. 148: 410–414.
[38] Rahaman, T., Vasiljevic, T. and Ramchandran, L. 2016. Effect of processing on conformational changes of food proteins related to allergenicity. Trends in Food Science & Technology. 49: 24-34.
[39] Todorov, S. D., Stojanovski, S., Iliev, I., Moncheva, P., Nero, L. A. and Ivanova, I. V. 2017. Technology and safety assessment for lactic acid bacteria isolated from traditional Bulgarian fermented meat product “lukanka”, Brazilian Journal of Microbiology. 48(3): 576-586.
[40] Cao, P., Wu, L., Wu, Z., Pan, D., Zeng, X., Guo, Y. and Lian, L. 2019. Effects of oligosaccharides on the fermentation properties of Lactobacillus plantarum. J of Dairy Sci.102(4): 2863-2872.
[41] Nami, Y., Bakhshayesh, R. V., Manafi, M. and Hejazi, M. A. 2019. Hypocholesterolaemic activity of a novel autochthonous potential probiotic *Lactobacillus plantarum* YS5 isolated from yogurt. LWT. 111: 876-882.