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Scientific Research

Investigating the antioxidant activity of sesame meal protein hydrolysate produced with fermentation by *Bacillus* species

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ABSTRACT

Protein hydrolysate is a valuable source of bioactive peptides. The production of protein hydrolysate through the fermentation process is an environmentally friendly approach that is preferred over enzymatic and chemical hydrolysis in most cases. In this research, *Bacillus* species, including *Bacillus pumilus*, *Bacillus coagulans*, *Bacillus licheniformis*, and *Bacillus subtilis*, were employed to hydrolyze sesame meal protein. The investigated tests included measuring the concentration of peptides by the OPA method, DPPH radical inhibition, and iron ion reducing power, total antioxidant activity, and iron ion chelating activity. The concentration of peptides was evaluated after 24 h for *Bacillus* species. The lowest peptide concentration (0.656 mg/mL) was associated with the fermented treatment by *B. licheniformis*, while the highest value (1.38 mg/mL) was observed for the hydrolyzed treatment with *B. subtilis*. A significant difference ($p<0.05$) was observed among all the treatments. Results of DPPH radical inhibition showed the highest inhibition was associated with the samples hydrolyzed by *B. coagulans* (76.6%), and the lowest value was attributed to the hydrolysate by *B. licheniformis* (57.36%). The sample fermented with *B. pumilus* exhibited higher reducing power (0.992 absorbance at 700 nm) with a significant difference ($p<0.05$) observed between the treatments. The highest chelating activity (85.6%) was observed in the sample fermented with *B. subtilis*. The total antioxidant activity demonstrated that the protein hydrolysate with fermentation by *B. coagulans* had the highest absorbance value at 695 nm with a significant difference between the treatments ($p<0.05$). In conclusion, the fermentation of sesame meal protein by *Bacillus* species resulted in the production of protein hydrolysate with substantial antioxidant activity, positioning it as a promising source for inclusion in food formulations.

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1- Introduction

Currently, numerous diseases significantly influence people's quality of life. The World Health Organization's study identifies cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes as the main causes of mortality. [1]. Oxidative damage to lipids, proteins, and nucleic acids can lead to various diseases. This damage occurs when there is an imbalance between antioxidants and reactive oxygen species (ROS) in living organisms [2]. ROS production is an inherent byproduct of respiration and is counteracted by the body's endogenous antioxidant defense mechanism or external antioxidant consumption. Antioxidants have the ability to neutralize free radicals and protect human cells against oxidative stress; however, sometimes, due to various factors, ROS are excessively produced and cause severe diseases [4]. Protein hydrolysates have become more important recently because of their nutritional characteristics and bioactive effects. Peptides are biological molecules that are obtained from the hydrolysis of certain proteins and usually consist of 2-20 amino acid subunits [5]. Nevertheless, there are exceptions like Lunasin, a bioactive peptide made up of 44 amino acids with various biological activities, including immunomodulatory, antihypertensive, anticancer, anti-inflammatory, antioxidant, and antimicrobial [6]. Protein hydrolysates, including bioactive peptides, are derived from animal and plant protein sources. Sesame (*Sesamum indicum*) is a plant seed rich in lipids, dietary fiber, vitamins, antioxidants, and a high protein content. There are current findings on protein hydrolysates and bioactive peptides derived from sesame protein through enzymatic hydrolysis [7]. Fermentation is one of the methods of protein hydrolysis. This method involves using microorganisms to produce protein hydrolysates and bioactive peptides. Peptidases are secreted by bacteria during fermentation and break down proteins. The special advantages of this method compared to the enzymatic hydrolysis method are the existence of a greater variety of microbial proteases, and high levels of protease activity due to the activity of all proteases produced by the microorganism. It has a lower cost and is therefore affordable and enhances the value of the utilized substrate. In addition, it is considered an environmentally friendly method compared to enzymatic hydrolysis [8]. The selection of the microorganism is important in this process because it is responsible for the production of the proteolytic enzyme. However, suitable environmental conditions for its growth (temperature, time, water activity, pH) should be provided. *Bacillus* species are able to produce a significant amount of non-specific proteases to enhance hydrolysis. Furthermore, their effective growth performance is attributed to their ability to utilize inexpensive carbon sources and grow in difficult conditions with inadequate nutrients. Most strains, including *B. subtilis*, are generally recognized as safe (GRAS) and generate peptides with numerous biological functions through the fermentation of soybeans [10]. Nevertheless, there are several *Bacillus* species with significant proteolytic activity that require evaluation for their capacity to produce protein hydrolysates or bioactive peptides. Species such as *B. thuringiensis*, *B. megaterium*, and *B. proteolyticus* have not been previously recorded. In general,

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the purpose of this research was to use 4 species of *B. pumilus*, *B. coagulans*, *B. licheniformis*, and *B. subtilis* to hydrolyze sesame protein isolate and evaluate their antioxidant activity (DPPH radical inhibition, iron ion reducing power, total antioxidant activity, and iron ion chelating activity).

2- Material and methods

2-1- Preparation of raw materials: Sesame meal was obtained from Keshavarz Food Industry Co, Qom, Iran. Then it was ground and dried in an oven at 30 ˚C until it reached a constant weight. Next, defatting was done with hexane, and the defatted meal was utilized for protein extraction.

2.2 Microbial strains: *Bacillus pumilus* PTCC 1319, *Bacillus coagulans* IBRC 10807, *Bacillus licheniformis* PTCC 1595 and *Bacillus subtilis* PTCC 1156.

2.3 Sesame meal protein extraction: At first, the sesame meal was mixed with distilled water at a ratio of 1 to 10. The pH was adjusted to 11 with 1 N NaOH, then stirred for 1 h at 50 °C, and centrifuged (Hanil Co, Combi 514R, South Korea) at 8000 rpm for 15 min. The supernatant was adjusted to 4 with 0.1 N HCl and stirred for 30 min, then centrifuged for 15 min. Protein isolate dried with a freeze dryer (Ningbo Dscientz Technology Co, Ltd, China) and stored at -20 °C [11].

2.4 Protein hydrolysis by fermentation: For protein hydrolysis by fermentation, *Bacillus* species were first cultivated in BHI medium. To perform fermentation, protein 3%, inoculum 2%, pH=7, and temperature of 37 °C were considered. Fermentation was done with each of the *Bacillus* species, and after 24 h of incubation, it was centrifuged at 5000 rpm for 10 min, then sterilized with a 0.45 µ filter and dried by a freeze dryer.

2.5 Peptide concentration: Peptide concentration was done by the OPA method. To make the OPA solution, 25 mL of 100 mM tetraborate, 2.5 mL of 20% SDS, 40 mg of OPA (dissolved in 1 mL of methanol), and 50 mg of DTT were mixed together. Subsequently, the volume was increased to 50 mL by adding distilled water. 36 µL of the sample and 270 µL of the OPA solution were added in a 96-well microplate, and the absorption of the samples was measured using a spectrophotometer (BMG Labtech, SPECTROstar Nano, USA). Different concentrations of L-serine amino acid were used to make the standard curve [12].

2.6 Antioxidant test

2.6.1 DPPH radical inhibition: For DPPH radical inhibition activity, at first 20 mg of sesame protein hydrolysate was dissolved in 1 mL of distilled water, and 1 mL of it was combined with 750 µL of absolute ethanol. Then, 250 µL of 0.15 mM DPPH solution was added to it and placed in a dark environment for 30 min, and then the absorbance of the samples was determined at 517 nm. The control sample contained distilled water and DPPH solution [13]. The inhibition percentage was determined through the following formula:

$$\text{Percentage of DPPH radical inhibition} = \frac{control\ absorbtion - sample\ absorbtion}{control\ absorbtion} \times 100$$

2.6.2 Iron ion reducing power: To perform this test, 20 mg of protein hydrolysate was dissolved in 1 mL of distilled water. 250 µL of protein hydrolysate was mixed with 250 µL of 0.2 M phosphate buffer and 250 µL of potassium ferricyanide and stirred for 1

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min. The resulting mixture was placed in an incubator at 50 ˚C for 30 min. Next, 10% TCA (Sigma, Aldrich) was added to the mixture and centrifuged at 12000 rpm for 10 min. The resulting supernatant was mixed with 0.1% ferric chloride w/v, and the absorbance of the sample was recorded at 700 nm [14].

2.6.3 Iron ion chelating activity: To measure iron ion chelating, at first 500 mL of the sample (20 mg/mL) was mixed with 25 µL of 2 mM $FeCl_2$ solution and 900 µL of distilled water. Then 50 µL of 5 mM ferrozine solution was added and stirred. After 10 min of storage at ambient temperature, the absorbance was read at 562 nm. Distilled water was utilized as a control sample [15]. The chelating activity was determined through the following formula:

$$\text{Iron ion chelating activity} = \frac{control\ absorbtion - sample\ absorbtion}{control\ absorbtion} \times 100$$

2.6.4 Total antioxidants: At first, 50 µL of the sample dissolved in distilled water (concentration 20 mg/mL) with 500 µL of the reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were mixed and placed in a water bath at 90 °C for 90 min. Then it was centrifuged at 5000 rpm for 10 min, and the absorbance of the samples was recorded at 695 nm. The control sample contained reagents and distilled water [16].

2.7 Statistical analysis: Data analysis was done by SPSS version 26 software. A comparison of average data was done with Duncan's test at a 95% confidence level. All tests were carried out in triplicate.

3- Result and discussion

3.1 Peptide concentration: In this study, the proteolytic activity of 4 species of *Bacillus* was evaluated by OPA method to determine the concentration of peptides. Enzyme production by microorganisms depends on pH, temperature, amount of inoculum, incubation time, and type of nutrient source [17]. The source of carbon and nitrogen is an important factor for the production of protease in the fermentation medium [18]. According to Figure 1, *B. coagulans* and *B. subtilis* showed the highest absorption at the wavelength of 340 nm. And the peptide concentration was 1.33 and 1.38 mg/mL, respectively. The lowest peptide concentration (0.656 mg/mL) was related to *B. licheniformis*. Jensen et al. (2010) stated that the difference in the protease activity of probiotics is due to the difference in genetic characteristics between different species. Based on this, each species of *Bacillus* needs different optimal conditions for protease activity, and the hydrolysis pattern of proteinases may be due to the specificity of gene expression and also gene mutation [19]. Sesame protein hydrolysis and its conversion into peptides and free amino acids are needed for cell growth. Fang et al. (2017) investigated the hydrolysis of fish skin protein and reported an increase in the concentration of peptides during the fermentation process by *Aspergillus oryzae* [20]. Also, Jamil et al. (2014) reported that *Bacillus* had a good ability to hydrolyze a type of fish protein [21].

3.2 DPPH radical scavenging activity: DPPH is a stable free radical that exhibits maximum absorption at 517 nm. By the collision of free radicals with a proton-donating substrate such as an antioxidant, the radicals are destroyed and absorption decreases. DPPH radical scavenging activity of protein hydrolysate obtained from 4 *Bacillus* species was evaluated. The

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results are given in Figure 2. The highest percentage of DPPH radical inhibition (76.6%) was related to protein hydrolysis with *B. coagulans*, and the lowest percentage (57.36%) was related to *B. licheniformis*. The ability to inhibit DPPH radical depends on the type of protein substrate and the type of species used in the fermentation process, so that each species of *Bacillus* has a different mechanism of proteolytic activity and produces peptides with different sizes [22]. Hydrophobic and aromatic amino acids are considered to be the main amino acids responsible for oxidation resistance. Hence, increasing the number of these amino acids in the peptide sequence as a result of fermentation may improve the oxidation resistance of the hydrolysate [23]. It is also reported that methionine has a higher potential for reducing fat oxidation. Margo et al. (2019) stated that lentil protein hydrolysate with *Aspergillus niger* and *Aspergillus oryzae* has high antioxidant activity [24]. Turino et al. (2013) investigated the antihypertensive antioxidant activity of lentil protein hydrolysate by *Lactobacillus plantarum* and *Bacillus*. Furthermore, it has been proposed that microbial proteases might produce bioactive peptides during fermentation, hence enhancing the biological characteristics of the fermented products [25]. Also, Wally et al. (2020) found an antioxidant peptide from chickpea sprout protein hydrolysate that showed high hydroxyl and DPPH radical scavenging activities [26].

3.3 Iron ion reducing power: Reducing power is utilized to assess the capacity of antioxidants for donating electrons or protons. Many studies have shown that there is a clear correlation between the antioxidant activity and the reducing power of some bioactive compounds [27]. In this method, the ability of sesame meal protein hydrolysate with 4 *Bacillus* species to reduce Fe^{3+} to Fe^{2+} was evaluated. The antioxidant property of sesame protein hydrolysate can reduce ferric iron to ferrous iron. Therefore, the presence of Fe^{2+} ions can be controlled by measuring the color change at a wavelength of 700 nm. Figure 3 shows the reducing power of sesame meal protein hydrolysate by *Bacillus* species. According to Figure 3, *B. pumilus* showed the highest reducing power (0.992), and *B. licheniformis* showed the lowest reducing power of iron ions (0.708) at the wavelength of 700 nm. The difference in the reducing power of protein hydrolysate with different species is probably due to the fact that the produced peptides may be different in terms of chain length and amino acid sequence, which is caused by the different enzymatic mechanisms of the species [21].

3.4 Iron ion chelating activity: Increasing the level of iron can lead to the formation of ROS, which significantly causes oxidative stress in the body. The antioxidant activity of sesame protein hydrolysate can be due to its ability to chelate metal ions to form stable and water-soluble components. Therefore, the chelating activity of iron ions (Fe^{2+}) in this study was performed using 20 mg/mL of sesame meal protein hydrolysate to investigate the potential metal chelating activity. According to Figure 4, protein hydrolysate with fermentation by *B. subtilis* had the highest chelating activity of 87.7% and the lowest chelating activity of 67.13%, related to hydrolysis by *B. licheniformis*. Fang et al. (2015) reported 87% chelating activity of fish protein hydrolysate by *Aspergillus oryzae* and stated that the high chelating activity of iron ions by *A. oryzae* is related to hydrolytic genes [29]. The difference in activity and specificity of

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bacterial proteolytic enzymes leads to the formation of different hydrolysis products. Also, the higher capacity of iron ion chelating by the protein hydrolysate of skim milk is the result of the presence of phosphopeptides derived from milk casein containing the amino acids cysteine, tryptophan, serine, and tyrosine by *B. subtilis*, which interact with metal ions. [30].

3.5 Total antioxidant capacity: The evaluation of total antioxidant activity is based on the reduction of molybdenum (6+) to molybdenum (5+), and the absorption value at 695 nm shows the high total antioxidant capacity. A quantitative method to assess the antioxidant power is associated with the formation of a green phosphomolybdenum complex in an acidic condition. According to Figure 5, the highest total antioxidant activity was related to *B. coagulans*, and the lowest total antioxidant activity was related to *B. subtilis*. Researchers reported that the type of strain producing the proteolytic enzyme as well as the protein source affect the antioxidant activity of the protein hydrolysate [31]. Cruz Casas et al. (2023) evaluated amaranth seed protein hydrolysate with fermentation by *Bacillus* species. The results of their research showed that *Bacillus* species produced enzymes with high proteolytic activity that broke the protein chain and produced peptides with high electron-donating properties, which was consistent with our research results [32].

4- Conclusion

In general, *Bacillus* species have high proteolytic activity, which can be hydrolyzed proteins of sesame meal with biological activity such as antioxidant activities (DPPH radical inhibition), iron ion reducing power, chelating activity, and total antioxidant activity. The results showed that *B. pumilus*, *B. coagulans*, *B. licheniformis*, and *B. subtilis* have protease activity, and based on their different enzyme mechanisms, they showed different antioxidant activity, which can be utilized in functional foods as alternative nutrients to prevent various diseases.

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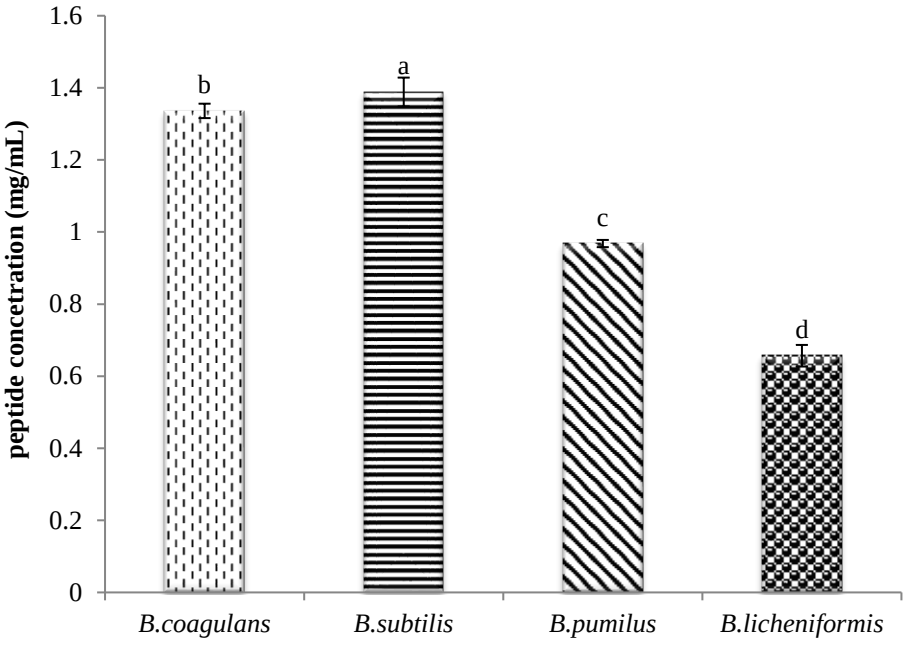

Figure 1: peptide concentration of the sesame meal protein hydrolysate produced by *Bacillus* specious in 24 h after fermentation.

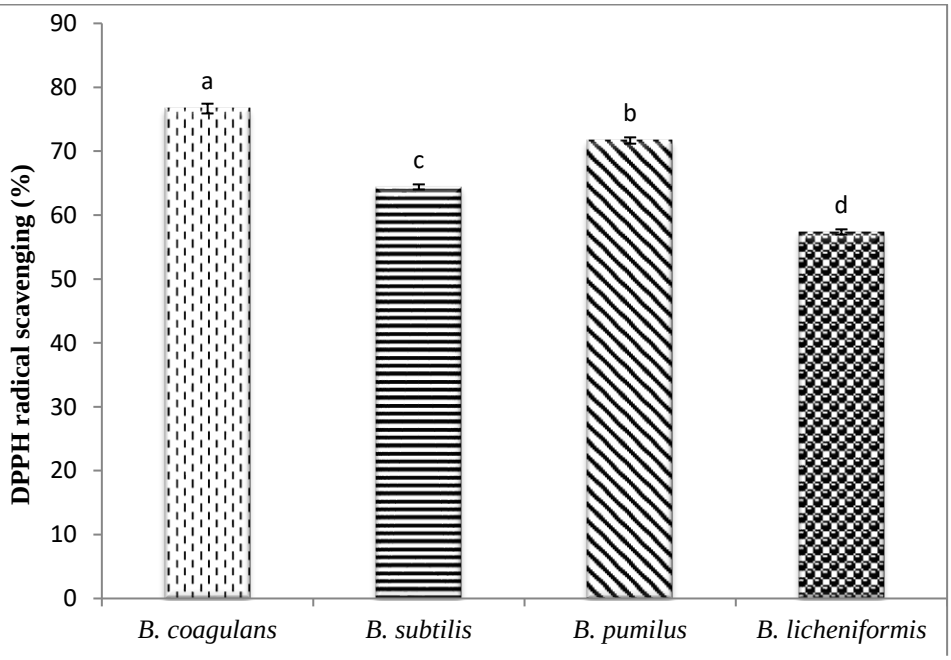

Figure 2: DPPH radical scavenging activity of the sesame meal protein hydrolysate produced by *Bacillus* specious in 24 h after fermentation.

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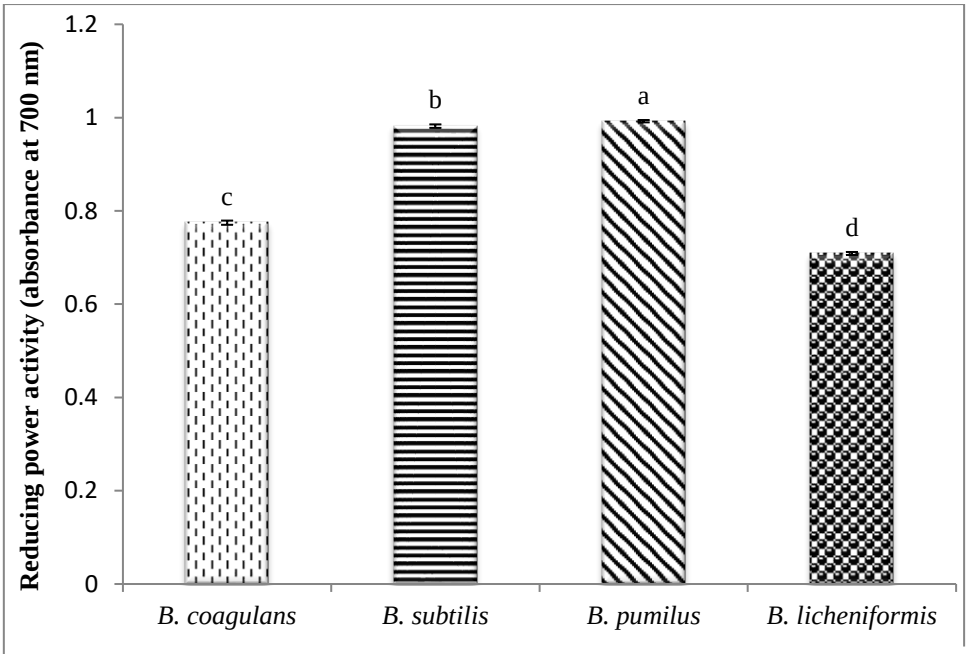

Figure 3: Reducing power activity of the sesame meal protein hydrolysate produced by *Bacillus* specious in 24 h after fermentation.

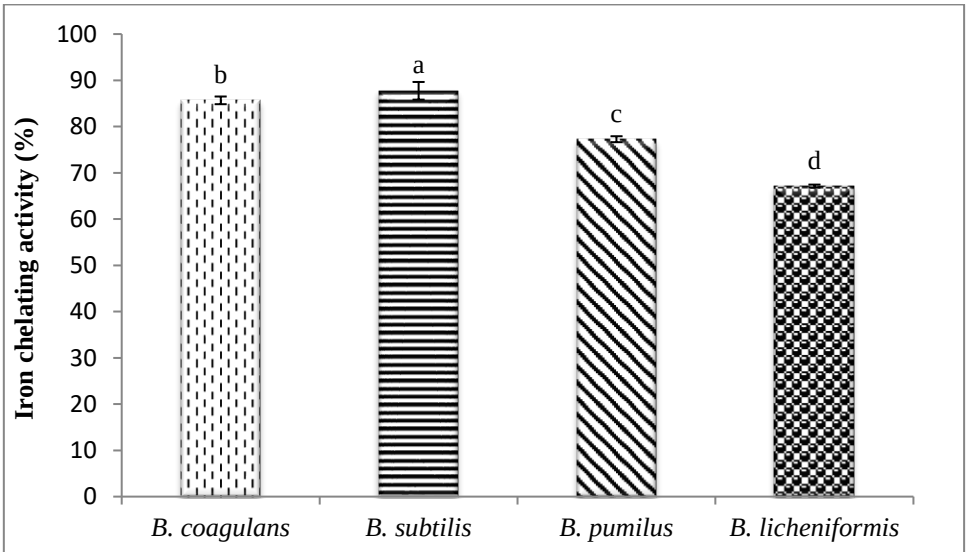

Figure 4: Iron chelating activity of the sesame meal protein hydrolysate produced by *Bacillus* specious in 24 h after fermentation.

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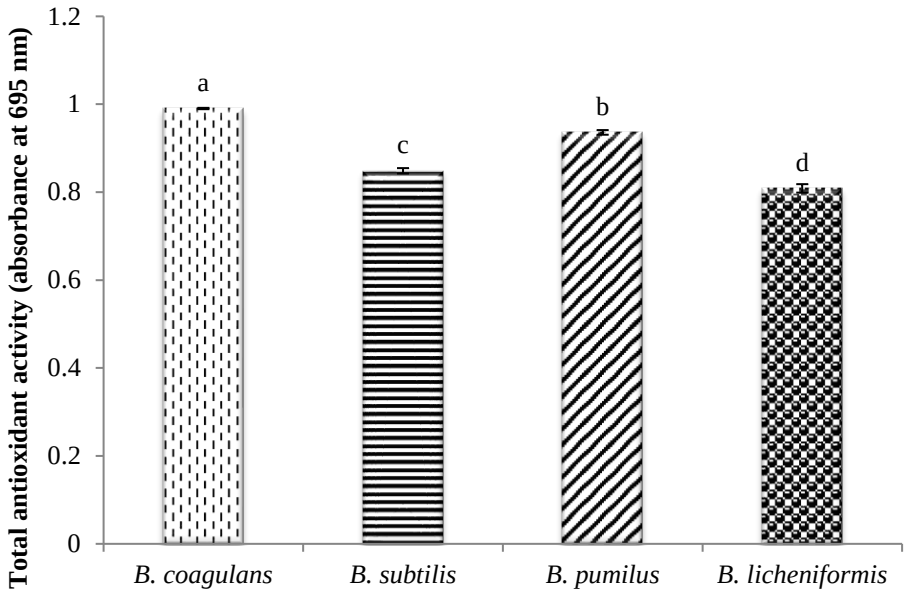

Figure 5: Total antioxidant activity of the sesame meal protein hydrolysate produced by *Bacillus* specious in 24 h after fermentation.

5- References

[1] Organisation mondiale de la santé. 2018. *World Health Statistics 2018:* Monitoring Health for the SDGs Sustainable Development Goals. World health organization.

[2] Nwachukwu, I.D. and Aluko, R.E. 2019. Structural and functional properties of food protein-derived antioxidant peptides. Journal of food biochemistry, 43(1), p.e12761.

[3] Ahmed, M., Verma, A. K., & Patel, R. 2020. Collagen extraction and recent biological activities of collagen peptides derived from sea-food waste: A review. Sustainable Chemistry and Pharmacy, 18, 100315. https://doi.org/10.1016/j.scp.2020.100315.

[4] Leung, R., Venus, C., Zeng, T., & Tsopmo, A. 2018. Structure-function relationships of hydroxyl radical scavenging and chromium-VI reducing cysteine-tripeptides derived from rye secalin. Food Chemistry, 254, 165-169. https://doi.org/10.1016/j.foodchem.2018.01.190.

[5] Chalamaiah, M., Ulug, S. K., Hong, H., & Wu, J. 2019. Regulatory requirements of bioactive peptides (protein hydrolysates) from food proteins. Journal of Functional Foods, 58, 123-12. https://doi.org/10.1016/j.jff.2019.04.050.

[6] Mudgil, P., Omar, L. S., Kamal, H., Kilari, B. P., & Maqsood, S. 2019. Multi-functional bioactive properties of intact and enzymatically hydrolysed quinoa and amaranth proteins. Lwt, 110, 207-213. https://doi.org/10.1016/j.lwt.2019.04.084.

[7] Lu, X., Zhang, L., Sun, Q., Song, G., & Huang, J. 2019. Extraction, identification and structure-activity relationship of antioxidant peptides from sesame (*Sesamum indicum L.*) protein hydrolysate. Food Research International, 116, 707-716. https://doi.org/10.1016/j.foodres.2018.09.001.

[8] CK Rajendran, S. R., Mohan, A., Khiari, Z., Udenigwe, C. C., & Mason, B. 2018. Yield, physicochemical, and antioxidant properties of Atlantic salmon visceral hydrolysate: Comparison of lactic acid bacterial fermentation with Flavourzyme proteolysis and formic acid treatment. Journal of food processing and preservation, 42(6), e13620.

[DOI: 10.22034/FSCT.21.156.80]

[9] Jemil, I., Abdelhedi, O., Mora, L., Nasri, R., Aristoy, M. C., Jridi, M., & Nasri, M. 2016. Peptidomic analysis of bioactive peptides in zebra blenny (Salaria basilisca) muscle protein hydrolysate exhibiting antimicrobial activity obtained by fermentation with *Bacillus mojavensis* A21. Process Biochemistry, 51(12), 2186-2197. https://doi.org/10.1016/j.procbio.2016.08.021.

[10] Jiang, X., Cui, Z., Wang, L., Xu, H., & Zhang, Y. 2020. Production of bioactive peptides from corn gluten meal by solid-state fermentation with Bacillus subtilis MTCC5480 and evaluation of its antioxidant capacity in vivo. Lwt, 131, 109767. https://doi.org/10.1016/j.lwt.2020.109767.

[11] Chatterjee, R., Dey, T. K., Ghosh, M., & Dhar, P. 2015. Enzymatic modification of sesame seed protein, sourced from waste resource for nutraceutical application. *Food and Bioproducts Processing*, *94*, 70-81. https://doi.org/10.1016/j.fbp.2015.01.007.

[12] Muhialdin, B. J., Rani, N. F. A., & Hussin, A. S. M. 2020. Identification of antioxidant and antibacterial activities for the bioactive peptides generated from bitter beans (*Parkia speciosa*) via boiling and fermentation processes. Lwt, 131, 109776. https://doi.org/10.1016/j.lwt.2020.109776.

[13] Jemil, I., Jridi, M., Nasri, R., Ktari, N., Salem, R. B. S.-B., Mehiri, M., Nasri, M. 2014. Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis* A26. Process Biochemistry, 49(6), 963-972. https://doi.org/10.1016/j.procbio.2014.03.004

[14] Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y., & Nasri, M. 2009. Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by gastrointestinal proteases. Food chemistry, 114(4), 1198-1205. https://doi.org/10.1016/j.foodchem.2008.10.075.

[15] Jamdar, S. N., Rajalakshmi, V., Pednekar, M. D., Juan, F., Yardi, V., & Sharma, A. 2010. Influence of degree of hydrolysis on functional properties, antioxidant activity and ACE inhibitory activity of peanut protein hydrolysate. Food chemistry, 121(1), 178-184. https://doi.org/10.1016/j.foodchem.2009.12.027.

[16] Prieto, P., Pineda, M., & Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry, 269(2), 337-341.

[17] Sanjukta, S., Padhi, S., Sarkar, P., Singh, S. P., Sahoo, D., & Rai, A. K. 2021. Production, characterization and molecular docking of antioxidant peptides from peptidome of kinema fermented with proteolytic *Bacillus* spp. Food Research International, 141, 110161. https://doi.org/10.1016/j.foodres.2021.110161.

[18] Nasri, R., Abdelhedi, O., Nasri, M., & Jridi, M. 2022. Fermented protein hydrolysates: biological activities and applications. Current Opinion in Food Science, 43, 120-127. https://doi.org/10.1016/j.cofs.2021.11.006.

[19] Jensen, M. P., & Ardö, Y. 2010. Variation in aminopeptidase and aminotransferase activities of six cheese related Lactobacillus helveticus strains. International Dairy Journal, 20(3), 149-155.

[20] Fang, Y., Xu, Z. I., Shi, Y., Pei, F., Yang, W., Ma, N., & Hu, Q. 2017. Protection mechanism of Se-containing protein hydrolysates from Se-enriched rice on Pb2+-induced apoptosis in PC12 and RAW264. 7 cells. Food Chemistry, 219, 391-398.

[21] Jemil, I., Jridi, M., Nasri, R., Ktari, N., Salem, R. B. S.-B., Mehiri, M., Nasri, M. 2014. Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by *Bacillus subtilis* A26. Process Biochemistry, 49(6), 963-972. https://doi.org/10.1016/j.procbio.2014.03.004.

[22] Zhi, T., Li, X., Sadiq, F. A., Mao, K., Gao, J., Mi, S., & Sang, Y. 2022. Novel antioxidant peptides from protein hydrolysates of scallop (Argopecten irradians) mantle using enzymatic and microbial methods: Preparation, purification, identification and characterization. LWT, 164, 113636. https://doi.org/10.1016/j.lwt.2022.113636.

[23] Hwang, H. S., & Winkler-Moser, J. K. 2017. Antioxidant activity of amino acids in soybean oil at frying temperature: Structural effects and synergism with tocopherols. Food Chemistry, 221, 1168-1177. https://doi.org/10.1016/j.foodchem.2016.11.042 .

[24] Magro, A. E. A., Silva, L. C., Rasera, G. B., & de Castro, R. J. S. 2019. Solid-state fermentation as an efficient strategy for the biotransformation of lentils: enhancing their antioxidant and antidiabetic potentials. Bioresources and Bioprocessing, 6(1), 1-9.

[DOI: 10.22034/FSCT.21.156.80]

[25] Torino, M. I., Limón, R. I., Martínez-Villaluenga, C., Mäkinen, S., Pihlanto, A., Vidal-Valverde, C., & Frias, J. 2013. Antioxidant and antihypertensive properties of liquid and solid state fermented lentils. Food chemistry, 136(2), 1030-1037. https://doi.org/10.1016/j.foodchem.2012.09.015.

[26] Wali, A., Mijiti, Y., Yanhua, G., Yili, A., Aisa, H. A., & Kawuli, A. 2021. Isolation and identification of a novel antioxidant peptide from chickpea (*Cicer arietinum L.*) sprout protein hydrolysates. International Journal of Peptide Research and Therapeutics, 27, 219-227.

[27] Khantaphant, S., & Benjakul, S. 2008. Comparative study on the proteases from fish pyloric caeca and the use for production of gelatin hydrolysate with antioxidative activity. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 151(4), 410-419. https://doi.org/10.1016/j.cbpb.2008.08.011.

[28] Mandel, S., Weinreb, O., Reznichenko, L., Kalfon, L., & Amit, T. 2006. Green tea catechins as brain-permeable, non toxic iron chelators to" iron out iron" from the brain. Journal of Neural Transmission-Supplements only, (71), 249-258.

[29] Fang, Y., Wang, S., Liu, S., Lu, M., Jiao, Y., Chen, G., & Pan, J. 2015. Solid-state fermentation of Acanthogobius hastaprocessing by-products for the production of antioxidant protein hydrolysates with *Aspergillus oryzae*. Brazilian Archives of Biology and Technology, 58, 343-352. https://doi.org/10.1590/S1516-8913201500297.

[30] Corrêa, A. P. F., Daroit, D. J., Fontoura, R., Meira, S. M. M., Segalin, J., & Brandelli, A. 2014. Hydrolysates of sheep cheese whey as a source of bioactive peptides with antioxidant and angiotensin-converting enzyme inhibitory activities. Peptides, 61, 48-55. https://doi.org/10.1016/j.peptides.2014.09.001.

[31] Mansor, A. B., Kahar, A. A., Rahman, S. A., Lelamurni, D., & Razak, A. 2014. Distribution and characterization of indigenous microbes from Malaysian fermented fish products. Functional Food Culture.

[32] Cruz-Casas, D. E., Aguilar, C. N., Ascacio-Valdés, J. A., Rodríguez-Herrera, R., Chávez-González, M. L., & Flores-Gallegos, A. C. 2023. Bioactive protein hydrolysates obtained from amaranth by fermentation with lactic acid bacteria and *Bacillus* species. Heliyon, 9(2).

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بررسی فعالیت آنتی‌اکسیدانی پروتئین هیدرولیز شده کنجاله کنجد تولیدی با تخمیر توسط گونه‌های *باسیلوس*

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چکیده

پروتئین هیدرولیز شده منبع ارزشمندی از پپتیدهای زیست فعال است. تولید پروتئین هیدرولیز شده از طریق فرآیند تخمیر یک رویکرد سازگار با محیط زیست است که در اغلب موارد نسبت به هیدرولیز آنزیمی و شیمیایی ترجیح داده می‌شود. در این پژوهش از ٤ گونه *Bacillus pumilus* PTCC 1319، *Bacillus coagulans* IBRC 10807، *Bacillus licheniformis* PTCC 1595 و *Bacillus subtilis* PTCC 1156 جهت هیدرولیز پروتئین کنجاله کنجد استفاده شد. آزمون‌های مورد بررسی شامل اندازه‌گیری غلظت پپتیدها با روش OPA، اندازه‌گیری فعالیت آنتی‌اکسیدانی شامل مهار رادیکال آزاد DPPH، قدرت احیاء کنندگی یون آهن، فعالیت آنتی‌اکسیدانی کل و قدرت شلاته کنندگی یون آهن بود. غلظت پپتیدها پس از مدت زمان ٢٤ ساعت برای ٤ گونه *Bacillus* اندازه‌گیری شد. کمترین غلظت پپتید (mg/mL ۰/٦٥٦) مربوط به تیمار تخمیر شده با *B. licheniformis* و بیشترین مقدار (mg/mL ۱/۳۸) مربوط به تیمار هیدرولیز شده با *B. subtilis* بود؛ بطوری‌که بین همه تیمارها اختلاف معنی‌داری ($p<۰/۰۵$) مشاهده شد. نتایج درصد مهار رادیکال آزاد DPPH نشان داد که بیشترین درصد مهار (٪۷٦/٦) مربوط به نمونه هیدرولیز شده توسط *Bacillus coagulans* و کمترین مقدار (٪۵۷/۳٦) مربوط به تیمار هیدرولیز شده با *B. licheniformis* بود به طوری‌که بین تیمارها اختلاف معنی‌داری ($p<۰/۰۵$) مشاهده شد. نمونه تخمیر شده با *Bacillus pumilus* قدرت احیاء کنندگی بیشتری (۰/۹۹۲ جذب در ۷۰۰ نانومتر) نشان داند. همچنین بین تیمارها اختلاف معنی‌داری ($p<۰/۰۵$) مشاهده شد. بیشترین درصد شلاته کنندگی یون آهن (٪۸۵/٦) در نمونه تخمیر شده با *B. subtilis* مشاهده شد. فعالیت آنتی‌اکسیدانی کل نشان داد که پروتئین هیدرولیز شده حاصل از تخمیر توسط *Bacillus coagulans* دارای بیشترین مقدار جذب در ٦۹۵ نانومتر می‌باشد و بین تیمارها اختلاف معنی‌داری ($p<۰/۰۵$) مشاهده شد. بطور کلی تخمیر پروتئین کنجد توسط گونه‌های *Bacillus* منجر به تولید پروتئین‌های هیدرولیز شده با فعالیت آنتی‌اکسیدانی بالایی شد که می‌تواند به عنوان منبعی بالقوه در فرمولاسیون مواد غذایی مورد استفاده قرار گیرد.

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