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Research Internship TEC 21: Analysis of okara soybean by-product peptides with antimicrobial properties for food industry

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Abstract– ***Tec 21 is an educational model from Tec de Monterrey that offers students 7th semester an optional a Research Internship as part of his educational development in "cross-functional skills" such as Innovation, Scientific Thinking and Written Language. This article is generated by a student pursuing a degree in Biotechnology Engineering as part of their research internship program, serving as evidence of their competencies in innovation, scientific thinking, and written language. One-quarter of total food production is lost globally due to food spoilage and contamination. Furthermore, synthetic food preservatives have increased their use in the food industry to address this issue even though they have been related to potential health risks. In this study, okara, a by-product waste from the soymilk production process was evaluated as a source of bioactive peptides which could act as a natural alternative to synthetic food preservatives. An experimental design of two kinds of okara were generated to compare the peptide profile through the time. One in which protein extraction happened immediately after okara generation, and another in which microbial growth was allowed for a week before protein extraction. Protein quantification assay and SDS-PAGE analysis were used to evaluate the protein composition changes. Antimicrobial analysis using disk diffusion susceptibility test against common foodborne pathogens was used. The protein quantification assay indicated a ten-fold decrease in protein content for the batch with microbial growth. SDS-PAGE analysis showed the presence of low molecular weight peptides when microbial growth occurs in okara, which is attributed to the proteolytic activity on enzymes during okara fermentation. Furthermore, antimicrobial analysis demonstrated that protein extract from okara with microbial growth has more antimicrobial effects against Staphylococcus aureus compared to food preservative sodium benzoate. In this regard, okara promises to be a valuable source of bioactive peptides when endogenous microorganisms are allowed to grow, but further studies and methods are needed to characterize okara derived peptides. This research exemplifies the student's integration of Innovative Entrepreneurship, Reasoning for Complexity, and Communication competencies, thereby advancing knowledge for future challenges.***

Keywords—Educational innovation, higher education, antimicrobial peptides, okara, natural preservatives, soybean, food waste.

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I. INTRODUCTION

Integrating theoretical knowledge with practical skills, the TEC 21 educational model at Tecnológico de Monterrey cultivates a holistic approach to learning, empowering students like myself to thrive in an ever-changing landscape. Embracing competencies such as Innovative Entrepreneurship (C2), Reasoning for Complexity (C5), and effective Communication (C6), I embark on a scientific journey where I not only expand the boundaries of knowledge but also develop critical skills essential for addressing complex challenges. Through this article, I present findings from my research endeavors, showcasing the transformative impact of the TEC 21 model on my academic and professional growth, while highlighting the intersection of innovative entrepreneurship, reasoning for complexity, and effective communication in shaping my scientific trajectory.

Regarding the problem subject, it is estimated that about 25% of the total food production globally is lost due to microbial contamination and 1.3 billion tons of food per year are lost due to spoilage [1]. Preservatives are substances that increase the shelf life of products and have been a food additive of great relevance in the food industry given that they allow the retarding of microbial contamination as well as natural spoilage processes, preventing foodborne illness and economical losses [2]. Synthetic or chemical preservatives such as benzoates, sorbates, and nitrites are commonly used in food production, but they pose a health hazard for consumers as they can be toxic or have detrimental side effects such as carcinogenicity, hypersensitivity, and asthma [2, 3]. Conversely, natural preservatives are derived from plant, animal, or microbial origin [4], and they are preferred by consumers for their non-toxicity. Among natural preservatives are essential oils, spices, extracts, enzymes, natural polymers, bacteriocins, and peptides [5]. The latter refer to fragments of proteins that are released by proteolytic enzymes and that are characterized by their wide range of action including

antioxidant, anticancer, antimicrobial, antifungal, and antiviral activity [6, 7].

Amidst the different natural sources of peptides, okara, the insoluble residue from soybean milk production, represents a potentially valuable option. This matrix holds many nutritional properties, being composed in dry base of 15.2-33.4% protein which contains all essential amino acids [8, 9], 10-15% oil, and 40-60% of crude fiber in the form of cellulose and hemicellulose [10, 11] while also containing isoflavones, minerals, vitamins, saponins and phytates [12, 13]. Additionally, peptides isolated from soybean have displayed antioxidant activity [6], anti-hypertension effects [10], anticancer, antiobesity, antidiabetic, and antimicrobial activity [13]. Moreover, 1.4 billion tons of okara are produced worldwide every year as a waste by-product of the soybean milk production process and most of it has disposal and reutilization issues, being either discarded or used as live-stock feed, resulting in a waste of a valuable resource at best and in environmental pollution at worst [8, 9, 11]. Therefore, the high protein content of okara and its by-product nature makes it a natural low-cost source of peptides with bioactive properties, including potential antimicrobial activity. In this sense, several pretreatments have been explored in okara with the goal of increasing the release of bioactive substances and the utilization of its nutrients, which involve fermentation with either yeast, fungus or bacteria, enzymatic treatment, high-pressure homogenization, or the combination of several treatments which can result in the generation of peptides from okara protein and other valuable bioactive compounds [6].

As for antimicrobial peptides (AMPs), these are characterized by features such as low molecular weight, short chain sequences of less than 50 amino acids, cationic charge, heat stability, and amphipathic nature with both hydrophilic and hydrophobic amino acids [14]. Their mechanism of action to inhibit microbial growth is related with the electrostatic interaction with the cell membrane as they can permeate through it and disrupt it [5], affecting the pH gradient, membrane potential, and osmotic regulation [14]. Additionally, AMPs can also have intracellular activity by inhibiting DNA, RNA, and protein synthesis, amongst other effects [15]. While there are few studies on the activity of okara derived peptides, some examples of their use include applications as anti-fungal compounds for crop protection, animal nutrition, ecological materials, functional foods for humans, and production of bioactive compounds through fermentation [8]. Furthermore, soy derived peptides have been shown to have antimicrobial activity against *Pseudomonas aeruginosa* and *Listeria monocytogenes* [14], as well as with E*scherichia coli, Staphylococcus aureus,* and *Micrococcus luteus* [16].

Still, the use of okara peptides as antimicrobial agents in the food industry hasn't been explored, which could provide a natural preservative that derives from a circular economy, in which the by-product of a process are the primary resources of another process [17], saving economical resources and providing an outlet for the tons of okara produced annually. Particularly, some factors that have limited the development of AMPs from okara are the need of preservation methods due to the quick contamination of okara due to its high moisture content, the difficulties associated with the scale-up of the protein extraction and peptide generation process, and the lack of characterization of the bioactive peptides from okara [6, 8, 14]. Okara is nutrient rich matrix that contains 70-80% moisture content [11], creating susceptible conditions for the growth of microorganisms. Therefore, two different batches of okara were prepared to test differences in protein and peptide profile when microbial growth is present. The first batch was immediately dried and stored after preparation while the second batch was left wet during a week at room temperature, allowing growth of endogenous microorganisms. After protein extraction resulting in a lyophilizate for each batch, protein quantification assay was performed. By these means, there's a need to develop a profitable and scalable process that results in AMPs for their use in the food industry so that okara waste product can be exploited. In this work, the potential antimicrobial activity of peptides obtained from soybean okara is evaluated against common foodborne pathogens, as well as the effects of allowing endogenous microbial growth before protein extraction.

II. MATERIALS AND METHODS

A. Research Proposal for Student

The research internship program, aligned with the TEC 21 model, aims to develop competencies in Innovative Entrepreneurship (C2), Reasoning for Complexity (C5), and Communication (C6) in which student selects the project named "antimicrobial properties of okara-derived peptides" proposed by advisor. The student was selected based on their academic performance and interest in food preservation, and was advised on innovation, problem-solving challenges, research methodology training, and scientific writing. The experimental phase includes preparing okara, extracting and characterizing bioactive peptides, and assessing their antimicrobial activity against *S. aureus*. Throughout the program, the student draft, review, and refine this scientific manuscript, integrating their developed competencies. The program requires the submission of the manuscript to a scientific journal, demonstrating the practical application of the TEC 21 model in fostering innovation, complex problem-solving, and effective communication.

B. Microorganisms and media

Escherichia coli ATCC 8739, *Listeria monocytogenes*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were cultivated for their use in the antimicrobial disk diffusion susceptibility test since these bacteria are reported as common foodborne pathogens [18, 19, 20, 21, 22, 23]. All microorganisms were obtained from laboratory stocks. E. coli ATCC 8739 and P. aeruginosa were

grown on Difco ™ Malt Extract Broth (Becton Dickinson & Company, 211320) supplemented with 4 g/L of peptone from casein (Merck, 1.07213) and 15 g/L of bacteriological agar (Becton Dickinson & Company, 215000) for solid media. L. monocytogenes and S. aureus were grown on Difco ™ Tryptic Soy Broth (Becton Dickinson & Company, 211825) for liquid media and Difco ™ Tryptic Soy Agar (Becton Dickinson & Company, 236950) for solid media. S. *enterica* was grown on Difco ™ Nutrient Broth (Becton Dickinson & Company, 234000) and supplemented with 15 g/L of bacteriological agar (Becton Dickinson & Company, 215000) for solid media. Culture conditions were in agitation at 37 ºC.

C. Preparation of okara

Whole soybeans were acquired from a local supermarket and utilized to prepare soymilk according to the initial steps of the Illinois process [44]. This involved soaking the whole soybeans in a distilled water solution containing 0.5% $NaHCO_3$ (Desarrollo de Especialidades Químicas S.A. de C.V., 120314-01) (in a ratio of 1 part beans to 3 parts solution) for 17 hours, followed by draining the soybeans. Subsequently, the soybeans were soaked again for 30 minutes in a fresh distilled water solution of 0.5% $NaHCO_3$, drained once more, and ground using a Moongiantgo® Mini Spice Coffee Grinder with enough distilled water to achieve a soybean solids concentration of 12%. Afterwards, the generated slurry was filtered using a cheesecloth to obtain soymilk and the okara by-product. Two batches of okara were produced using the same process. The first batch underwent immediate drying at 60 ºC for 20 hours, followed by pulverization to create okara flour. In contrast, the second batch was left undried in a covered bowl at room temperature for one week to encourage the growth of endogenous microorganisms. Both samples were immediately stored at -20 ºC for further use.

D. Protein extraction

Okara flour from the batch without microbial growth and wet okara from the batch with microbial growth were defatted with hexane (1:10 w/v) for 30 minutes following [12] protocol. Afterwards, protein extraction was made following [24] methodology. Distilled water was added to defatted okara in a ratio of 1:20, pH was adjusted to 12 using NaOH 50% w/v, and the suspension was stirred for 1 hour at 50 ºC, following centrifugation at 8000 rpm for 20 minutes. Subsequently, the supernatant was acidified to pH 4.2 using HCl 2M and a second centrifugation step was performed at 8000 rpm for 20 minutes. The pellet was recollected and resuspended in deionized water in a ratio of 1:5, and pH was neutralized to 7 using NaOH 50% w/v. Finally, solutions of extracted protein were freeze dried for 5 days and then stored at -80 ºC until further use.

E. Protein quantification

The Pierce™ BCA Protein Assay Kit from Thermo Fisher Scientific was used to quantify protein following the protocol provided by the manufacturer (Thermo Fisher Scientific). Absorbance at 562 nm was measured using a microplate reader (Synergy HT Bio Tek). For this assay, a calibration curve was made with bovine serum albumin protein (BSA) included in the kit in a concentration range of 25-2000 µg/mL. Protein sample solutions of each okara batch were prepared by adding 0.5 mg of protein lyophilizate in 1 mL of MilliQ water. All measurements were done by triplicate.

F. SDS-PAGE

For the analysis of okara protein extracts, a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) 12% polyacrylamide gel was prepared following the [43] methodology. Preparation of samples consisted of adding 5 µl of 6X loading buffer and 10 µl of protein extract solution (3 mg/mL) from each okara batch, then heated at 99ºC for 10 min, allowed to stand for 5 minutes at room temperature and frozen until use. Gels were run at 80 V, 50 mA and 80 W. Precision Plus Protein™ Dual Xtra Prestained Protein Standard (Bio-Rad, 1610377) was used as molecular weight marker.

G. Antimicrobial disk diffusion susceptibility test

Protein extracts from okara were tested for antimicrobial activity using the Kirby-Bauer disk diffusion susceptibility test protocol [25], with modifications to culture conditions. Inoculums were prepared by incubating 1 mL of bacterial stocks in 10 mL of their respective culture media at 37 ºC in agitation for four days and subsequently adjusting turbidity to 0.5 McFarland standard which was prepared in-house by mixing 0.5 mL of a 1.175% w/v solution of $BaCl_2$ (Sigma-Aldrich, 529591) with 99.5 mL of a 1% v/v H2SO4 (Desarrollo de Especialidades Químicas S.A. de C.V., 140907-02) solution. Optical density was measured at 600 nm using a UV-visible light spectrophotometer (Thermo Scientific GENESYS™ 10S). Agar plates were inoculated using a sterile loop by streaking three times over all the agar surfaces, and blank sterile antibiotic sensitivity disks (Innovating Science, IS35040) were impregnated with 50 µl of sample. Two disks impregnated with a 3 mg/mL solution of each batch of okara protein extract, and a disk impregnated with a 3 mg/mL solution of sodium benzoate (Diquítra, 502295) as a positive control was placed in each of the plates using forceps. Concentration of the positive control was defined according to the reported minimum inhibitory concentration (MIC) of sodium benzoate against several of the bacteria used in this experiment [26]. Plates were incubated for 24 hours at 37 ºC and inhibition zones were measured with a ruler. For each bacterium experiments were done by triplicate.

III. RESULTS AND DISCUSSION

A. Okara protein extraction and quantification

The protein concentration of extract solutions in each of the lyophilizates was 255.33 ± 10.47 µg/mL and 26.00 ± 8.67 µg/mL for okara without and with microorganism growth, respectively (Table 1). Furthermore, the protein content in the lyophilizate represents 51.11% and 5.20% in okara without and with microorganism growth, respectively. This represents a reduction of nearly 90% in the protein concentration due to the microorganism activity. According to [24], a total protein content of 68.86% was reported with microbial growth, which could be a consequence of the difference in soybean variety and the method of okara generation.

To determine the protein profile shift in both samples, an SDS-PAGE analysis was performed. The okara without microbial growth contains peptides and proteins in the range of 2 kDa up to 75 kDa with defined bands in the 50 kDa mark, below the 75 kDa and 25 kDa mark, and above the 2 kDa mark (Figure 1). On the other hand, okara batch with microbial growth exhibited a less distributed profile, with a concentration of bands below the 25 kDa, particularly over the 2 kDa range. These results suggest that microorganism present in okara transformed the proteins, from 50 kDa and 75 kDa mostly, into peptides below the 2 kDa. Notably, on this batch there's also a decreased amount of protein from the 25 kDa mark upwards, which could relate to high molecular weight proteins being broke down into low molecular weight peptides. This could be attributed to the proteolytic activity of enzimes from soy endogenous microorganisms during fermentation [13, 27]. By these means, the batch with microbial growth decreased protein concentration measurement since most of the protein content was hydrolyzed into low molecular weight peptides. Several Bacillus subtilis strains have been isolated from soybeans and fermented soy products [13]. These enzymes have been reported to secrete enzymes which break down macromolecules present in okara, including proteins, and increase its antioxidant activity [28]. Furthermore, lactic acid bacteria have also been identified as endogenous microorganisms from soybean okara, particularly strains belonging to the genera *Lactobacillus* such as *Lactobacillus spentosus* and *Lactobacillus plantarum* [29, 30]. These studies proved that certain strains could generate bioactive peptides from soybeans [31]. Further studies must be performed regarding the isolation and identification of the microorganisms present in the produced okara batch a type of peptides is dependent on the variety of soybean and of the microorganism strain present in it [13].

TABLE I
PROTEIN QUANTIFICATION OF OKARA EXTRACTS.

Sample	Lyophilizate (µg)	Volume (mL)	Protein concentration (µg/mL)	Protein in the lyophilizate (%)
Okara without microorganism growth	500.00	1.00	255.33 ± 10.47[a]	51.11[a]
Okara with microorganism growth			26.00 ± 8.67[b]	5.20[b]

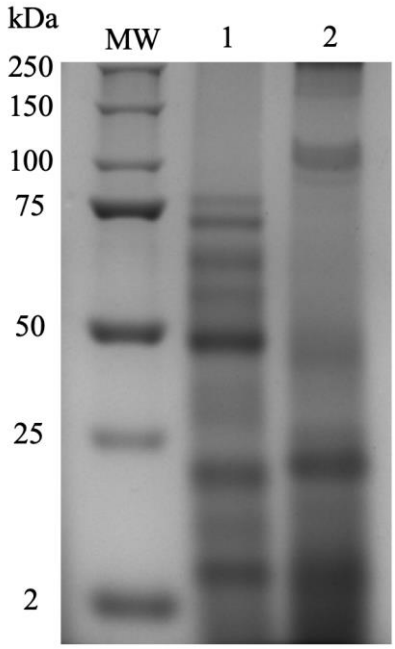

Fig. 1 SDS-PAGE analysis of protein extraction samples from okara (3 mg/mL). Lane 1: Okara batch without microbial growth. Lane 2: Okara batch with microbial growth. MW: Molecular Weight Marker. kDa: Kilodaltons.

B. Antimicrobial activity of okara protein extracts

S. aureus was the only bacteria with measurable inhibition diameters (Figure 2). The okara extract has a higher antimicrobial activity against S. aureus with a 20 mm inhibition of microbial growth compared to 11 mm for the sodium benzoate. Similar studies have reported an increased or equivalent antimicrobial activity of peptides against S. aureus in comparison to sodium benzoate [32, 33, 34]. In the other hand, okara protein extract from the batch without microbial growth exhibited a concentrated bacterial growth, a phenomenon that occurred with most tested bacteria. Therefore, the availability of nutrients in the batch without microbial could be a contributing factor that, instead of inhibiting bacterial growth, promotes it since free carbohydrates such as arabinose, glucose, fructose, and sucrose, as well as protein that contains all essential amino acids, and fatty acids including linoleic, palmitic, and linolenic acid are present in okara [11].

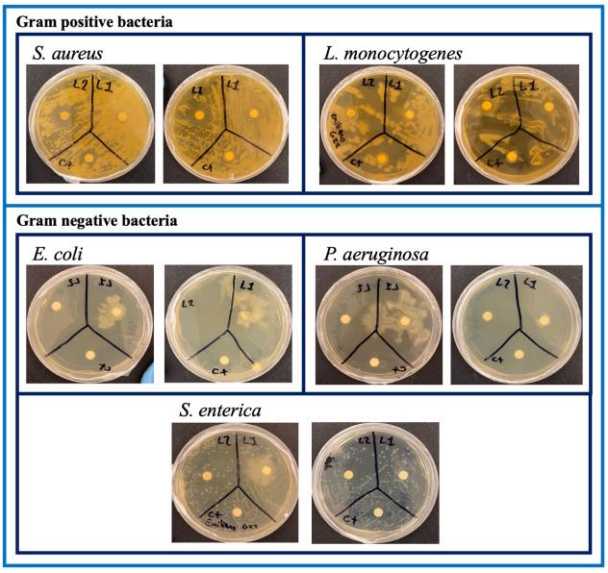

Fig. 2 Antimicrobial disk diffusion susceptibility test of okara protein extracts against gram positive and gram-negative bacteria. Top left disk contains okara protein extract (3 mg/mL) from batch with microbial growth. Top right disk contains okara protein extract (3 mg/mL) from batch without microbial growth. Bottom center disk contains sodium benzoate (3 mg/mL) as positive control.

As for *L. monocytogenes*, *E. coli*, *P. aeruginosa*, and *S. enterica*, poor microbial growth was achieved on the plates. This could be attributed to culture conditions that could have affected proper growth such as the use culture media other than Müller-Hinton agar which is indicated in the Kirby-Bauer protocol. Particularly in the case of *E. coli* and *P. aeruginosa* which had the poorest growth and were cultivated on Malt Extract Agar that, according to the manufacturer, is a media suited for yeasts and molds that restricts bacterial growth. Also, there was excess moisture is some of the plates, which is a factor that can disturb the assay's results [35]. Additionally, there are several parameters dependent upon the operator such as plate streaking that can potentially influence the accuracy of the method [36].

Furthermore, it is to be noted that the disk diffusion susceptibility assay may not be the most adequate method for testing the antimicrobial activity of bioactive peptides. Several aspects from the method could underestimate the susceptibility of microorganisms to peptides such as the fact that negatively charged components in agar can interact with positively charged peptides and neutralize them, having a detrimental effect on their activity [37]. Moreover, the choice of media could alter antimicrobial activity results since Müller-Hinton agar as well as all the media used in this study contain peptone from casein which has free amino acids and short peptide chains [37, 38], some of which could be anionic and interfere with cationic peptides to be tested, which happen to be the bioactive peptides with the highest reported antimicrobial activity [39]. Hence, various factors need to be taken into account when assessing the antimicrobial susceptibility of peptide-containing extracts, as even the choice of plasticware can introduce interference [37]. Consequently, the necessity arises for the development of a novel standardized method tailored specifically for peptides in future investigations.

Regarding the antimicrobial activity of okara protein extracts, the presence of other compounds in addition to bioactive peptides that may enhance or diminish the antimicrobial effect, particularly on the batch with microbial growth since soy fermentation creates secondary metabolites other than peptides [13]. In the case of *B. subtillis* strains isolated from fermented soybean products, bioactive peptides generated by this bacterium have demonstrated antimicrobial activity against foodborne pathogens like *Clostridium botulinum*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, but other compounds such as organic acids or bacteriocins are also produced by this microorganism and have antimicrobial effects against pathogens [13]. This could also be the case with *Lactobacillus* strains isolated from soybeans, which have shown antimicrobial activity against pathogens Escherichia coli 0157, Staphylococcus aureus ATCC 25923 and Listeria monocytogenes EP01, but lactic acid is the primary antibiotic compound generating this susceptibility rather than bioactive peptides [29].

Therefore, it remains relevant to further characterize the okara protein extracts and assess whether bioactive peptides alone are responsible for the antimicrobial effect of S. aureus. Also, the identification and characterization of the peptides present in the extract could determine if the bioactive peptides are either produced by an endogenous microorganism of okara or if they come directly from soybeans given that peptides isolated from soybeans have also exhibited antimicrobial activity [13]. In addition, once the bioactive peptides are identified, their sequence could give further insight on their mechanism of action. Currently, cationic, and amphiphilic peptides of low molecular weight have been identified by several studies as the natural bioactive peptides with the highest antimicrobial activity [39, 40, 41]. The mechanism of action of peptides could be explained by the membrane model in which cationic peptides increase the permeability of the negatively charged bacterial membrane, leading to cell lysis [42]. Gram positive bacteria tend to be more susceptible to bioactive peptides since they only have a peptidoglycan and lipoteichoic acid layer before the plasma membrane, as opposed to the gram-negative bacteria which, in addition to the peptidoglycan layer, also have an outer membrane which restricts the access of bioactive peptides to the inner plasma membrane [41, 42]. Still, antimicrobial peptides also have intracellular mechanisms of action including inhibition of protein and nucleic acid synthesis, degradation of enzymes, inhibition of cell wall synthesis, among others [40, 42].

IV. CONCLUSION

The results obtained from this study indicate the potential use of protein extract of okara with microbial growth as a food preservative to inhibit the growth of S. aureus. The repetition of the antimicrobial susceptibility test with proper culture conditions for *L. monocytogenes, E. coli*, *P. aeruginosa*, and *S. enterica* is required to demonstrate whether or not the okara protein extract has any growth inhibition for these foodborne pathogens. Once this is determined, further characterization experiments will be required to identify peptide fractions, calculate both their minimum inhibitory concentration and minimum bactericidal concentration, and test for their antioxidant and cytotoxic activity in order to confirm their use as safe natural food preservatives. Additionally, for okara bioactive peptides to be a viable alternative in food industry, several challenges such as prevention of contamination in okara batches, optimization and scale-up of the protein extraction process and decrease in costs need to be addressed.

Given the global threat of food contamination and spoilage towards world food security as well as the potential health risks associated with synthetic preservatives, this study aims to provide a natural and sustainable alternative of natural preservatives from bioactive peptides derived from soybean okara. The findings suggest that the proliferation of indigenous microorganisms in soybean-derived okara enhances the concentration of bioactive peptides. Moreover, the protein extract obtained from this process demonstrates inhibitory effects against the gram-positive bacterium S. *aureus*, hinting at the potential for developing an okara-based product as a natural preservative. This presents a valuable opportunity to utilize this typically discarded by-product. Furthermore, it was determined that further characterization of peptide fractions from the okara extract is necessary, along with the development of standardized methods tailored specifically for assessing the antimicrobial susceptibility of bioactive peptides.

Finally, this research conducted on natural preservatives in the food industry not only addresses the pressing need for safer alternatives but also exemplifies the integration of key competencies such as Innovative Entrepreneurship, Reasoning for Complexity, and Communication during the research internships of the student. By developing innovative solutions to replace artificial preservatives, the student demonstrates their ability to adapt to changing environments and create value with a positive impact on society. Moreover, the process of analyzing complex problems, synthesizing information, and continuously learning throughout the research journey reflects his commitment to reasoning for complexity. Additionally, effectively communicating these findings through various channels showcases his proficiency in communication, essential for professional and personal interactions. Overall, this scientific endeavor not only contributes to advancing knowledge in the field but also enhances the student's transversal competencies, preparing them for future challenges and opportunities in the food industry and beyond.

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REFERENCES

[1] Petruzzi, L., Corbo, M. R., Sinigaglia, M., & Bevilacqua, A. (2017). Microbial Spoilage of Foods: Fundamentals. In The Microbiological Quality of Food: Foodborne Spoilers (pp. 1–21). Elsevier Ltd. https://doi.org/10.1016/B978-0-08-100502-6.00002-9

[2] Barberis, S., Quiroga, H. G., Barcia, C., Talia, J. M., & Debattista, N. (2018). Natural Food Preservatives Against Microorganisms. In Food Safety and Preservation (pp. 621–658). Elsevier Inc. https://doi.org/10.1016/b978-0-12-814956-0.00020-2

[3] Kamala Kumari, P. v., Akhila, S., Srinivasa Rao, Y., & Rama Devi, B. (2019). Alternative to artificial preservatives. Systematic Reviews in Pharmacy, 10(1), S13–S16. https://doi.org/10.5530/srp.2019.1s.17

[4] Sharif, Z., Mustapha, F., Jai, J., Yosof, N., & Zaki, N. A. M. (2017). Review on methods for preservation and natural preservatives for extending the food longevity. Chemical Engineering Research Bulletin, 19.

[5] Amiri, S., Moghanjougi, Z. M., Bari, M. R., & Khaneghah, A. M. (2021). Natural protective agents and their applications as bio-preservatives in the food industry: An overview of current and future applications. Italian Journal of Food Science, 33(SP1), 55–68. https://doi.org/10.15586/ijfs.v33iSP1.2045

[6] Fang, J., Lu, J., Zhang, Y., Wang, J., Wang, S., Fan, H., Zhang, J., Dai, W., Gao, J., & Yu, H. (2021). Structural properties, antioxidant and immune activities of low molecular weight peptides from soybean dregs (Okara). Food Chemistry: X, 12, 100175. https://doi.org/10.1016/j.fochx.2021.100175.

[7] Tkaczewska, J. (2020). Peptides and protein hydrolysates as food preservatives and bioactive components of edible films and coatings - A review. Trends in Food Science and Technology, 106, 298–311. https://doi.org/10.1016/j.tifs.2020.10.022

[8] Colletti, A., Attrovio, A., Boffa, L., Mantegna, S., & Cravotto, G. (2020). Valorisation of by-products from soybean (Glycine max (L.) Merr.) processing. Molecules, 25(9), 2–33. https://doi.org/10.3390/molecules25092129

[9] de Benedetti, S., Girlando, V., Pasquali, M., Scarafoni, A., Trombetta, D., & Smeriglio, A. (2021). Valorization of Okara by Enzymatic Production of Anti-Fungal Compounds for Plant Protection. https://doi.org/10.3390/molecules26164858

[10] O'Toole, D. K. (1999). Characteristics and use of okara, the soybean residue from soy milk production - A review. Journal of Agricultural and Food Chemistry, 47(2), 363–371. https://doi.org/10.1021/jf980754l

[11] Vong, W. C., & Liu, S. Q. (2016). Biovalorisation of okara (soybean residue) for food and nutrition. Trends in Food Science and Technology, 52, 139–147. https://doi.org/10.1016/j.tifs.2016.04.011

[12] Eze, O. F. (2019). Extraction of Proteins from Soybean Residue (Okara) and Investigation of their Physicochemical Properties and their Application as Emulsifiers. University of Reading.

[13] Mora-Escobedo, R., del Carmen Robles-Ramírez, M., Delia Román-Gutiérrez, A., Castro-Rosas, J., Baruchs Muñoz-Llandes, C., & Araceli Guzmán-Ortiz, F. (2019). Peptides and Microorganisms Isolated from

Soybean Sources with Antimicrobial Activity. Soybean - Biomass, Yield and Productivity. https://doi.org/10.5772/intechopen.81243
[14]Dhayakaran, R., Neethirajan, S., & Weng, X. (2016). Investigation of the antimicrobial activity of soy peptides by developing a high throughput drug screening assay. Biochemistry and Biophysics Reports, 6, 149–157. https://doi.org/10.1016/j.bbrep.2016.04.001
[15]Li, Y., Xiang, Q., Zhang, Q., Huang, Y., & Su, Z. (2012). Overview on the recent study of antimicrobial peptides: Origins, functions, relative mechanisms and application. Peptides, 37(2), 207–215. https://doi.org/10.1016/j.peptides.2012.07.001
[16]Mcclean, S., Beggs, L. B., & Welch, R. W. (2014). Antimicrobial activity of antihypertensive food-derived peptides and selected alanine analogues. Food Chemistry, 146, 443–447. https://doi.org/10.1016/j.foodchem.2013.09.094
[17]Millati, R., Cahyono, R. B., Ariyanto, T., Azzahrani, I. N., Putri, R. U., & Taherzadeh, M. J. (2019). Agricultural, industrial, municipal, and forest wastes: An overview. In Sustainable Resource Recovery and Zero Waste Approaches. Elsevier B.V. https://doi.org/10.1016/B978-0-444-64200-4.00001-3
[18]Arslan, S., Eyi, A., & Özdemir, F. (2011). Spoilage potentials and antimicrobial resistance of Pseudomonas spp. isolated from cheeses. https://doi.org/10.3168/jds.2011-4676
[19]Bintsis, T. (2017). Foodborne pathogens. AIMS Microbiology, 3(3), 529. https://doi.org/10.3934/MICROBIOL.2017.3.529
[20]Food & Drug Administration. (2022). Foodborne Pathogens. https://www.fda.gov/food/outbreaks-foodborne-illness/foodborne-pathogens
[21]Fung, F., Wang, H. S., & Menon, S. (2018). Food safety in the 21st century. Biomedical Journal, 41(2), 88. https://doi.org/10.1016/J.BJ.2018.03.003
[22]Lorenzo, J. M., Munekata, P. E., Dominguez, R., Pateiro, M., Saraiva, J. A., & Franco, D. (2018). Main Groups of Microorganisms of Relevance for Food Safety and Stability: General Aspects and Overall Description. Innovative Technologies for Food Preservation, 53. https://doi.org/10.1016/B978-0-12-811031-7.00003-0
[23]Won, G., & Lee, J. H. (2017). Salmonella Typhimurium, the major causative agent of foodborne illness inactivated by a phage lysis system provides effective protection against lethal challenge by induction of robust cell-mediated immune responses and activation of dendritic cells. Veterinary Research, 48(1), 66. https://doi.org/10.1186/S13567-017-0474-X
[24]Tao, X., Cai, Y., Liu, T., Long, Z., Huang, L., Deng, X., Zhao, Q., & Zhao, M. (2019). Effects of pretreatments on the structure and functional properties of okara protein. Food Hydrocolloids, 90, 394–402. https://doi.org/10.1016/J.FOODHYD.2018.12.028
[25]Hudzicki, J. (2012). Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society For Microbiology, December 2009, 1–13. https://www.asm.org/Protocols/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Pro
[26]Oladapo, A. S., Akinyosoye, F. A., & Abiodun, O. A. (2014). The inhibitory effect of different chemical food preservatives on the growth of selected food borne pathogenic bacteria. African Journal of Microbiology Research, 8(14), 1510–1515. https://doi.org/10.5897/ajmr2013.6370
[27]Qin, P., Wang, T., & Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. Journal of Agriculture and Food Research, 7, 100265. https://doi.org/10.1016/J.JAFR.2021.100265
[28]Mok, W. K., Tan, Y. X., Lee, J., Kim, J., & Chen, W. N. (2019). A metabolomic approach to understand the solid-state fermentation of okara using Bacillus subtilis WX-17 for enhanced nutritional profile. AMB Express, 9(1). https://doi.org/10.1186/S13568-019-0786-5
[29]Aritonang, S. N., Roza, E., Rossi, E., Purwati, E., & Husmaini. (2017). Isolation and identification of lactic acid bacteria from okara and evaluation of their potential as candidate probiotics. Pakistan Journal of Nutrition, 16(8), 618–628. https://doi.org/10.3923/pjn.2017.618.628
[30]Gudisa, A., & Yenasew, A. (2022). Isolation, Identification, and Biochemical Characterization of Lactic Acid Bacteria from Okara. Food Science and Quality Management, August. https://doi.org/10.7176/fsqm/117-03
[31]Chatterjee, C., Gleddie, S., & Xiao, C. W. (2018). Soybean Bioactive Peptides and Their Functional Properties. Nutrients, 10(9). https://doi.org/10.3390/NU10091211
[32]Heymich, M. L., Friedlein, U., Trollmann, M., Schwaiger, K., Böckmann, R. A., & Pischetsrieder, M. (2021). Generation of antimicrobial peptides Leg1 and Leg2 from chickpea storage protein, active against food spoilage bacteria and foodborne pathogens. Food Chemistry, 347(July 2020). https://doi.org/10.1016/j.foodchem.2020.128917
[33]Hou, L., Shi, Y., Zhai, P., & Le, G. (2007). Inhibition of foodborne pathogens by Hf-1, a novel antibacterial peptide from the larvae of the housefly (Musca domestica) in medium and orange juice. Food Control, 18(11), 1350–1357. https://doi.org/10.1016/J.FOODCONT.2006.03.007
[34]Tang, W., Yuan, H., Zhang, H., Wang, L., Qian, H., & Qi, X. (2015). An antimicrobial peptide screened from casein hydrolyzate by Saccharomyces cerevisiae cell membrane affinity method. Food Control, 50, 413–422. https://doi.org/10.1016/J.FOODCONT.2014.09.030
[35]European Committe on Antimicrobial Susceptibility Testing. (2022). Antimicrobial susceptibility testing EUCAST disk diffusion method. In European Society of Clinical Microbiology and Infectious Deseases (Vol. 0, Issue January, pp. 1–21). www.eucast.org
[36]Hombach, M., Maurer, F. P., Pfiffner, T., Böttger, E. C., & Furrer, R. (2015). Standardization of operator-dependent variables affecting precision and accuracy of the disk diffusion method for antibiotic susceptibility testing. Journal of Clinical Microbiology, 53(12), 3864–3869. https://doi.org/10.1128/JCM.02351-15/ASSET/2F82F73C-4B9F-4A7A-B92B-D5F1EADBBFD9/ASSETS/GRAPHIC/ZJM9990946400001.JPEG
[37]Mercer, D. K., Torres, M. D. T., Duay, S. S., Lovie, E., Simpson, L., von Köckritz-Blickwede, M., de la Fuente-Nunez, C., O'Neil, D. A., & Angeles-Boza, A. M. (2020). Antimicrobial Susceptibility Testing of Antimicrobial Peptides to Better Predict Efficacy. Frontiers in Cellular and Infection Microbiology, 10(July), 1–34. https://doi.org/10.3389/fcimb.2020.00326
[38]Link, H., & Weuster-Botz, D. (2011). Medium Formulation and Development. Comprehensive Biotechnology, 199–213. https://doi.org/10.1016/B978-0-444-64046-8.00074-4
[39]Arulrajah, B., Muhialdin, B. J., Zarei, M., Hasan, H., & Saari, N. (2020). Lacto-fermented Kenaf (Hibiscus cannabinus L.) seed protein as a source of bioactive peptides and their applications as natural preservatives. Food Control, 110(September 2019), 106969. https://doi.org/10.1016/j.foodcont.2019.106969
[40]Lee, J., Kang, H. K., Cheong, H., & Park, Y. (2021). A Novel Antimicrobial Peptides From Pine Needles of Pinus densiflora Sieb. et Zucc. Against Foodborne Bacteria. Frontiers in Microbiology, 12(May). https://doi.org/10.3389/fmicb.2021.662462
[41]Luo, Y., & Song, Y. (2021). Mechanism of Antimicrobial Peptides: Antimicrobial, Anti-Inflammatory and Antibiofilm Activities. International Journal of Molecular Sciences 2021, Vol. 22, Page 11401, 22(21), 11401. https://doi.org/10.3390/IJMS222111401
[42]Zhang, Q. Y., Yan, Z. bin, Meng, Y. M., Hong, X. Y., Shao, G., Ma, J. J., Cheng, X. R., Liu, J., Kang, J., & Fu, C. Y. (2021). Antimicrobial peptides: mechanism of action, activity and clinical potential. Military Medical Research 2021 8:1, 8(1), 1–25. https://doi.org/10.1186/S40779-021-00343-2
[43]LAEMMLI, U. K. (1970). Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. Nature, 227(5259), 680–685. https://doi.org/10.1038/227680a0
[44]Nelson, A. I., Steinberg, M. I., & Wei, L. S. (1976). Illinois Process for Preparation of Soymilk. Journal of Food Science, 41(1), 57–61. https://doi.org/10.1111/j.1365-2621.1976.tb01100.x145–153. https://www.banglajol.info/index.php/CERB/article/view/33809.