INSIGHTS-JOURNAL OF
HEALTH AND REHABILITATION

INSIGHTS-JHR
INSIGHTS-JOURNAL OF HEALTH
AND REHABILITATION

EXPLORING PROTEIN-RICH PLANT SUBSTRATES FOR SINGLE-CELL PROTEIN PRODUCTION

Original Research

Maria Ghani[1], **Tayyaba Asif**[1]*, **Tahmina Maqbool**[2]
[1]Department of Applied Sciences, Faculty of Engineering Sciences and Technology, Hamdard University, Pakistan.
[2]Department of Pharmaceutics, Faculty of Pharmacy, Hamdard University, Pakistan.
Corresponding Author: Tayyaba Asif, Department of Applied Sciences, Faculty of Engineering Sciences and Technology, Hamdard University, Pakistan, Tayyaba.asif@hamdard.edu.pk

Acknowledgement: The authors express sincere gratitude to all laboratory members for their technical assistance and support throughout this study.

Conflict of Interest: None Grant Support & Financial Support: None

ABSTRACT

Background: Protein deficiency remains a global nutritional challenge, particularly in developing countries where malnutrition and food insecurity are prevalent. According to UNICEF, one in five children in Pakistan suffers from acute malnutrition, manifesting as stunted growth, muscular weakness, skin lesions, and hormonal imbalances. With increasing economic instability and reduced accessibility to conventional protein sources, alternative nutrient-rich and low-cost substrates such as *Moringa oleifera*, curry leaves, and pea plant waste are being explored for single-cell protein (SCP) production. SCP, derived from microbial biomass, provides a sustainable protein source capable of addressing dietary deficiencies and supporting public health.

Objective: The study aimed to evaluate the potential of *Moringa oleifera*, curry leaves, and pea plant waste as plant-derived substrates for the production of single-cell protein using *Saccharomyces cerevisiae* as a microbial model.

Methods: Vegetable waste from pea plants and leaf samples of *Moringa oleifera* and curry leaves were collected, shade-dried, powdered, and hydrolyzed using 10% hydrochloric acid. The filtrates were utilized as substrates for yeast fermentation under controlled conditions (pH 5.5; 25–28°C for 8 days). The *S. cerevisiae* culture was centrifuged at 4,000 rpm for 15 minutes at 4°C before protein estimation. Protein concentration was determined using the Bradford assay with Bovine Serum Albumin (2.0 mg/mL) as the standard, and absorbance was measured at 595 nm.

Results: Dried plant substrates showed higher protein concentrations than fresh ones. *Moringa oleifera* exhibited the highest protein yield of approximately 28%, followed by pea plant waste at 25%, while curry leaves recorded 18%. Increasing substrate hydrolysate concentration from 2% to 4% enhanced protein production by 35%. Pea waste and *Moringa oleifera* demonstrated well-defined exponential growth phases, while curry leaves showed prolonged lag phases due to inhibitory phytochemicals.

Conclusion: The findings suggest that *Moringa oleifera* and pea plant waste are effective, sustainable, and low-cost substrates for SCP production, offering potential solutions to protein deficiency and waste management challenges. The study emphasizes the value of optimizing plant-derived substrates to improve microbial protein yield for human and animal nutrition.

Keywords: Amino acids; Malnutrition; *Moringa oleifera*; Protein deficiency; *Saccharomyces cerevisiae*; Single-cell protein; Sustainable nutrition.

INTRODUCTION

Protein deficiency represents one of the most critical nutritional challenges worldwide, particularly in developing regions where access to high-quality dietary proteins remains limited. With the global population increasing exponentially, the demand for conventional animal-based protein sources is placing immense pressure on agricultural and feed industries to sustain food security (1). The imbalance between protein demand and supply has driven researchers to explore alternative, sustainable, and cost-effective protein sources that can bridge this nutritional gap. One promising solution gaining global attention is *Single Cell Protein* (SCP), a microbial-derived protein source that offers both nutritional and environmental advantages. Single Cell Protein refers to the biomass obtained from the cultivation of microorganisms such as bacteria, fungi, yeast, and algae on various organic substrates (2-4). These microorganisms are capable of converting inexpensive and renewable materials—such as agricultural residues, fruit and vegetable waste, and even industrial byproducts—into protein-rich biomass through fermentation processes. SCP can be derived from dead, dried microbial cells or purified protein extracts and is recognized for its high protein content, rich amino acid profile, and essential vitamins and minerals. Owing to these attributes, it serves as an excellent dietary supplement, especially for malnourished populations and in conditions where conventional protein sources are scarce or expensive (5-7). The growing acceptance of microorganisms as a food source is not new; they have long been used in fermentation industries for the production of bread, cheese, yogurt, beer, and soy sauce. However, modern advancements in biotechnology and waste valorization have expanded their role toward sustainable protein generation (8).

Utilizing agro-industrial waste and plant-based substrates not only minimizes environmental waste but also contributes to circular bioeconomy initiatives by converting low-value biomass into high-value nutritional products. Moreover, SCP production can play a pivotal role in reducing the ecological footprint of livestock farming by substituting or supplementing traditional feed ingredients such as soybean meal and fishmeal (9-11). The medical and nutritional significance of SCP lies in its ability to combat protein-energy malnutrition and support balanced nutrition. SCP provides essential amino acids like lysine and methionine, which are often limited in plant-based diets (12). Its inclusion in human diets or animal feeds enhances growth, immune function, and overall health outcomes. In the context of increasing global hunger and environmental degradation, SCP represents a scientifically validated approach to achieving food sustainability and nutritional equity Therefore, this study aims to explore the potential of Single Cell Protein derived from microbial and agro-waste sources as a sustainable, safe, and nutritionally viable alternative to conventional protein supplies. The objective is to evaluate its production, nutritional value, and feasibility for addressing the growing global protein deficiency problem through biotechnological innovation and waste utilization.

METHODS

This experimental study was conducted to evaluate the production of single-cell protein (SCP) using *Saccharomyces cerevisiae* cultivated on plant-derived waste substrates. The study followed a laboratory-based design comprising the collection, preparation, fermentation, and protein estimation phases. Ethical approval for the research protocol was obtained from the Institutional Review Board (IRB) of the hosting institution and all experimental procedures were performed in accordance with biosafety and ethical research standards. Vegetable waste from *Pisum sativum* (pea plant) was collected from the local market, thoroughly washed to remove adhering soil and impurities, and subsequently air-dried under shade to preserve nutrient content. The dried plant material was crushed into fine powder and sieved to ensure uniform particle size for consistent fermentation results. In addition, fresh *Moringa oleifera* and curry leaves were collected from local gardens, washed, shade-dried, and ground into fine powder to be used as supplemental nutrient sources. A commercially available culture of *Saccharomyces cerevisiae* was employed as the microbial source of protein due to its high growth rate, safety for consumption, and established use in food biotechnology. For substrate processing, 40 grams of pea powder were hydrolyzed using 10% hydrochloric acid to break down complex carbohydrates and release accessible nutrients. The mixture was incubated in a water bath at 60°C for one hour to enhance hydrolysis, followed by cooling and filtration through Whatman filter paper. The obtained filtrate served as a carbon and nitrogen source for yeast growth (13,14). Separately, 1 gram each of *Moringa oleifera* and curry leaf powders was homogenized in distilled water using a sterile mortar and pestle.

The homogenates were centrifuged at 4,000 rpm for 10 minutes, and the resulting supernatants were collected for use as nutrient supplements in the fermentation medium. For fermentation, a 100 mL nutrient medium was prepared containing ammonium sulfate (1%), magnesium sulfate heptahydrate (0.1%), monopotassium phosphate (1%), sodium chloride (1%), and calcium chloride (1%). The pH of the medium was adjusted to 5.5 using dilute acid or alkali as required. The inoculum of *S. cerevisiae* was introduced aseptically, and fermentation was carried out under controlled conditions at 25–28°C for eight days. After incubation, the culture was centrifuged at 4,000 rpm for 15 minutes at 4°C to separate the biomass for subsequent protein estimation. Protein quantification was performed using the Bradford method, a colorimetric assay based on the binding of Coomassie Brilliant Blue dye to protein molecules. Bovine Serum Albumin (BSA) at a concentration of 2.0 mg/mL served as the standard. The optical density (OD) of each sample was measured spectrophotometrically at 595 nm to determine the protein concentration. Data were analyzed using standard analytical procedures, and results were expressed in milligrams of protein per milliliter of sample.

RESULTS

The experimental investigation demonstrated that different plant-derived substrates exerted significant effects on the growth and protein yield of *Saccharomyces cerevisiae*. Protein estimation using the Bradford assay revealed that the protein concentration varied substantially among *Moringa oleifera*, pea plant waste, and curry leaves under both fresh and dried conditions. Dried samples consistently showed higher protein concentrations compared to their fresh counterparts. Among the dried samples, *Moringa oleifera* exhibited the highest protein concentration of approximately 28%, followed by pea plant waste with around 25%, and curry leaves with 18%. In contrast, fresh samples of these substrates displayed lower protein yields, with *Moringa oleifera*, pea plant waste, and curry leaves containing approximately 20%, 17%, and 12% protein, respectively. The results indicated that drying plant substrates enhanced protein availability, possibly by reducing moisture content and concentrating nutrients. Pea plant waste hydrolysate yielded the highest protein output among all substrates, highlighting its efficiency in supporting yeast biomass production. The presence of readily fermentable carbohydrates, amino acids, and nitrogenous compounds in peas likely facilitated microbial growth. *Moringa oleifera* also demonstrated considerable potential for single-cell protein production due to its rich composition of amino acids, minerals, and vitamins, while curry leaves exhibited the lowest efficiency, possibly due to the presence of inhibitory secondary metabolites and antimicrobial compounds.

Comparative analysis of substrate efficiency revealed that the protein yield of *S. cerevisiae* improved with increasing substrate concentration. Hydrolysates prepared at 4% concentration produced higher protein content compared to 2%, confirming that substrate optimization directly influenced SCP yield. Additionally, the growth of *S. cerevisiae* varied with substrate composition, showing distinct lag, log, and stationary growth phases. Cultures grown on pea waste displayed a well-defined log phase with robust multiplication, while *Moringa oleifera* supported moderate growth. Curry leaves, however, resulted in a prolonged lag phase and reduced exponential growth, indicating nutrient limitations and inhibitory effects. The control sugar substrate demonstrated the highest growth rate, reflecting the optimal conditions provided by simple carbohydrates. The growth pattern observations suggested that the nutrient availability and chemical composition of each substrate were crucial determinants of yeast performance. Pea waste and *Moringa oleifera* supported consistent and sustainable growth, indicating their suitability for microbial protein production. Curry leaves, despite containing beneficial phytochemicals, were less effective due to their low sugar content and antimicrobial compounds. Overall, the study confirmed that plant-derived substrates, particularly pea waste and *Moringa oleifera*, could serve as efficient and low-cost feedstocks for SCP production. The data supported the hypothesis that substrate type and processing significantly influence protein yield and microbial growth kinetics.

Table 1: Protein Concentration (%) of Fresh and Dried Plant Substrates

Substrate	Protein (%) – Fresh	Protein (%) – Dried
Moringa oleifera	20	28
Pea plant waste	17	25
Curry leaves	12	18

Table 2: Comparative Substrate Efficiency at Different Hydrolysate Concentrations

Hydrolysate Concentration	Relative Protein Yield (%)	Relative Growth Efficiency (%)
2%	100	90
4%	135	125

Table 3: Growth Phases and Efficiency of *Saccharomyces cerevisiae* on Different Substrates

Substrate	Lag Phase (hrs)	Log Phase Duration (hrs)	Stationary Phase (hrs)	Relative Growth (%)
Sugar (Control)	4	24	30	100
Pea waste	6	20	28	85
Moringa oleifera	8	16	26	70
Curry leaves	12	10	22	40

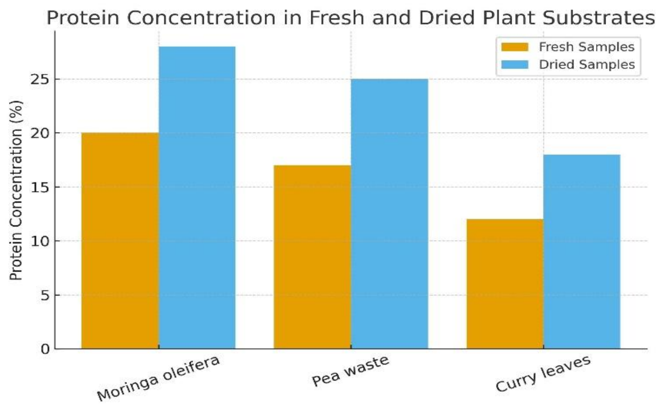

Figure 1 Protein Concentration in Fresh and Dried Plant Substrates

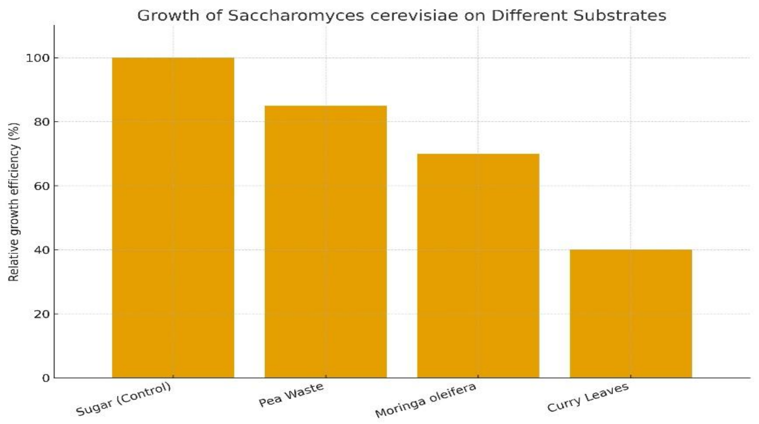

Figure 1 Growth of Saccharomyces cerevisiae on Different Substrates

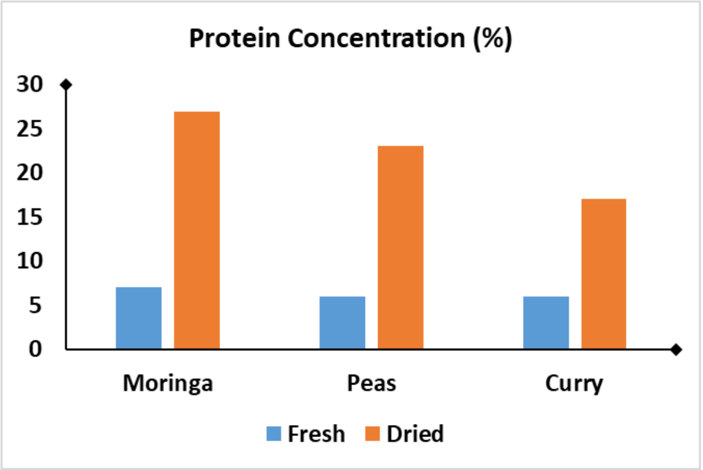

Figure 3 Protein Concentration (%)

DISCUSSION

The findings of this study confirmed that different plant-derived substrates had a substantial impact on the growth performance and protein yield of *Saccharomyces cerevisiae*, supporting the potential of plant-based wastes as low-cost raw materials for single-cell protein (SCP) production. The observation that dried samples exhibited higher protein concentrations than fresh ones aligns with previous studies that emphasized moisture reduction as a critical step for enhancing nutrient density and microbial accessibility. The superior protein yield in dried *Moringa oleifera* (28%) and pea plant waste (25%) suggested that substrate pre-processing improves protein availability and metabolic efficiency during fermentation. This outcome is consistent with earlier reports indicating that plant-derived substrates with higher carbohydrate and nitrogen content promote enhanced microbial biomass formation during yeast fermentation (12-14). Pea plant waste proved to be an efficient substrate, most likely due to its balanced amino acid profile, moderate sugar levels, and nitrogenous compounds that facilitate optimal yeast metabolism. Its growth kinetics showed a well-defined logarithmic phase and consistent stationary phase, indicating a stable nutrient-to-growth relationship. *Moringa oleifera* also demonstrated significant potential, as its nutritional composition—including essential amino acids, vitamins, and minerals—supports microbial protein synthesis. However, the slightly lower growth rate compared to pea waste might be attributed to the presence of phenolic and tannin compounds that can inhibit yeast activity. This observation is supported by earlier findings that secondary metabolites in *Moringa* can restrict microbial proliferation unless subjected to pre-treatment such as blanching or enzymatic degradation (15,16). In contrast, curry leaves exhibited poor substrate efficiency, with low protein yield (18%) and a prolonged lag phase. This can be attributed to their high concentration of antimicrobial alkaloids, essential oils, and phenolics that impede microbial growth. Similar inhibitory effects have been reported in studies utilizing other aromatic plants, suggesting that these natural compounds reduce yeast growth through membrane disruption or enzyme inhibition mechanisms (17). Despite this limitation, the mild protein content of curry leaves still supports their potential for co-fermentation if blended with other nutrient-rich substrates, which could minimize inhibitory effects and improve overall yield.

The study also revealed that increasing the substrate concentration from 2% to 4% resulted in higher protein output, demonstrating the influence of substrate optimization on SCP yield. This aligns with prior experimental observations where elevated substrate concentrations enhanced biomass yield by improving carbon and nitrogen availability (18). However, excessive substrate loading can sometimes lead to substrate inhibition, nutrient diffusion limitations, or pH fluctuations that adversely affect microbial metabolism. Thus, future studies should focus on optimizing substrate concentrations and hydrolysis conditions to balance nutrient availability with microbial tolerance. In terms of growth patterns, the yeast cultures grown on pea waste exhibited a longer and more productive log phase compared to those grown on *Moringa* or curry leaves, confirming the superior fermentable carbohydrate composition of pea waste. The control sugar substrate showed the highest growth rate, validating its role as an ideal carbon source (19,20). The distinct differences in growth dynamics across substrates emphasize that carbon source type and nutrient complexity govern yeast metabolism and SCP yield efficiency. The implications of these findings extend beyond laboratory-scale production. Utilizing plant residues such as pea waste and *Moringa oleifera* for SCP generation offers a sustainable and eco-friendly solution to the global protein deficiency challenge. The integration of agricultural waste management with biotechnological fermentation addresses both environmental and nutritional goals. SCP production through yeast fermentation also offers advantages such as short generation time, high protein yield per biomass unit, and minimal land requirement compared to conventional livestock-based protein production. These advantages make it a promising alternative for addressing malnutrition, especially in resource-constrained regions where animal protein sources are limited or unaffordable (21,22).

One of the notable strengths of this study lies in its application of commonly available agro-wastes as substrates, demonstrating practical feasibility and environmental relevance. The use of *S. cerevisiae* as the microbial strain provided additional advantages due to its established safety, ease of cultivation, and high protein digestibility. The study design effectively compared multiple plant substrates under controlled fermentation conditions, allowing for a clear understanding of substrate-specific effects. However, several limitations were observed. The study lacked quantitative data on dry biomass yield, cell count, and nitrogen assimilation rate, which are critical for determining fermentation efficiency. Additionally, the experiment did not include a detailed compositional analysis of the produced SCP to assess amino acid balance or possible toxin formation. The absence of statistical analysis also limited the ability to draw definitive conclusions about the significance of the observed differences. Future research should incorporate replicative statistical validation, compositional analysis using chromatographic methods, and molecular evaluation of yeast metabolic pathways under varying substrate conditions. Future studies should analyze SCP safety parameters (such as nucleic acid content and potential toxin accumulation) and conduct amino acid profiling to ensure suitability for human consumption (23). Additionally, energy efficiency and cost-effectiveness analyses are recommended to determine the feasibility of industrial-scale implementation. In summary, the study demonstrated that pea plant waste and *Moringa oleifera* represent efficient, nutrient-rich, and sustainable substrates for SCP production, while curry leaves showed limited suitability due to inhibitory phytochemicals. The observed variations in growth patterns and protein yields highlighted the importance of substrate optimization and pre-treatment in microbial protein synthesis. These findings contribute to the growing evidence supporting the valorization of agricultural byproducts as alternative protein sources and provide a foundation for scaling up SCP production for food and feed industries.

CONCLUSION

This study concluded that plant-based substrates such as pea plant waste and *Moringa oleifera* serve as effective, sustainable, and low-cost sources for single-cell protein production using *Saccharomyces cerevisiae*. The findings emphasized that these substrates provide essential nutrients that enhance microbial growth and protein synthesis, offering a promising alternative to conventional protein sources. The approach not only addresses global nutritional challenges but also contributes to waste valorization by converting agricultural residues into valuable protein biomass. The study reinforces the potential of microbial biotechnology in achieving food security and sustainable agro-waste management, while highlighting the need for future research to optimize fermentation conditions and expand industrial applications of single-cell protein for human and animal nutrition.

AUTHOR CONTRIBUTION

Author	Contribution
Maria Ghani	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Tayyaba Asif*	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Tahmina Maqbool	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published

REFERENCES

1. MANNA TK. UTILIZATION OF FERMENTED MORINGA OLEIFERA LEAF MEAL IN THE DIET OF LABEO ROHITA (HAMILTON, 1822) FINGERLINGS: CENTRAL INSTITUTE OF FISHERIES EDUCATION; 2020.

2. El-Badawi A, Hassan A, Yacout M, Khalel M, EL Naggar S. To what extent dry moringa oleifera leaves supplementation couldinfluence growth performance of sheep and affect methane production. Egyptian Journal of Nutrition and Feeds. 2022;25(3):269-81.

3. Totakul P, Viennasay B, Sommai S, Matra M, Infascelli F, Wanapat M. Supplemental effect of Chaya (Cnidoscolus aconitifolius) leaf pellet on rumen fermentation, nutrients digestibility and microbial protein synthesis in growing crossbred bulls. Italian Journal of Animal Science. 2021;20(1):279-87.

4. Wada OZ, Vincent AS, Mackey HR. Single-cell protein production from purple non-sulphur bacteria-based wastewater treatment. Reviews in Environmental Science and Bio/Technology. 2022;21(4):931-56.

5. Suryapratama W, Suhartati FM. Reduction in methane productions from sheep by supplemented of moringa oleifera leaf extract in the ration. Animal Production. 2022;24(3):161-71.

6. Harahap M, Rahayu YP, Lubis MS, Yuniarti R. Produksi Protein Sel Tunggal dari Kultur Saccharomyces cerevisiae dengan Medium Limbah Cair Tahu. Vitalitas Medis: Jurnal Kesehatan dan Kedokteran. 2024;1(2):109-28.

7. Carranza-Méndez R, Chávez-González M, Sepúlveda-Torre L, Aguilar C, Govea-Salas M, Ramos-González R. Production of single cell protein from orange peel residues by Candida utilis. Biocatalysis and agricultural biotechnology. 2022;40:102298.

8. Kiran N, Nadeem M, Manzoor F, Nelofar R, Sarwar A, Ullah N, et al. Potato peel as substrate for Single Cell Protein in animal feed by submerged fermentation. Italian Journal of Food Science. 2024;36(4):57.

9. Uppala R, Muthukumaran A. Management of biodegradable waste through the production of single-cell protein. Cost Effective Technologies for Solid Waste and Wastewater Treatment: Elsevier; 2022. p. 39-49.

10. Direkvandi E, Mohammadabadi T, Salem A. Influence of three microbial feed additives of Megasphaera elsdenii, Saccharomyces cerevisiae and Lactobacillus sp. on ruminal methane and carbon dioxide production, and biofermentation kinetics. Journal of Applied Microbiology. 2021;131(2):623-33.

11. Knorr D, Augustin MA. Expanding our food supply: underutilized resources and resilient processing technologies. Journal of the Science of Food and Agriculture. 2025;105(2):735-46.

12. de Souza Anastácio AS. ENRIQUECIMENTO PROTEICO DA MORINGA PARA SUSTENTABILIDADE AGROPECUÁRIA NO SEMIÁRIDO: Universidade Federal de Campina Grande.

13. Abd El Latif MA. EFFECTIVENESS OF CERTAIN NATURAL FEED SUPPLEMENTS ON PRODUCTIVE PERFORMANCE, NUTRIENTS DIGESTION COEFFCIENT, CARCASS CHARACTERISTICS, AND SOME BLOOD PARAMETERS OF BROILER CHICKS SHORT TITLE: EFFECT OF NATURAL FEED ADDITIVES ON BROILER. Egyptian Poultry Science Journal. 2022;42(1):77-93.

14. Kewan K, Ali M, Ahmed B, El-Kolty SA, Nayel U. The effect of yeast (saccharomyces cerevisae), garlic (allium sativum) and their combination as feed additives in finishing diets on the performance, ruminal fermentation, and immune status of lambs. Egyptian Journal of Nutrition and Feeds. 2021;24(1):55-76.

15. Feng X, Ng K, Ajlouni S, Zhang P, Fang Z. Effect of solid-state fermentation on plant-sourced proteins: A review. Food Reviews International. 2024;40(9):2580-617.

16. Shi H, Yang E, Li Y, Chen X, Zhang J. Effect of solid-state fermentation on nutritional quality of leaf flour of the drumstick tree (Moringa oleifera Lam.). frontiers in bioengineering and biotechnology. 2021;9:626628.

17. Alarape OL, Migwi PK, Ondiek JO. Effect of replacing alfalfa hay with dried Moringa oleifera leaves on rumen fermentation, rumen microbial protein synthesis and methane production in lactating dairy cows. International Congresses of Turkish Science and Technology Publishing. 2023:276-82.

18. Hashem NM, Hosny NS, El-Desoky NI, Shehata MG. Effect of nanoencapsulated alginate-synbiotic on gut microflora balance, immunity, and growth performance of growing rabbits. Polymers. 2021;13(23):4191.

19. Du W, Xu W, Hu Y, Zhao S, Li F, Song D, et al. Crosslinking of feed nutrients, microbiome and production in ruminants. Frontiers Media SA; 2025. p. 1610490.

20. del Carmen Carranza-Méndez R, Sepúlveda-Torre L, Ramos-González R, Verma DK, Baranwal D, Aguilar CN, et al. Agro-industrial wastes for production of single-cell protein (SCP). Innovations in Fermentation and Phytopharmaceutical Technologies: Elsevier; 2022. p. 381-96.

21. Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022). Single Cell Protein Production Using Different Fruit Waste: A Review. Separations, 9(7), 178. Thiviya, P., Gamage, A., Kapilan, R., Merah, O., & Madhujith, T. (2022). Single Cell Protein Production Using Different Fruit Waste: A Review. Separations, 9(7), 178.

22. Yamaguchi, N. U., Cusioli, L. F., Quesada, H. B., Ferreira, M. E. C., Fagundes-Klen, M. R., Vieira, A. M. S., ... & Bergamasco, R. (2021). A review of Moringa oleifera seeds in water treatment: Trends and future challenges. Process Safety and Environmental Protection, 147, 405-420.

23. Daba, S. D., & Morris, C. F. (2022). Pea proteins: Variation, composition, genetics, and functional properties. Cereal Chemistry, 99(1), 8-20.