Archives of Ecotoxicology, Vol. 4, No. 3, pp. 90-93, 2022

Archives of Ecotoxicology

Journal homepage: https://office.scicell.org/index.php/AE

Novel mechanisms of Ɛ-poly lysine as an antimicrobial agent and its food preservative application

Monica Puttaswamy, Nagashree Nagaraj Rao, Ashwani Sharma*

Department of Biotechnology, RV College of Engineering, Bengaluru-560059. Karnataka, India

Article info
Received 04 October 2022
Revised 13 October 2022
Accepted 14 October 2022
Published online 11 December 2022

Mini Review

Keywords:
Ɛ-poly lysine, Biopolymers,
Natural preservative,
Antimicrobial agent Biodegradable

Abstract

In the present situation, it is very important to know about the awareness of healthy eating habits and the consumers have less time for food preparation. Due to this the research is going on the production of fresh cut fruits. In common fruits will be undergoing several microbial contaminations, by reducing its shelf life period epsilon poly lysine is used as natural preservatives in food production. It is safe for human consumption and biodegradable. This epsilon poly lysine has several characteristics like water soluble, thermo stable and cationic homo-biopolymers between Ɛ-amino groups and α-carboxyl groups of 25-35-L-Lysine residues. This review article addresses the Ɛ- poly lysine as natural preservative, antimicrobial agent, and potential applications of Ɛ-poly lysine.

1. Introduction

Homo poly amino acids has a unique features of repeating units of amino acids. It is a linear synthetic polymer and as four types of homo poly amino acids which is secreted by the microorganisms such as poly (Ɛ- L -lysine), poly (ɣ-glutamic acid), poly (L- diaminopropionic acid) and ploy (ɣ-L-diaminobutanoic acid). Out of all these types, Ɛ-poly lysine is most commonly used for the several applica (**Xu *et al.,* 2016**)

It is estimated that 5 to 13 million deaths are caused by the gastrointestinal tract inflammation due to the impact of food poisoning organisms. Since past few years the question has been raised about the safety of traditional preservatives to reach the consumer demands and food safety, the food industries are circumvallating to natural preservatives Ɛ- poly lysine shows a propitious natural antimicrobial which as wide range of activity against those food poisoning bacteria in vitro, food matrices and food spoilage process (**Hyldgaard *et al.,* 2014**)

1.1 Structure and properties of Ɛ- Poly Lysine

In the archival period Ɛ- poly lysine was expediently discovered by Shima and Sakai which was produced by filamentous fungus of *Streptomyces albulus346* as an extracellular matrix from soil, which has 25 - 35 lysine residues with linkages in the middle of α - carboxyl and Ɛ- amino groups and it is called as dragendroff - positive material. In detail, Ɛ- poly lysine obtained by the polymerization of lysine monomer, which is the most reviewed poly-amino acids. This poly lysine has classified in two categories α- poly lysine and Ɛ- poly lysine. It is synthesised artificially by 50 lysine residues which has been linked by the - carboxyl and Ɛ - amino groups. Due to its steep toxicity, it shows limited practical applications. The naturally occurring Ɛ- poly lysine has prominent chemical and biological features with much safety relevance gaining more attention from researchers in various sector. Ɛ- Poly lysine possess many salient features as follows, it is biodegradable, water-soluble substances and used as antibacterial property over the bacterial pathogens which includes both gram positive and gram-negative bacteria. Ɛ- poly lysine also shows antibacterial effects against fungi, yeast, and phage. Ɛ-poly lysine antimicrobial activity is allied to the electrostatic adsorption to its cell surface, which results in the stripping of the external membrane and uneven distribution of the cytoplasm which is finally leading to physiological cell damages. Pharmacokinetic studies revealed that Ɛ- poly lysine is not collocated efficiently in certain organs or tissues analysed. It is observed that Ɛ- poly lysine can be excreted within the duration of 168 hours after administration by demonstrating it is safe to humans. Ɛ- Poly lysine is eco-friendly in nature, based on these research and observations it is analysed that Ɛ- poly lysine and its components have a variety of application in several industries such as food, medicine, and pharmaceuticals and also on electronic products (**Shi *et al.,* 2015**)

1.2 Ɛ- Poly Lysine as a food preservative

For nourishment protection, Ɛ- poly lysine can be utilized alone or in blend with other nourishment added substances, for example, glycine, vinegar, ethanol, what's more, thiamine lauryl sulfonate. Ɛ- poly lysine caused no danger in generation, neurological or immunological capacities, early stage what's more, foetal turn of events, development of posterity, and advancement of incipient organisms or embryos for two ages. A synergistic impact on the hindrance of microbial development was seen when Ɛ- poly lysine and glycine were utilized to safeguard consolidated milk. The synergistic impact brought

*Corresponding author: ashwanisharma@rvce.edu.in
https://doi.org/10.36547/ae.2022.4.3.90-93

about a diminishing in the aggregate sum of additive that should have been added to the nourishment. **Nishikori (2000)** contemplated the combinatorial bactericidal movement of Ɛ-poly lysine and citrus seed extricates with a segment extricated from citrus organic product strips. These bactericidal pieces were seen as viable against numerous disease-causing microbes, for example, *Listeria monocytogenes, E. coli O-157:H7 Salmonella typhimurium, Vibrio cholera, Vibrio parahaemolyticus*, what's more, *S. aureus* could securely be added straightforwardly to nourishments. These palatable bactericidal organizations have been marketed for use in an assortment of utilizations, for example, sterilization of surfaces and articles where the nearness of microbes is suspected. They can be securely utilized in washing dishes, kitchen utensils and restrooms (**Pandey and Kumar, 2014**)

1.3 Applications of Ɛ-Poly Lysine

Generally, Ɛ-poly lysine is almost utilized in food additive method. Which can also use with several food additive to increase its preservative activity against microorganisms. This food preservative is more seen in the Japan country in various food types like boiled rice and other foods. Ɛ-poly lysine can be used as nano particle for biomedical applications, Ɛ-poly lysine can be utilized as drug carriers which can be applied in the treatment of cancer. Gene delivery has discovered for the introducing the genes in to the mammalian cell culture which can be done by using Ɛ-poly lysine as a gene carrier application. To increase the cellular uptake by liposomes of a biocompatible and biodegradable delivery system, in this we can use Ɛ-poly lysine as a liposome. Other applications are used to remove the endotoxin from cell products and also been incorporated in several industries and implemented in suitable reactor are studied for future commercial applications in upcoming days (**Bankar and Singhal, 2013**)

2. Review of Literature

2.1 Microbial synthesis of Ɛ- poly lysine

The enhancement of Shelf life of a Protein containing beverage system, HMP with Ɛ-PL.HCL is used to obtain the poly electrolyte complexes for delivering systems its antimicrobial and physiochemical properties are revealed. It can be implemented in soya milk systems without depleting the antimicrobial activity of Ɛ-PL.HCL by controlling the undesirable precipitation **(Lv *et al.,* 2020)**. The isolation of new strains which are capable of producing Ɛ- poly lysine, have screened Ɛ- poly lysine from the soil by novel methods which contain 3 major steps- 1) Enhancement the culture of Ɛ- poly lysine tolerating strains. 2) Nishikawas's screening methodology. 3) Selecting strain which has high Ɛ- poly lysine tolerant capacity and the results showed that TUST-2 a new strain which was able to produce Ɛ- poly lysine and also its chemotaxonomic and morphological features were typical to the genus *Streptomyces* and undergoes analysis of 16S r-RNA sequence then 1H NMR spectrum, 13C NMR spectrum and MALDI-TOF-MS tests and this novel strain was confirmed as *Streptomyces diastato-chromogenes* TUST-2 (**Jia *et al.*, 2010**)

After the discovery of *Streptomyces* species, there is another novel mechanism used to isolate several streptomycetaceae and filamentous fungi. Kitasatospora was isolated from soli PL6-3 is the strain which was further identified as Kitastatospora species. Under continuous pH control about 4.0 and 120 hours of cultivation in fed batch fermentation the yield of Ɛ- poly lysine from this strain have reached 13.9 g/L during the fermentation process. It was observed that there were no mycelium pellets present in this strain which signifies that PL3-6 in culture broth were different from streptomycetaceae strain. This helpful in the administration of nutrition and secretion of products, it undergoes analysis of SDS-PAGE to the purified Ɛ- poly lysine products from PL3-6 which showed 5.01 kDA by gel permeation chromatography, it indicates 40 lysine residues are composed of PL3-6, generally Ɛ- poly lysine with higher number of lysine shows higher the effect of antimicrobial activity. So this study give the conclusion that PL3-6 strain has higher residues compare to other strains which is potential to use in the field of food preservatives (**Ouyang *et al.,* 2006**). The methods involved in the adsorption and desorption circumstances are single factor test, response surface procedure, orthogonal design. The resin and Ɛ- poly lysine complexes was characterized by SEM AND FTIR, which shows the best Ɛ- poly lysine adsorption performances in D155 resin which is further selected for the separation and purification of Ɛ- poly lysine. D155 resin was optimized by maintaining pH 8.5 and adsorption period was maintained for 14 hours then the optimization of adsorption efficiency of Ɛ- poly lysine was 96.84% and the desorption efficiency was about 97.57%. The overall recovery of Ɛ- poly lysine was 94.49% was obtained under optimal conditions. This D155 resin was analysed as high adsorption and easy desorption ability which also include high selectivity and high stability which can be used for the isolation of Ɛ- poly lysine from fermentation broth (Zhu *et.al* 2016). PLD enzyme is composed of 2 homogenous substances, the Characterization method of PLD enzyme from a species MY5-36 was studied for the purification of enzyme by anion exchange chromatography technique, it includes DEAE-Sepharose, Mono Q and 15 Q, the purification multiple was 500 with an 40.7% recovery rate of enzyme activity was observed, PLD enzyme molecular weight of subunits was 43.6 and holo enzymes was 87.0 kDA which was detected by SDS-PAGE and gel filtration chromatography under the optimal pH 7.0 and temperature 30°C. But then PDL enzyme was treated within 20°C - 40°C it declines rapidly from 50°C to 60°C temperature. The Km value obtained was 0.216 mmol/(L) and Vmax was 0.112 mmol/(L-min). Which shows this enzyme has a behaviour of metalloenzymes which can be activated by Co^{2+} and inhibited by Ca^{2+} (**Feng *et al.,* 2007**).

2.2 Mechanism of Poly Lysine antimicrobial activity and used as natural food preservative

L lysine is used as a food preservative, biosynthesis of Ɛ- poly lysine in the newly isolated strain Bacillus cereus was studied by the Isolation method it is used for the production of Ɛ- poly lysine, optimization of Ɛ- poly lysine by using the most significant media components which affects its production under shake flask conditions. It involves orthogonal array method was used for the determination of maximum consumption rate of several components. The optimized media contains 5% glucose, 1% yeast extract powder 1.5% of ammonium sulphate, 0.004% $ZnSo_4$ and 0.05% $MgSo_4$ which results in the yield of Ɛ- poly lysine from 36.29 mg/L to 83.49 mg/L in basal media. These results 2-fold increase in Ɛ- poly lysine production and further changes can be leads to a potential commercial strain **(Chheda and Vernekar, 2014)**. Synthesis and mechanisms of antimicrobial peptides on nisin. Protein fragments are also called as antimicrobial peptides which are present in the innate immune system and act as host defence molecules. These protein fragments have unique characteristics which can be used as antimicrobial, anti-fungal, antitumor and anti-viral roles. In further incorporation of the antimicrobial peptides in to polymer matrices as delivery system which will be used in medicine, pharmaceuticals and food packaging fields. As consumer demands for natural food products, it increases the challenge in various food industries to explore a novel technique for food preservation methods. Antimicrobial are prominent

approach to prevent spoilage of food and to increase its shelf life and food safety (**Santos *et al*., 2018**) The antimicrobial activity and Synthesis of GML - (glycerol mono-laurate) was done by the Esterification of glycerol and lauric acid under the condition of 120°C - 140°C within 7 hours by using zeolite Y as heterogeneous catalyst. In the absence of catalyst dealumination the acid conversion was 78% and with GML contained in sample was 38.6% which is obtained at 140°C. The molar ration of glycerol and lauric acid was 8%, by using delaminated catalyst acid converts reaches to 98% and which GML in the sample was 50.4% this examines that GML has antimicrobial activity against *Bacillus cereus* and *Sacharomyces aureus* gram positive bacteria (**Setianto *et al*., 2001**). The Ɛ- poly lysine antimicrobial and high temperature stable in nature which helps to study the Ɛ- poly lysine producing strains screening technique, synthesis and production of its antimicrobial activity (**Chheda and Vernekar, 2015**). The edible coating combination of lipids, proteins and polysaccharides are known as biopolymers. Edible coatings are thin layers which are derived from the biopolymers. Its function is to protect a food product from all barriers for storage and distribution. Loss of horticultural products are including the post-harvest spoilage because of an immense moisture content. These coatings on fresh cut fruits and vegetables showed prominent results by extending the shelf life (**Galus, 2019**)

2.3 Ɛ- poly lysine as edible coating and food packaging

Ɛ- poly lysine as natural antimicrobial activity and commonly used as a food preservative. By in vitro Ɛ- poly lysine is capable of inhibiting the germination and elongation of spores tubes and mycelial growth of Botrytis which is also involved in the controlling grey mould in harvested jujube fruit effectively. Ɛ- poly lysine acts against B. Cinerea which involves - 1) Stimulation of ROS (reactive oxygen species) by lowering the pathogenesis expression which are related to the pathogen genes. 2) Results in the nucleic acids and soluble carbohydrates leaking, plasma membrane damage of the fungal cells. 3) RBOH (respiratory oxidase homology) genes induction in Ɛ- poly lysine treated fruit (**Li *et al.,* 2019**). PG-PL are used for the active food packaging materials against the Bacillus cereus and Escherichia coli which causes food borne diseases. The main aim is to develop a modified poly glutamic and poly lysine films using 0,2,4 and 6 % of poly lysine contents which act as a microbioreactor for growing and protecting GABA (gamma amino butyric acid) producing bacterial isolates. FTIR is used for the addition of poly lysine groups between the poly glutamic acid. SEM showed the huge differences in the microstructure of poly glutamic acid films by poly lysine. Thickness, opacity, moisture content witness index etc at break it has increased with its poly lysine content which simultaneously reduced in the tensile strength and total colour difference (ΔE). The conversion from yellowish-blueness to reddish-greenness showed independent value of poly glutamic acid-poly lysine from the poly lysine content. This PG-PL film can be used as edible coating with more antimicrobial properties against pathogens without any harmful effects on probiotic viability (**Karimi *et al.,* 2020**). Nissin, Ɛ- poly lysine and natamycin are known as biological preservatives. It is analysed that ion exchange chromatography and solid liquid separations are the two key unit operation which is used to propose for the Ɛ- poly lysine separation and purification from the fermentation broth. Under the pH 1.5 80 mg/L of sodium polyacralyte is flocculated by adding in to the filtration for fermentation broth and solid liquid separation was achieved. Optimization of Amberlite IRC-50 resin by optimal conditions like 15 g/L initial poly lysine concentration, pH 8.5, adsorption flow rate was 1.5 (BV)/h and 2.5 M HCL, desorption flow rate was 7.0 (BV)/h. This confirmed that the extract of poly lysine was attained 90.2% purity and 75% of recover. This was the first report to show the high concentration of Ɛ- poly lysine from the separation and purification from fermentation broth, which gives the guidance to the industry (**Chen *et al.,* 2016**). Nano biopolymer is also used as the edible coating for food preservative techniques, with the high ratio of solvent mixture of nanocomposite's active agent was incorporated with the PHBV-CS (poly (3-hydroxy butyrateco-3-hydroxyvalerate)-Chitosan) by ultra-sonication in the absence of coupling agent use to produce the new degradable biopolymer nanocomposite by method called solvent casting process which increases the antimicrobial and mechanical properties to enhance the shelf life of poultry materials. ZnO-Ag NCs is a nano active agent used in the safety packaging of the preserved foods to avoid spoilage of the food. Various characterization techniques are conducted for nanocrystals, degradable biopolymer nanocomposite used for the sensory evaluation test for chicken breast which was the kept in refrigerator for 15 days and it showed good antimicrobial activity which offers the traditional petrochemical-based polymers replacement currently used for food packaging of poultry material (**Zare *et al.,* 2019**).

In worldwide ready to eat foods are consumed and minimally processed fresh cut fruits and vegetables has the higher demands which is increasing day by day, so there is need to bring the novel techniques and mechanisms to preserve these fruits by providing safe food products for consumers. Usually microbial degradation, ethylene production, enzymatic browning, respiration rate are major factors which is responsible for the spoilage of the fresh-cut fruits due to its external surface is exposed to the environment which results in reduction of their shelf life and the nutritional quality. Antimicrobial agents and organic acids can be extracted from the plant to preserve them, there are advance ideas have emerged which are better than conventional process which includes aloe vera coating, antibrowning agents and MAP (modified atmosphere packaging) are incorporated in industries. Pulsed light technology, multi layered edible coating, cold plasma technology has considered as the most advanced process, which has been used in food preservatives (**Ergun *et al.,* 2006**). The green technology effects of AEW (acidic electrolysed water) on physiochemical and micro biological features of fresh cut vegetable was investigated by taking fresh cut red cabbages as an example. Salmonella typhimurium DT104 was inoculated with the fresh cut red cabbages then it is washed with distilled water and chlorine with different concentrations of AEW for several times. The treatment of AEW was gradually decreases the populations of yeasts, aerobic bacteria, moulds which were artificially inoculated by Salmonella typhimurium DT104 and it was compared with one treated with distilled water and also red cabbage samples which has not treated, this enhances the AEW treatment effectively. After the treatment of red cabbages by AEW the bacterial species was not found. But the pH, colour and phenolic contents was not varied when the cabbages were washed in distilled water after treated with the AEW which was containing 100 mg/L for 3 minutes. Both antioxidant and anthocyanin contents were decreased by 18.5 % for cyanidin. And 11.2% for 2,2-DPPH (diphenyl-1-picrylhydrazyl) and 22.1% for pelargonidin. The result of nutritional value was limited and considerable. The condition of optimal process of AEW for red cabbage showed 100mg/mL ACC for three minutes which despite results in the inactivation of microflora with minimal loss of nutritional value and antioxidant activity (**Qadri *et al.,* 2015**).

3. Conclusion

Novel uses of Ɛ- poly lysine has been created because of its properties, for example, water solvency, nontoxicity, biodegradability and expansive range antibacterial exercises. It likewise can possibly be a proficient and earth well-disposed

bioremediatory, which will ideally be acknowledged soon. Broad research has been directed, and numerous reports are accessible that unmistakably show that Ɛ- poly lysine is an entirely significant homo-biopolymer with different applications in the nourishment business, medication, farming, hardware, and bioremediation. Notwithstanding all these potential applications, broad research, and huge scope business creation are definitely not at the necessary level. Investigations of the atomic component of biosynthesis and metabolic building of its administrative pathways are required so that hereditarily and metabolically changed Ɛ- poly lysine makers can be developed and its creation can be upgraded. It is ok for human ingestion and might be utilized more in nourishment protection. The current creation framework for Ɛ- poly lysine ought to be improved, what's more, new creation procedures ought to be intended for its copious supply with the goal that its application can be augmented later. The current data about the known wellsprings of Ɛ- poly lysine and expanded accessibility of Ɛ- poly lysine would widen the extent of its application in the future.

Declaration of interest

The authors report no conflicts of interest.

References

1. Bankar, S. B., & Singhal, R. S. (2013). Panorama of poly-ε-lysine. *RSC advances*, *3* (23), 8586-8603. https://doi.org/10.1039/C3RA22596H
2. Chen, X. S., Gao, Y., Zhen, B., Han, D., Zhang, J. H., & Mao, Z. G. (2016). Separation and purification of ϵ-poly-l-lysine from fermentation broth. *Process Biochemistry*, *51*(1), 134-141. https://doi.org/10.1016/j.procbio.2015.11.010
3. Chheda, A. H., & Vernekar, M. R. (2014). Improved production of natural food preservative ε-poly-l-lysine using a novel producer Bacillus *cereus. Food Bioscience, 7*, 56-63. https://doi.org/10.1016/j.fbio.2014.05.005
4. Chheda, A. H., & Vernekar, M. R. (2015). A natural preservative ε-poly-L-lysine: ermentative production and applications in food industry. *International Food Research Journal, 22*(1).
5. Ergun, M., Huber, D. J., Jeong, J., & Bartz, J. A. (2006). Extended shelf life and quality of fresh-cut papaya derived from ripe fruit treated with the ethylene antagonist 1-methylcyclopropene. *Journal of the American Society for Horticultural Science*, *131*(1), 97-103. https://doi.org/10.21273/JASHS.131.1.97
6. Feng, X. H., Xu, X. Y., Yao, Z., & Xu, H. (2007). Purification and Properties of epsilon-Poly-L-lysine-degrading Enzyme from Kitasatospora sp. MY5-36. *Chinese journal of process engineering, 7*(6), 1211.
7. Galus, S. (2019). Development of Edible Coatings in the Preservation of Fruits and Vegetables. In *Polymers for Agri-Food Applications* (pp. 377-390). Springer, Cham.
8. Hyldgaard, M., Mygind, T., Vad, B. S., Stenvang, M., Otzen, D. E., & Meyer, R. L. (2014). The antimicrobial mechanism of action of epsilon-poly-l-lysine. *Appl. Environ. Microbiol., 80*(24), 7758-7770. https://doi.org/10.1128/AEM.02204-14
9. Jia, S., Xu, C., Tan, Z., Cao, W., Ou, H., He, X., & Deng, Z. (2010). Isolation and characterization of a new epsilon-poly-L-lysine-producing strain TUST-2. *Acta microbiologica Sinica, 50*(2), 191-196.
10. Karimi, M., Yazdi, F. T., Mortazavi, S. A., Shahabi-Ghahfarrokhi, I., & Chamani, J. (2020). Development of active antimicrobial poly (L-glutamic) acid-poly (l-lysine) packaging material to protect probiotic bacterium. *Polymer Testing*, 106338. https://doi.org/10.1016/j.polymertesting.2020.106338
11. Li, H., He, C., Li, G., Zhang, Z., Li, B., & Tian, S. (2019). The modes of action of epsilon-polylysine (ε-PL) against Botrytis cinerea in jujube fruit. *Postharvest biology and technology*, *147*, 1-9. https://doi.org/10.1016/j.postharvbio.2018.08.009
12. Lv, J. M., Meng, Y. C., Shi, Y. G., Li, Y. H., Chen, J., & Sheng, F. (2020). Properties of epsilon-polylysine· HCl/high-methoxyl pectin polyelectrolyte complexes and their commercial application. *Journal of Food Processing and Preservation, 44*(2), e14320. https://doi.org/10.1111/jfpp.14320
13. Ouyang, J., Xu, H., Li, S., Zhu, H., Chen, W., Zhou, J., ... & Ouyang, P. (2006). Production of ϵ-poly-L-lysine by newly isolated Kitasatospora sp. PL6-3. *Biotechnology Journal: Healthcare Nutrition Technology, 1*(12), 1459-1463. https://doi.org/10.1002/biot.200600079
14. Pandey, A. K., & Kumar, A. (2014). Improved microbial biosynthesis strategies and multifarious applications of the natural biopolymer epsilon-poly-l-lysine. *Process Biochemistry, 49*(3), 496-505. https://doi.org/10.1016/j.procbio.2013.12.009
15. Qadri, O. S., Yousuf, B., & Srivastava, A. K. (2015). Fresh-cut fruits and vegetables: Critical factors influencing microbiology and novel approaches to prevent microbial risks—A review. *Cogent Food & Agriculture*, *1*(1), 1121606. https://doi.org/10.1080/23311932.2015.1121606
16. Santos, J. C., Sousa, R. C., Otoni, C. G., Moraes, A. R., Souza, V. G., Medeiros, E. A., ... & Soares, N. F. (2018). Nisin and other antimicrobial peptides: Production, mechanisms of action, and application in active food packaging. *Innovative Food Science & Emerging Technologies, 48*, 179-194. https://doi.org/10.1016/j.ifset.2018.06.008
17. Setianto, W. B., Wibowo, T. Y., Yohanes, H., Illaningtyas, F., & Anggoro, D. D. (2017, May). Synthesis of glycerol mono-laurate from lauric acid and glycerol for food antibacterial additive. In IOP Conference Series*: Earth and Environmental Science* (Vol. 65, No. 1, p. 012046). IOP Publishing. https://doi.org/10.1088/1755-1315/65/1/012046
18. Shi, C., He, Y., Feng, X., & Fu, D. (2015). ε-Polylysine and next-generation dendrigraft poly-L-lysine: chemistry, activity, and applications in biopharmaceuticals. *Journal of Biomaterials Science, Polymer Edition, 26*(18), 1343- 1356. https://doi.org/10.1080/09205063.2015.1095023
19. Xu, Z., Xu, Z., Feng, X., Xu, D., Liang, J., & Xu, H. (2016). Recent advances in the biotechnological production of microbial poly (ε-l-lysine) and understanding of its biosynthetic mechanism. *Applied microbiology and biotechnology, 100*(15), 6619- 6630. https://doi.org/10.1007/s00253-016-7677-3
20. Zare, M., Namratha, K., Ilyas, S., Hezam, A., Mathur, S., & Byrappa, K. (2019). Smart Fortified PHBV-CS Biopolymer with ZnO–Ag Nanocomposites for Enhanced Shelf Life of Food Packaging. *ACS applied materials & interfaces*, *11*(51), 48309-48320. https://doi.org/10.1021/acsami.9b15724
21. Zhu, M., Zhang, Z., Liu, Y., Wang, F., Xia, L., Xia, J., & Guo, H. (2016). Optimization of process parameters for ε-Polylysine production by response surface methods. *International Journal of Polymer Science,* 2016. https://doi.org/10.1155/2016/3785036