# ANIMAL PROTEIN BIOPLASTICS

**Abstract**

Plastic and resulting environmental pollution is the well debated talk of today's scenario. We all are dependent on plastics for daily activities and this is the reason why we continue to use it even after being well acquainted with the environmental threats that it poses. While looking for alternatives to synthetic plastic, bioplastics are the material of the future. The bioplastics are formed from natural materials like polyesters, carbohydrates and proteins among others. These bioplastics are biodegradable hence are not detrimental to the natural ecosystem. This chapter discusses in detail the bioplastics made up of different animal protein sources, their physicochemical properties and their potential applications.

**Keywords:** Bioplastics, protein, biodegradable, biofilms, collagen, keratin

**Authors**

**Anand Shubha**
Department of Botany, Faculty of Science
Dayalbagh Educational Institute
Dayalbagh, Agra, Uttar Pradesh, India
shubhaanand@dei.ac.in

**Gupta Sharmita**
Department of Botany, Faculty of Science
Dayalbagh Educational Institute
Dayalbagh, Agra, Uttar Pradesh, India
sharmitagupta@dei.ac.in

The usage of synthetic plastics is expanding in last few years and our dependence on it for the day to day life cannot be ignored. Synthetic plastics are the material of choice to make daily use products owing to their physiochemical, mechanical properties and their ability to be molded and extruded in a variety of ways. Although of great use, synthetic plastics are the biggest threat to the environment because of their non biodegradable nature. The non biodegradability of plastics makes them the causative agent of environmental pollution and climate change. Plastics are accumulating in water ecosystems as each year millions of tons of plastic waste, including micro and macro debris enters into lakes, ponds and oceans. This debris is life threatening hazard for marine creatures like fish, turtle, and tortoise among many others. Many studies have reported the presence of nano plastics inside the bodies of fishes, shrimps and lobsters. Consuming the contaminated seafood or water also poses serious threat to human health. In fact latest studies by Heather A. Leslie, Amsterdam University in 2022 have shown the presence of plastics such as Polyethylene tetra phthalate, polyethylene, Polystyrene in human blood [1]. Mean quantity of 1.6 µg/ml of plastic particles has been reported by the researchers. An estimated increase of 40% is expected by the year 2025 to Asia Pacific Coral Reefs [2].

Combating the problem of plastic pollution has been sought by various methods including chemical degradation and/ or recycling. Chemical degradation leads to an incomplete degradation of plastics and also results in the release of toxic gases, so it is not a favorable alternative. Other alternates include pyrolysis, catalytic and enzymatic degradation which have their own limitations. In order to tackle the above mentioned issues, plastics can be produced from biopolymers or polyesters that are safer for both environment and mankind. Various sources of biopolymers suitable for the production of bioplastics include polyesters, proteins and carbohydrates. Bioplastics are suitable alternatives as they are biodegradable and have promising mechanical properties.

This chapter will cover the proteins from various animal sources that are currently being explored by researchers for the production of bioplastics. Proteins can serve as an excellent source of various functional groups present in their side chains that could participate in the formation of intermolecular bonds. The steps involved in the preparations of protein based bioplastics include denaturing, cross-linking and plasticization. The above properties make protein a promising candidate for the preparation of bioplastics of the future. (Fig 1.)

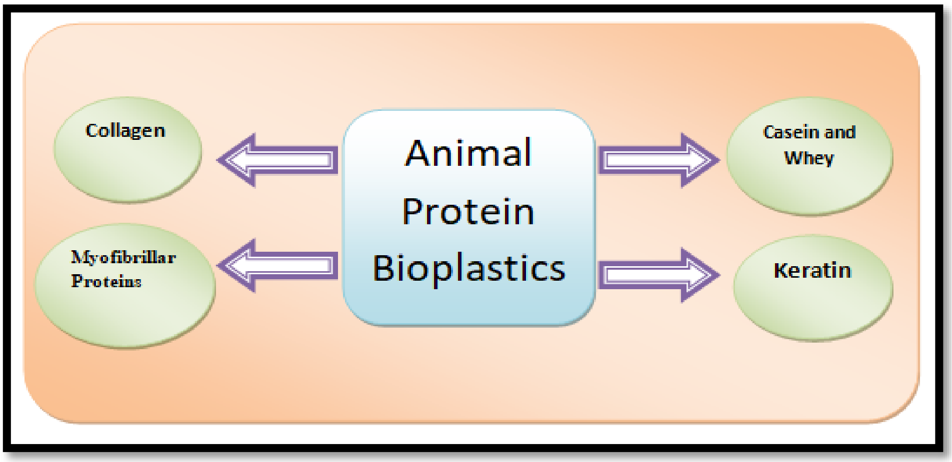


**Figure 1: Animal Protein sources of Bioplastics**

 

1. **Collagen:** Collagen protein is animal protein present in bone, muscle, skin and blood vessels of mammals. It is the most abundant protein in our body representing one-third of the total protein component. In vertebrates, a total of twenty-six different types of collagen made up of forty-six different polypeptide chains are present[3].Collagen has the ability to self assemble under laboratory conditions, in a solution to form aggregates employing the intermolecular attraction forces. Collagen bioplastics have been formed by the process of extrusion alone and in combinations with other material in order to improve the physical properties of the films (Fig. 2).[Table1]

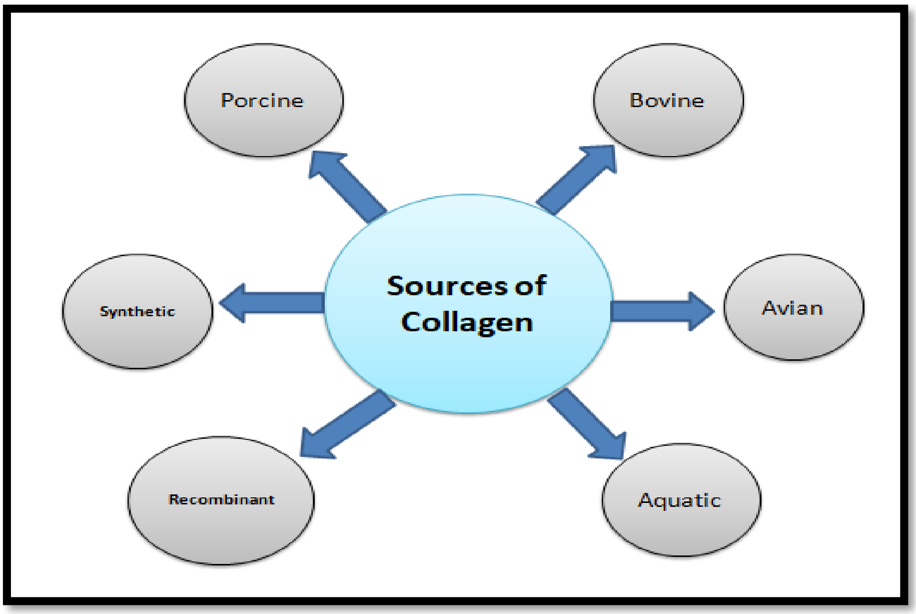


**Figure 2: Sources of Collagen**

**Table 1: Collagen Bioplastics**

| Sl.no | Composition | Properties | Applications | Reference |
|---|---|---|---|---|
| 1 | **Collagen and Starch** | The films composed of starch and collagen were reported to posses' rough surface as compared to the pure collagen films. The former was reported to become smoother upon heat treatment. The authors have reported improved tensile strength then the pure collagen films in both wet and dry state upon heat treatment. Addition of starch also resulted in improved thermal stability. Heating also resulted in increased intermolecular interactions between starch and collagen. | Biodegradable Packaging | [4] |
| 2 | **Transglutaminase crosslinked Collagen** | The researchers reported a decrease in film thickness and increase in thermal stability of the collagen films upon cross linking with TGase at 45 °C and 65 °C. | Biodegradable and Edible packaging | [5] |

| | | | | |
|---|---|---|---|---|
| 3 | **Collagen , Soy Protein and NaCl** | Addition of NaCl resulted in higher tensile strength and elasticity in the films. Addition of soy protein alone decreased gel and film properties whereas soy protein and NaCl together produced better film properties. | Food and Pharmaceutical industry | [6] |
| 4 | **Collagen and Polycaprolact one** | Collagen biopolymeric fibers with PCL depicted increased tensile strength. | Drug delivery, Enzyme immobilization and Advanced Biomedical Applications | [7] |

2. **Myofibrillar Proteins**: These proteins involve actin and myosin components of the skeletal muscle of animals. The Myofibrillar proteins from fish and chicken have been reported for the formation of edible films [8] [9]. The myofibrillar bioplastic films with different combinations are summarized in Table2. The fish waste has also been used for the formation of bioplastics (Fig3)

**Figure 3: Bioplastic from fish waste .Ref [14]**

**Table 2: Myofibrillar Bioplastics**

| Sl. no | Composition | Properties | Applications | Reference |
|---|---|---|---|---|
| 1 | Myofibrillar Proteins and Microbial Transglutaminase | More compact and homogeneous film structure, improved mechanical properties, tensile strength and water vapour permeability. These films exhibited decreased solubility. | Edible Films | [10] [11] |
| 2 | mTGAse modified Fish myofibrillar Protein and Montmorillonite (MMT) nano clay | Improved water gain, water vapor permeability, and solubility of the films are reported by the researchers | Packaging Biofilms, Nanocomposites | [12] |
| 3 | Crayfish Flour with Sodium sulphite/Urea | Increased torque value upon SS and Urea addition | biodegradable plastic materials | [13] |
| 4 | Fish byproduct | Myofibrillar films from fish biproduct with plasticizers (40%) have been reported to posses homogenous, translucid, resistant and flexible properties. These bioplastics exhibit low water solubility and water vapour permeability | Food packaging | [14][15] |
| 5 | Fish byproduct and passion fruit pectin | Optimum films were formed with 5% protein and 3 % pectin. The films exhibited better thermal properties and biodegradability. | Food packaging | [16] |

3. **Milk Proteins:** The major components of milk include: water, fat, protein, lactose and minerals. The proteins present in milk are casein and whey. Protein constitutes approximately 3.5 % bovine milk, with slight variations within different breeds.

   - **Casein:** Casein is present only in milk and is present in the form of calciumcaseinate-phosphate complex. It forms more than 8% of the total protein component of milk and is present in the colloidal state. Casein is composed of three separate fractions viz. α,$^{\beta}$ and $^{\gamma}$ in the form of micelle. The casein isolated from bovine milk has been studied for the formation of bioplastics films as summarized in Table3 (Fig4).

**Figure 4: SEM Images of casein gelatin composite film [19]**

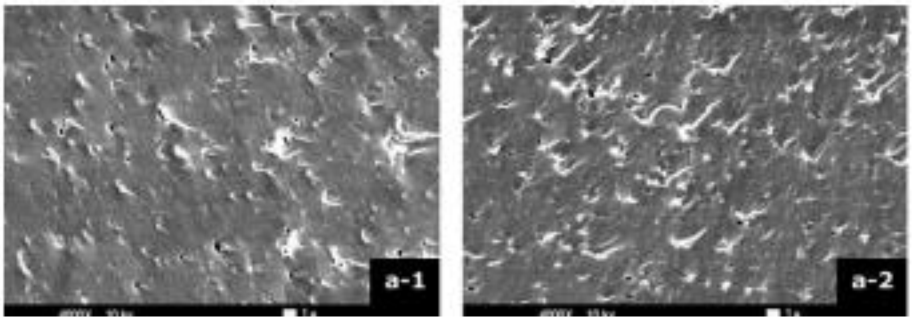


**Table 3: Casein Bioplastics**

| Sl.no | Composition | Properties | Applications | Reference |
|---|---|---|---|---|
| 1 | **Casein Phosphopeptides (CPP) and gelatin films** | Addition of CPP resulted in a decrease in water solubility, water vapour activity and light transmittance of the gelatin films as stated by the authors. The composite films exhibited good tensile properties. Lower concentrations of CPP ( approximately 0.1-0.2%) is reported to be the most suitable. | Food packaging | [17] |
| 2 | **Starch and Casein** | The researchers reported antimicrobial properties against E. coli, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Pseudomonas spp., and Salmonella of starch- casein edible films in the presence or absence of neem. It was also found out that the heat pressing resulted in decrease in water vapour transmissibility rate and slight change in oxygen transmission rate. Heating lead to toughening of the edible film with improved tensile properties. | Breathable films for MAP ( modified atmosphere packaging) | [18] |
| 3 | **Casein and gelatin cross-linked with Transglutaminase** | The authors have reported the formation of casein and gelatin films alone and as blends , modified by transglutaminase. The blend films were reported to have greater elongation values compared to the pure casein or gelatin films. There was no effect on the tensile and water barrier properties of the film in the blends although except casein: gelatin (75:25) formulation which is reported to possess lowest WVPV. (Fig.3) | Edible Films | [19] |
| 4 | **Sodium Caseinate** | Glycerol is reported to be more effective as plasticizer then sorbitol in the sodium caseinate films. Films with 90% sorbitol had comparable tensile properties with 40-50% glycerol. Oleic acid and beeswax have also been reported to have plasticizing effects and improved elasticity, flexibility and stretchability, and reduces water vapor permeability of these films as stated by the authors. | Edible Films | [20], [21] |

 

- **Whey:** β- lactoglobulins and α- lactalbumin constitute the whey proteins or serum proteins of milk. These proteins are present in the colloidal form and can be easily heat coagulated. These proteins have found to possess an excellent film forming properties [23]. Films based on whey proteins have good flexibility, transparency and gas barrier properties (Fig 5). Various whey protein based films that are studied have been summarized in Table4.

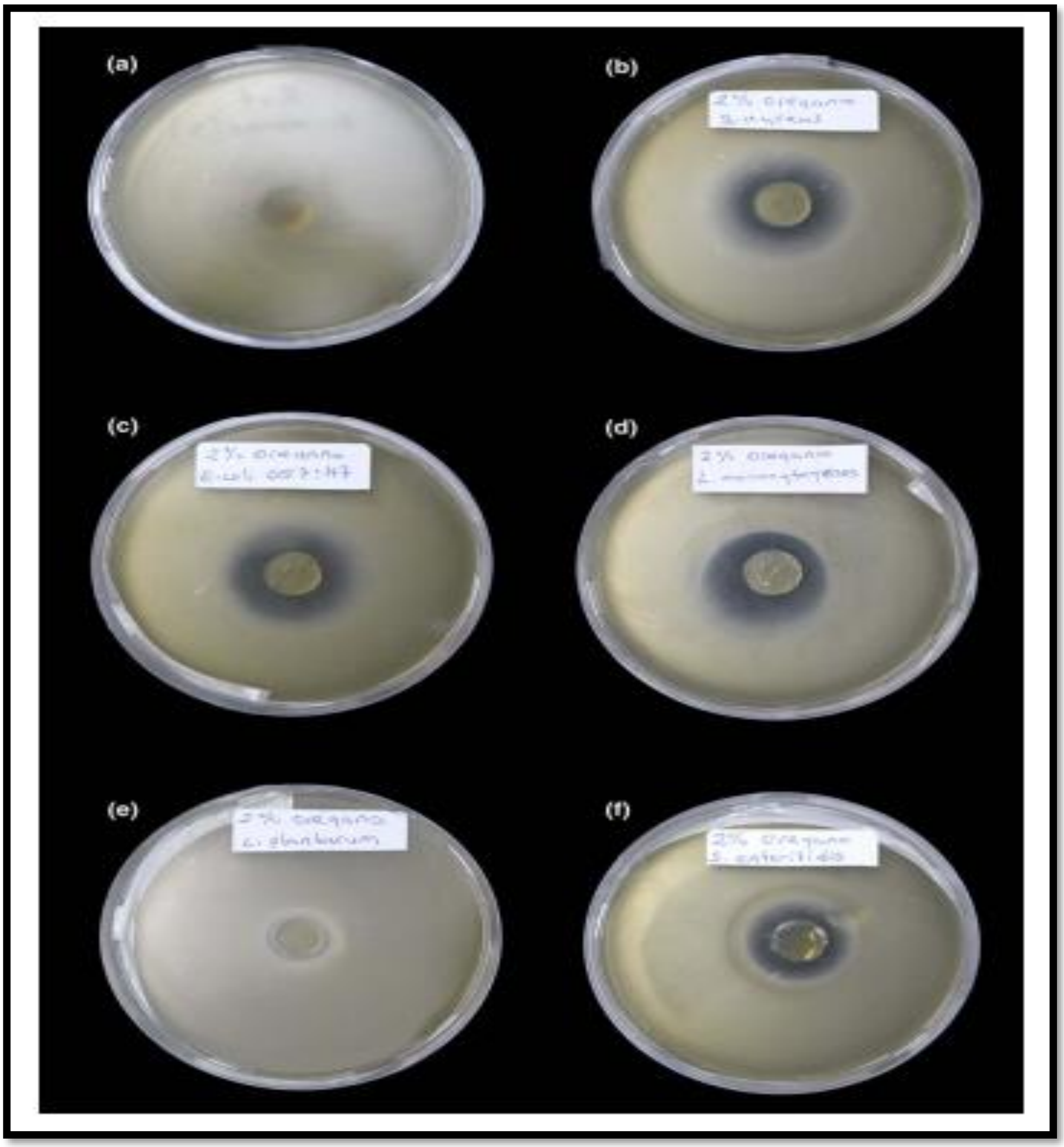


**Figure 5: Inhibitory zones of WP films with 2% oregano essential oils [26]**

**Table 4: Whey Protein Biofilms**

| Sl no. | Composition | Properties | Applications | Reference |
|---|---|---|---|---|
| 1 | **Whey Protein** | Whey Protein films formed by ultrasonic treatment have slightly less water vapour permeability then the films produced by heat treatment. Ultrasound also improved tensile and puncture properties of the films. Although heat treated films are reported to possess better mechanical and tensile properties, thus better suitable for cheese slice separation material. | Cheese Slice Packaging | [22],[23] |
| 2 | **Whey Protein Concentrate (WPC)** | Formation of WPC films by casting in the presence of glycerol and sorbitol has been reported. Film thickness increased from 0.168 to 0.305mm (with glycerol) and from 0.251 to 0.326 mm (with sorbitol) with maximum plasticizer concentrations. | Cheddar Cheese Packaging | [24],[25] |
| 3 | **Whey Protein and Essential Oils** | Antimicrobial properties of oregano, rosemary and garlic essential oils incorporated in whey protein edible films have been reported. Among all these, oregano essential oil is reported to be most effective (at concentration of2%). Although WP films showed poor water barrier properties but it was improved by incorporation of essential oils. | Active Packaging | [26],[27] |
| 4 | **WPI and Pullulan** | WPI and pullulan films in different ratios were formed and it was found that pullulan at low concentrations was effective in improving the properties of Whey Protein films | Food packaging | [28] |
| 5 | **WPI and Gluconal Cal ( mixture of calcium lactate and gluconate/α-tocopheryl acetate (VE)** | Addition of Gluconal Cal and α-tocopheryl acetate resulted in increased film elongation at break | Wrapping or coating to enhance the nutritional value of foods. | [29] |

4. **Keratin:** Keratin is a fibrous protein present in hair, horns, feathers, nails of animals. Human hair is 80% keratin and chicken feather is almost 90% keratin. The keratinous materials are often discarded as waste. The keratin from sheep wool, chicken feather waste and human hair can be used for the production of bioplastics. The Hydrogels, films and fibers produced have been known for various biomedical applications like wound healing, autonomous nerve regeneration, drug delivery systems among others. Table 5 summarizes various bioplastics based on keratin from various sources (Fig6).

 

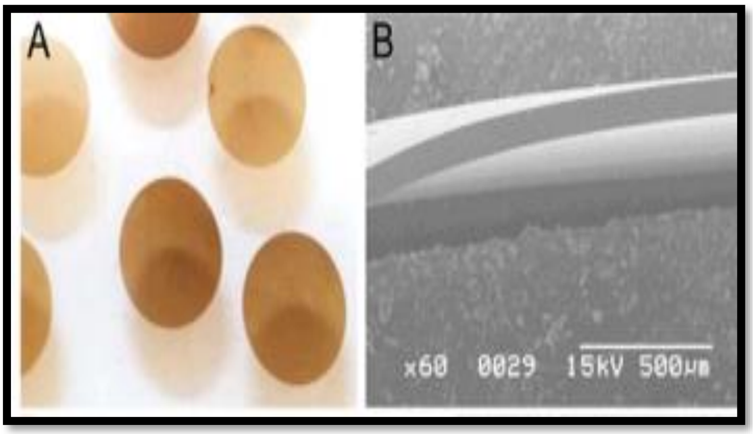


**Figure 6: Keratin Bioplastic and its SEM image [30]**

**Table 5: Keratin Bioplastics**

| Sl no | Composition | Properties | Applications | Reference |
|---|---|---|---|---|
| 1 | **Aqueous and alkaline keratin dialysate** | Glycerol used as plasticizer at 1% concentration followed by curing of the films at 100 °C for 2 hrs. | Epithelial Wound Healing , Ocular Surface reconstruction | [30], [31] |
| 2 | **Keratin Crosslinked with TGase** | Treatment with TGase improved the tensile strength and decreased the elongation break of the keratin films. Authors have also reported an improved film stability in gastric juice and PBS. Although these films showed rougher surface but presence of higher molecular weight proteins as a result of crosslinking is seen. | Tissue Engineering | [32] |
| 3 | **Keratin, chitosan/ gelatin** | Improved Water uptake properties and porosity, thermal and physical stability has been reported by the authors . The scaffolds have shown biocompatibility | Tissue Engineering and wound healing | [33],[34],[35] |
| 4 | **Keratin and Polyvinyl alcohol(PVA)** | Authors have reported formation of nano fibers by electrospinning with higher mechanical strength and thermal stability. | Nano Fibers | [36] |
| 5 | **Keratin hydrogel** | Sheep wool keratin used with glycerol as plasticizer for the generation of hydrogel | Differentiation of odontoblast-like cells for pupal regeneration | [37] |

## CONCLUSION

Owing to the cross linking and biodegradable nature of proteins, these can effectively be used for the preparation of bio based plastics. These plastics have found diverse applications, including food packaging, wrapping, edible films from milk based and collagen proteins. The keratin based biomaterials have found utilization in various biomedical applications. Therefore, to summarize, in order to combat the environmental problems being faced by extensive use of petroleum based plastics, proteins from animal sources can serve as an excellent material for production of bio based plastics.

## REFERENCES

[1] Leslie, H. A., Van Velzen, M. J., Brandsma, S. H., Vethaak, A. D., Garcia-Vallejo, J. J., &Lamoree, M. H. (2022). Discovery and quantification of plastic particle pollution in human blood. Environment international, 163, 107199.
[2] J. B. Lamb, B. L. Willis, E. A. Fiorenza, C. S. Couch, R. Howard, D. N. Rader, J. D. True, L. A. Kelly, A. Ahmad, J. Jompa, and D. Harvell, Science 359, 460 (2018).
[3] Brinckmann J. Collagens at a glance. Top. Curr. Chem. 2005;247:1–-6.
[4] Kun Wang, Wenhang Wang, Ran Ye, Anjun Liu, Jingdong Xiao, Yaowei Liu, Yana Zhao,Mechanical properties and solubility in water of corn starch-collagen composite films: Effect of starch type and concentrations, Food Chemistry, Volume 216, 2017, Pages 209-216.
[5] Wang, K., Wang, W., Ye, R., Liu, A., Xiao, J., Liu, Y., Zhao, Y., Mechanical Properties and Solubility in Water of Corn Starch-Collagen Composite Films: Effect of Starch Type and Concentrations, Food Chemistry (2016)
[6] Anja Maria Oechsle, Tanita Julia Bugbee, Monika Gibis, Reinhard Kohlus, Jochen Weiss, Modification of extruded chicken collagen films by addition of co-gelling protein and sodium chloride, Journal of Food Engineering, Volume 207, 2017, Pages 46-55.
[7] DamodarDhakal, PisutKoomsap, Anita Lamichhane, Muhammad Bilal Sadiq, Anil Kumar Anal, Optimization of collagen extraction from chicken feet by papain hydrolysis and synthesis of chicken feet collagen based biopolymeric fibres, Food Bioscience, Volume 23, 2018, Pages 23-30.
[8] Shiku, Y.; Hamaguchi, P.Y.; Tanaka, M. Effect of pH on the preparation of edible films based on fish myofibrillar proteins. Fish. Sci. 2003, 69, 1026–1032
[9] Cercel, F.; Stroiu, M.; Alexe, P.; IaniGchi, D. Characterization of myofibrillar chicken breast proteins for obtain ˙ protein films and biodegradable coating generation. Agric. Agric. Sci. Proced. 2015, 6, 197–205.
[10] Yayli, D.; Turhan, S.; Saricaoglu, F.T. Edible packaging film derived from mechanically deboned chicken meat proteins: Effect of transglutaminase on physicochemical properties. Korean J. Food Sci. Anim. Resour. 2017, 37, 635–645.
[11] Kaewprachu, P.; Osako, K.; Tongdeesoontorn, W.; Rawdkuen, S. The effects of microbial transglutaminase on the properties of fish myofibrillar protein film. Food Packag. Shelf Life 2017, 12, 91–99.
[12] Rostamzad, H.; Paighambari, S.Y.; Shabanpour, B.; Ojagh, S.M.; Mousavi, S.M. Improvement of fish protein film with nanoclay and transglutaminase for food packaging. Food Packag. Shelf Life 2016, 7, 1–7.
[13] Felix, M.; Perez-Puyana, V.; Romero, A.; Guerrero, A. (2017). Development of protein-based bioplastics modified with different additives. Journal of Applied Polymer Science, 134(42), 45430.
[14] C.S. Araújo, A.M.C. Rodrigues, M.R.S. Peixoto Joele, E.A.F. Araújo, L.F.H. Lourenço, Optimizing process parameters to obtain a bioplastic using proteins from fish byproducts through the response surface methodology, Food Packaging and Shelf Life, Volume 16, 2018, Pages 23-30.

 

[15] Neves, E. M. P. X., Pereira, R. R., da Silva Pereira, G. V., da Silva Pereira, G. V., Vieira, L. L., &Lourenço, L. D. F. H. (2019). Effect of polymer mixture on bioplastic development from fish waste. *Boletim do Instituto de Pesca*, *45*(4).
[16] Florentino, G. I. B., Lima, D. A. S., Santos, M. M. F., da Silva Ferreira, V. C., Grisi, C. V. B., Madruga, M. S., & da Silva, F. A. P. (2022). Characterization of a new food packaging material based on fish by-product proteins and passion fruit pectin. *Food Packaging and Shelf Life*, *33*, 100920.
[17] Khedri, S., Sadeghi, E., Rouhi, M., Delshadian, Z., Mortazavian, A. M., de Toledo Guimarães, J., & Mohammadi, R. (2021). Bioactive edible films: Development and characterization of gelatin edible films incorporated with casein phosphopeptides. *LWT*, *138*, 110649.
[18] Jagannath, J. H., Radhika, M., Nanjappa, C., Murali, H. S., &Bawa, A. S. (2006). Antimicrobial, mechanical, barrier, and thermal properties of starch–casein based, Neem (Melia azardirachta) extract containing film. *Journal of Applied Polymer Science*, *101*(6), 3948-3954.
[19] Chambi, H., & Grosso, C. (2006). Edible films produced with gelatin and casein cross-linked with transglutaminase. Food research international, 39(4), 458-466.
[20] Fabra, M. J., Talens, P., &Chiralt, A. (2008). Tensile properties and water vapor permeability of sodium caseinate films containing oleic acid–beeswax mixtures. *Journal of Food Engineering*, *85*(3), 393-400.
[21] Chick, J., & Hernandez, R. J. (2002). Physical, thermal, and barrier characterization of casein-wax-based edible films. Journal of food science, 67(3), 1073-1079.
[22] Cruz-Diaz, K., Cobos, Á., Fernández-Valle, M. E., Díaz, O., &Cambero, M. I. (2019). Characterization of edible films from whey proteins treated with heat, ultrasounds and/or transglutaminase. Application in cheese slices packaging. Food Packaging and Shelf Life, 22, 100397.
[23] McHUGH, T. H., Aujard, J. F., &Krochta, J. M. (1994). Plasticized whey protein edible films: water vapor permeability properties. Journal of food science, 59(2), 416- 419.
[24] Wagh, Y.R., Pushpadass, H.A., Emerald, F.M.E. et al. Preparation and characterization of milk protein films and their application for packaging of Cheddar cheese. J Food Sci Technol 51, 3767–3775 (2014).
[25] Coupland, J. N., Shaw, N. B., Monahan, F. J., O'Riordan, E. D., & O'Sullivan, M. (2000). Modeling the effect of glycerol on the moisture sorption behavior of whey protein edible films. Journal of food engineering, 43(1), 25-30.
[26] Seydim, A. C., &Sarikus, G. (2006). Antimicrobial activity of whey protein based edible films incorporated with oregano, rosemary and garlic essential oils. Food research international, 39(5), 639-644.
[27] Çakmak, H., Özselek, Y., Turan, O. Y., Firatligil, E., &Güler, F. K. (2020). Whey protein isolate edible films incorporated with essential oils: Antimicrobial activity and barrier properties. Polymer Degradation and Stability, 109285.
[28] Gounga, M. E., Xu, S. Y., & Wang, Z. (2007). Whey protein isolate-based edible films as affected by protein concentration, glycerol ratio and pullulan addition in film formation. Journal of Food Engineering, 83(4), 521-530.
[29] Mei, Y., & Zhao, Y. (2003). Barrier and mechanical properties of milk protein-based edible films containing nutraceuticals. Journal of agricultural and food chemistry, 51(7), 1914-1918.
[30] Y. Feng, M. Borrelli, T. Meyer-ter-Vehn, S. Reichl, S. Schrader, G. Geerling, Epithelial wound healing on keratin film, amniotic membrane and polystyrene in vitro, Curr. Eye Res., 39 (6) (2014), pp. 561-570.
[31] M. Borrelli, N. Joepen, S. Reichl, D. Finis, M. Schoppe, G. Geerling, et al., Keratin films for ocular surface reconstruction: evaluation of biocompatibility in an in-vivo model, Biomaterials, 42 (2015), pp. 112-120.
[32] [32] L. Cui, J. Gong, X. Fan, P. Wang, Q. Wang, Y. Qiu, Transglutaminase-modified wool keratin film and its potential application in tissue engineering, Eng. Life Sci., 13 (2) (2013), pp. 149-155.

[33] Kakkar, P., Verma, S., Manjubala, I., & Madhan, B. (2014). Development of keratin–chitosan–gelatin composite scaffold for soft tissue engineering. Materials Science and Engineering: C, 45, 343-347.
[34] S. Singaravelu, G. Ramanathan, M. Raja, S. Barge, U.T. Sivagnanam, Preparation and characterization of keratin-based biosheet from bovine horn waste as wound dressing material, Mater. Lett., 152 (2015), pp. 90-93.
[35] C.E. Tanase, I. Spiridon, PLA/chitosan/keratin composites for biomedical applications, Mater. Sci. Eng. C, 40 (2014), pp. 242-247.
[36] J. Choi, G. Panthi, Y. Liu, J. Kim, S.-H. Chae, C. Lee, et al., Keratin/poly (vinyl alcohol) blended nanofibers with high optical transmittance, Polymer, 58 (2015), pp. 146-152.
[37] L. Ajay Sharma, M. Ali, R. Love, M. Wilson, G. Dias, Novel keratin preparation supports growth and differentiation of odontoblast-like cells, Int. Endod. J., 49 (5) (2016), pp. 471-482.

 