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Title:	**Gelatin Extracted from Rahu (*Labeo rohita*) and Silver Carp (*Hypophthalmichthys molitrix*) Scales and their Beads Efficacy as a Carrier of Inoculum and Secondary Metabolites**
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Gelatin Extracted from Rahu (*Labeo rohita*) and Silver Carp (*Hypophthalmichthys molitrix*) Scales and Their Beads Efficacy as A Carrier of Inoculum and Secondary Metabolites

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Abstract

Nowadays biological waste is increasing due to over population. At the same time, researchers are trying to use biological wastes in effective ways and trying to convert these wastes into useful products. In this study the scales of rohu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) were used for the extraction of gelatin. Chemical extraction method was mediated in the presence and absence of microbes to obtain gelatin from varying amounts of scales. Microbial isolates SCC5 from Silver carp and SEY from *Labeo rohita* were used for microbial extraction. Beads were prepared from extracted gelatin using three different types of oils and later used as a carrier of inoculum. Results showed that both isolates were positive for gelatin liquefaction and did not result in solidification even at low temperature when kept in the refrigerator. The amount of extracted gelatin from fish scales was less than 50 %. A pronounced increase was recorded when more than 30 gram of fish scales were used for extraction. It was noticed that beads prepared with natural gelatin were remained stable after washing and not degraded as compared to commercial gelatin. FTIR analysis of extracted gelatin showed the presence of peaks at 700, 1600 and 3400 nm range that were in line with peak trends of commercial gelatin. Beads were coated with antibiotic by soaking in the antibiotic dilution. From the zones formation of beads supplemented with the antibiotics it was observed that zone diameter increased as amount of antibiotic increased it means the beads have the capacity to carry the antibiotics. Moreover, to check the efficacy of these beads, plant growth experiments were performed and beads of extracted gelatin were injected with the different inoculum of bacterial isolates. It was concluded that efficacy of gelatin beads can also be used as carrier of fertilizers in agriculture, which is a recent advancement in the field of agriculture.

1. Introduction

Pakistan is a farming country having rich possessions of water. Major sources of aqua-farming are marine and freshwater [1]. From aqua-farming, major contributions are from the fisheries sector because it plays a significant role in the economy of Pakistan. Moreover, due to the increasing population, Pakistan is importing fish from Burma, Thailand, and China which is causing an increase in the prices of fish [2]. The poultry industry has shown tremendous progress over the last few years and become a source of employment in various countries incluing Pakistan [3]. Pakistan is also exporting meat and meat products from the poultry industry to Iran, Afghanistan, and Turkey [4]. Gelatin is a flashy, ordinary and vibrant protein and is capable of being decomposed. Gelatin is produced from different sources like fish scales, animal bones, and skin by collagen fractional hydrolysis [5]. In 1808 the first commercial gelatin was produced in the United States [6]. Gelatin is a bio-degradable and bio-compatible protein and also acts as a bio-polymer [7]. It is produced by the hydrolysis of collagen and consists of three alpha chains which ultimately form the triple helix building [8]. Depending on the collagen, gelatin has different types. Different animal products like cattle bones, goat, and pork skins are common sources of the production of gelatin. Almost 28 different types of collagens have been reported [9] and the main alternate sources are fish and poultry. Gelatin contains different components like Oxygen (O_2) 25.21 %, Nitrogen (N_2) 17.0 %, Hydrogen (H_2) 6.81 %, and Carbon (C) 50.52 %. It is a consequent of collagen which contains a high level of hydroxyproline and proline the cyclic amino acids. Three polypeptides chains which are helical are present in collagen [10]. The brownish colour of gelatin is due to Maillard reactions (reaction with sugars) in the process of gelatin extraction due to which some properties of the gel change [11].

Gelatin has different physical and chemical properties. It is flashy and fragrance-free, fragile and yellow in colour. The moisture content of gelatin is about 8-12 % and when particles of gelatin are drenched in water, they become swollen elements. When these swollen elements are warmed, they convert into solution form. It is soluble in propylene glycol and glycerol [12]. Gelatin may act as an acid or a base when in a solution meaning it is amphoteric in the nature. The point at which overall charge zero known as IEP (isoelectronic point) [13]. Gelatin is thermally labile molecule, and some bacteria also have ability to degrade the gelatin. But in powder form gelatin is stable and can be stored in containers for many years. Gelatin is the best medium for the growth of bacteria, but for growth, the pH is the most important factor [14]. Earlier in the 19th century, gelatin was used to make pharmaceutical products. This was mostly in the form of soft and elastic capsules of gelatin, hard capsules containing two pieces, coating of tablets and encapsulation [15]. Moreover, gelatin has also been used as a photographic emulsion since 1870 by Doctor Maddox of England [16]. Gelatin is also widely used for the purpose of coating, for example, to make playing cards, posters, glossy pages of magazines etc. For this purpose, gelatin may be used alone or along with the other materials and it creates a smooth surface [17], also films and coatings of gelatin used to preserve food [18]. Gelatin is also widely used in cosmetics like lotions, creams and most commonly in hair preparations [19]. Gelatin, which we obtain from mammalian sources, has many issues related to health

because it may cause some allergic reactions and cause other diseases [20]. Therefore, fish scales and poultry wastes are considered Halal and safer sources alternate than mammalian sources [21].

Mostly fish bones and scales are preferred for the extraction of gelatin because fish bones and scales produce a great amount of gelation having proline amino acid present abundantly than the skin of fish [22]. For the extraction of gelatin from fish skin, one of the conventional methods is used called CWB (conventional water bath) [23], these days an advanced method is used for the extraction of gelatin from fish without affecting the quality and reducing the time, which is ultrasound treatment [24]. Another method most commonly used is the chemical extraction method in which fish scales are treated with different chemicals to extract gelatin. This method is also effective and gives better yield than other methods. Moreover, the gelatin extracted from the fish scales rather than fish skin gives an even better yield still [25].

Gelatin in the form of beads also acts as fertilizers to enhance the growth of plants. Factors that bound the construction of agriculture are water and fertilizers. Nowadays the most recent advancement in the field of agriculture is hydrogels of gelatin, poly vinyl alcohol (PVA) and chitosan. These hydrogels have great potential for their use in agriculture like they can be used as a carrier and can also develop resistance in plants and are ultimately used for the protection of crops [26].

2. Materials and Methods

2.1 Collection and Preparation of Scales

The fish scales of rahu fish (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) were collected from the local market of Lahore, Pakistan. These scales were washed with tap water and dried. The dried scales were stored at 4 °C prior to use.

2.2 Chemical Extraction

For the extraction of gelatin from fish scales, the previously reported procedure of [27] were followed. Scales were treated with 5 % solution of NaCl in ratio of 1:10 and stirred for 30 minutes and the same step was repeated twice. In the second step, scales were treated with the 0.4 % solution of NaOH in the ratio of 1:10 for 30 minutes, and the step was also repeated twice. In the next step, scales were treated with a 10 % solution of hydrogen peroxide in a ratio of 1:4 volume by volume and repeated thrice. The next and final step was demineralization where scales were treated with 0.5 N EDTA solution with pH 7.66 for 16 hours in a shaking incubator, and scales were recovered by using sieve to remove the residues. After the filtration step, the scales were saturated in 0.05 M solution of acetic acid for 3 hours. Scales were washed with water and the water was added in ratio of 1:30 and kept at hot air oven for 24 hours. Obtained, dried extract of scales was kept at room temperature and finally ground using a grinder (RRH-250 Shanghai Yuanwo Industrial and Trade Co., Ltd.).

2.3 Gelatin Liquefaction Assay

Bacterial isolates were selected and serially diluted with the samples from fish scales solution up to 10^6 ml^{-1}. Bacterial colonies were purified and further screened for gelatin liquefaction assay following liquefaction assay of dela Cruz and Torres [28]. The nutrient gelatin broth media was prepared in test tubes and after cooling inoculated with bacterial cultures (cell densities adjusted at 0.5 at 600 nm) in each test tube and incubated for one week. During this interval test tubes were transferred to the refrigerator for 15-20

minutes to check the liquefaction of gelatin media. SCC 5 and SY isolates were used for further experiments.

2.4 Microbial Extraction of Gelatin from Fish Scales

For the microbe mediated extraction of gelatin from fish scales, chemical extraction method [27] was followed using bacterial inoculum to improve the efficacy on percentage yield of gelatin. For this extraction, 2 mL bacterial inoculum (cell densities adjusted at 0.5 at 600 nm) of SCC 5 and SY (previously isolated) were added to scales along with the chemicals, and yield of percentage was determined.

2.5 Utilization of Extracted Gelatin for Beads Formation

Gelatin bead formation was done by the method of [29] with some modification, namely that agar-agar was used in place of sodium alginate as previously reported. One gram of agar-agar was dissolved in 100 mL of distilled water, filter sterilized mineral oil (cooking oil 1, cooking oil 2, and Canola oil from sunflowers) was pre-cooled in refrigerator at 20° C for 2-3 hours before use. Then 10 grams of gelatin (commercial as well extracted gelatin) powder was added into the sterilized agar-agar media and dissolved at 40°C. Gelatin-agar-agar solution was added drop wise in pre-cooled, sterilized mineral oil with continuous stirring. Beads were washed using sterile water and dried on sterilized filter paper.

2.6 FTIR Spectrum of Extracted and Commercial Gelatin Beads

For FTIR analysis, samples were sent to Department of Chemical Engineering, University of Punjab, Lahore, Pakistan. FTIR spectra for both commercial and extracted gelatin beads were compared.

2.6.1 Preparation of Beads Containing Antibiotics using Disc Diffusion Assay

Luria broth (LB) agar plates were prepared. Fish pathogens previously isolated [30] Qureshi and Sabri culture was swabbed on each agar plate. In each plate one disc of pure antibiotic in different concentrations (400, 600 and 800 µl) was used as control. Amoxicillin Antibiotic was used to prepare different concentrations, and 30 µg per mL were dissolved into the 1 mL of sterile water. Prepared beads were loaded with different volumes (400, 600 and 800 µl) of concentrations (30 µg per mL) of antibiotic. Amoxicillin Antibiotic was used to make different concentrations, and 30 µg per mL were dissolved into the 1 mL of sterile water. These plates were incubated for 18-24 hours till zones of bacterial growth inhibition around the beads were observed.

2.6.2 Determination of beads efficacy as a carrier of inoculum

Beads were coated with inoculum and their efficacy was observed for plant microbial interaction.

2.6.2.1 Gelatin Beads Coated with Inoculum (Pathogens)

Prepared gelatin beads (prepared from commercial as well as extracted gelatin) were coated with fish pathogen culture using different adjusted cell densities of inoculum (200, 300, and 400 µl). Then beads were air dried and placed on LB-agar plates and incubated for 24 hours.

2.6.2.2 Determination of Beads Efficacy as a Carrier of Inoculum for Plant Growth by Plant Microbe Interaction

For the determination of beads efficacy as a carrier of inoculum for plant growth promotion, plant microbe interaction experiments were performed. The seeds of

wheat *Triticum aestivum Var* (Lasani) were sterilized using 0.1 % $HgCl_2$ solution for 1-2 minutes, followed by repeatedly washing of seeds with autoclaved water 67 times. Then seeds were soaked in autoclaved water for 15-20 minutes to remove the residual $HgCl_2$. Three experimental set ups were prepared with wheat seeds along with the un-inoculated beads as control. In another set inoculated beads (with bacterial culture with 0.5 OD at 600 nm) were used, while in third set, wheat seeds were inoculated under sterile conditions.

3. Results

Gelatin was extracted from fish scales by chemical as well as microbial extraction methods. It was seen that amount of gelatin increased with an increased amount of scales. The amount of extracted gelatin from fish scales was less than 50 % but a pronounced increase was noticed with more than 30 gram of fish scales (Fig. 1). Results showed that the yield of extracted gelatin was enhanced in the presence of microbial inoculum as compared to chemical extraction. The amount of extracted gelatin in the presence of microbial inoculum was greater than 50% even when initial concentration of substrate was less than 30 gram as observed in case of chemical extraction without inoculum (Fig.1).

3.1 Gelatin Liquefaction Assay

For liquefication assay nutrient gelatin media was inoculted with respective fish isolates i.e SEY and SCC5. It was noticed that the media containing the fish isolates were not solidified even when kept in refrigerator for 15-20 minutes which showed fish isolates were degrading the gelatin. This confirmed the gelatin degrading ability of bacterial isolates (Table 1).

Table 1. Comparative Analysis of Yield of Gelatin at Varying Amount of Fish Scales

Chemical mediated of Gelatin %Yield		Microbial mediated of Gelatin % Yield		
Amount of scales (grams)	**Yield (%)**	**Amount of scales (grams)**	**Bacterial Isolates**	**Yield (%)**
10	10.05	5	SCC5	58.00
30	15.56	5	SEY	50.01
40	45.41	-	-	-
60	47.44	-	-	-

3.2 Gelatin Beads Preparation from Commercial Gelatin

Commercial gelatin beads were prepared by using three different types of oils, cooking oil 1, cooking oil 2, sun flowers canola oil. It was noticed beads of commercial gelatin which formed using oil 2 were less stable and most of them were degraded during washing. However, the beads from commercial gelatin using oil 1 were more stable as compared to the beads in sun flowers canola oil. However, beads of fish extracted gelatin were prepared and found stable using all three oils. This showed beads formed from the fish extracted gelatin were more stable as compared to the commercial gelatin. It was

also noticed that beads prepared from extracted gelatin remained stable after washing and were not degraded compared to commercial gelatin.

3.3 FTIR Spectrum of Commercial and Extracted Gelatin

FTIR analysis of fish extracted gelatin was carried out which showed the presence of peaks at 700, 1600 and 3400 nm range and similar peak trend were also observed in the case of commercial gelatin.

The presence of peaks for the commercial gelatin at 3400 cm^{-1} are attributed to the presence of hydrogen bond water and amide-A as a functional group, (Figure 1) while peaks in the range of 1600 cm^{-1} peaks corresponds to the occurrence of amide group (Figure 2).

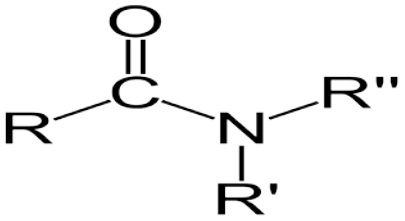

Figure 1. Amide as a Functional Group [31]

While sharp peaks at 700 showed the presence of amide III group. The same trend was recorded for extracted gelatin with the exception that peaks were broader at the expected peaks regions as compared to commercial gelatin (Figure 3).

3.4 Efficacy of Gelatin Beads as Carriers of Antibiotics using Disc Diffusion Assay

In this assay, different concentrations of antibiotics were injected using i.e. 200, 300, 600, and 800 µl for discs as well as for beads prepared from extracted gelatin. Out of these, 4 different concentration zone formations around beads was observed at concentrations of 300 and 600 µl. While, beads with concentration of 200 µl and 800 µl did not show any clear zone formation.

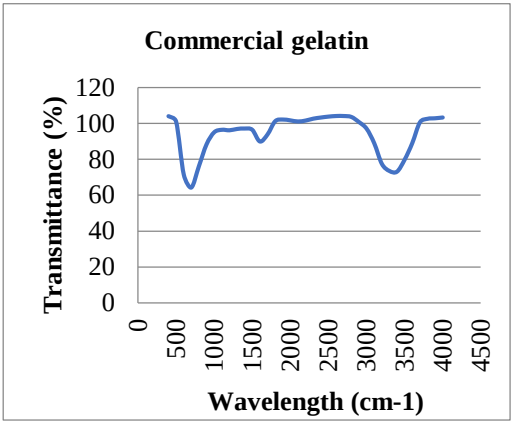

Figure 2. FTIR Spectrum of Commercial Gelatin

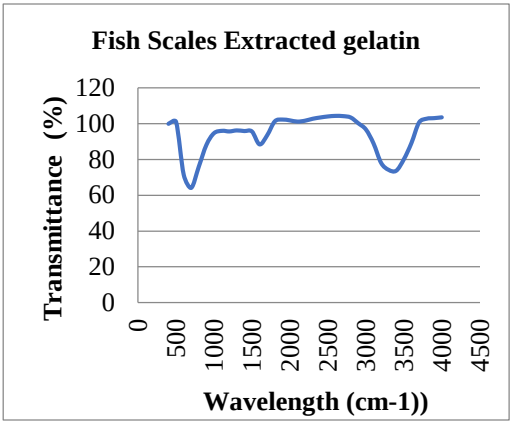

Figure 3. FTIR Spectrum of Extracted Gelatin

3.5 Efficacy of Gelatin Beads as Coated with Antibiotic

Gelatin beads were also coated with an antibiotic dilution of 300µl and it was observed that beads which were coated with the antibiotic dilution showed a greater diameter as compared to the

injected antibiotic of same concentration. However, the beads which were coated by soaking in the antibiotic dilution were degraded in case of commercial gelatin (Table 2). From the zones formation of beads supplemented with the antibiotics it was observed that zone diameter increased as the amount of antibiotic increased, it means they have the capacity to carry antibiotics.

Table 2. Zone Formation under Different Concentrations of Antibiotics

Antibiotic Concentrations (µl)	Growth inhibition zones (cm)		
	Diameter of disc zone	Diameter of bead	Diameter of bead zone
	Isolate SCC1	Isolate SCC4	Isolate SCC5
200	2±0.12	0.4±0.72	No zone
300	3±0.12	0.4±0.02	1.4±0.32
600	1.5 ±0.12	0.4±0.112	0.7±0.192
800	1±0.12	0.4±0.12	No clear zone

3.6 Determination of Beads Efficacy as a Carrier of Inoculum

Beads of extracted gelatin and commercial gelatin beads were injected and their efficacy was determined via inoculum.

3.6.1 Extracted Gelatin Beads Injected with Inoculum (Pathogens)

Beads of extracted gelatin were injected with different concentrations of pathogens, and it was obeserved that there was no specific zone fomation around the beads with the injected concentrations of 100 and 400 µl of inoculum. While the clear growth zones were formed around the beads injected with concentrations of 150, 200, and 300µl. It was also observed that growth-spreading zone diameter decreased when injected concentrations increased. This showed that they have less capacity to carry the increased amount of inoculum.

3.6.2 Commercial Gelatin Beads Coated with Inoculum (Pathogens)

When the beads (commercial grade) were coated with the inoculum, most beads were either degraded or they were not stable. Beads were kept in inoculum for 3-5 minutes, it was found that most beads did not retain their stability and degraded during soaking in inoculum. Although in case of coated beads, zones diameter was greater as compared to the injected beads. Injected beads can be used as a carrier of inoculum, but in case of coated beads they are not sufficient because microbes seems to degrade beads either by engulfing the beads, resulting in degradation. Only beads which were coated with the concentrations of 100 and 150 µl of inoculum remained stable while others were degraded (Table 3).

3.6.3 Determination of Beads Efficacy as a Carrier of Inoculum in Plant Growth Experiments

To check the efficacy of gelatin beads as a carrier of inoculum in plant growth experiments, seeds were either inoculated with microbes or with gelatin beads (inoculated and non-inoculated). Plant growth was determined in terms of root

length, shoot length, seedling length, seed germination, numbers of roots and shoots, dry weight, fresh weight and moisture content (Table 4).

Tabel 3. Diameter of Beads as a Carrier of Pathogens

Concentrations of fish pathogen (µl)	Diameter of injected beads zone(cm)	Diameter of coated beads zone(cm)
100	No specific zone	1
150	1.5	1.7
200	1.8	×
300	1	×
400	No specific zone	×

Diameter of each bead was 0.4 cm

It was observed that seed germination in pots that contained seeds with inoculated beads showed high germination (23 %) as compared to the control or seeds which were provided with only inoculum without beads. A similar response was recorded in the case of shoot length and moisture content 46% and 85%, respectively. A number of roots, shoots, and fresh weight were 56%, 12%, and 50%, respectively. These parameters were greater in seeds with only inoculum than control seeds and seeds with inoculated beads. The dry weight was the same in case of both inoculated beads provided to the seeds and seeds with only inoculum.

Table 4. Plant Growth by Plant Microbe Interaction

Seed	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling length (cm)	No. of Root	No. of shoot	Fresh weight (g)	Dry weight (g)	Moisture content (%)
Control	98	2.02 ±1.16	1.27 ±0.73	0.53 ±0.30	1.15 ±0.66	0.57 ±0.32	0.01 ± 0.005	0.05 ± 0.02	0.007 ±0.004
Seeds+ inoculated beads	75	1.08 ±0.62	2.11 ±1.21	0.72 ±0.41	0.94 ±0.54	0.57 ±0.32	0.01 ±0.005	0.0076 ±0.043	0.58 ±0.33
Inoculated seeds	100	3.72 ±1.86	1.78 ±0.89	1.37 ±0.68	0.5 ±0.25	0.5 ±0.25	0.005 ±0.002	0.0076 ±0.004	0.005 ±0.001

4. Discussion

Fish plays a vital role as source of food for humans and can contribute to the overall economy of the country [32]. Waste from fish industries can have a harmful effect on the environment. However, different practices can be adopted to reduce wastes produced from fish industy, such as making valuable products like gelatin that can further contribute to the country's economy [33].

Gelatin is actually a protein having a wide applications in various field like in photography, pharmaceuticals, and in the food industries [22]. Gelatin is obtained from different sources like cattle, pig [34], and chicken skin and bones [35]. Gelatin can also be obtained from fish skin, bones, and scales. The most plentiful sources of

gelatin is fish and fish bones, scales, and skin, which are considered as a halal sources of gelatin and these are widely used in drug and vaccine deliveries [36].

However, other gelatin sources (mammalian sources) are responsible for various diseases, and considering religious and social questions over mammalian sources, while, fish gelatin is good option as an alternative source [7].

In the present study gelatin was extracted form the scales of fish rahu (*Labeo rohita*) and silver carp (*Hypophthalmichthys molitrix*) by using both chemical and microbial mediated methods [27]. In the microbial method it was noticed that the gelatin yield enhanced by adding the microbes inoculum. This study showed that the scales of rahu and silver carp fish can be used as raw material to produce gelatin.

Gelatin assay identify the species producing the gelatinase, gelatinase terminates gel and ultimately liquification occurs. Gelatin liquification assay was performed by using already available isolates, such as SEY and SCC5 and both showed positove results. These showed the degradation of gelatin to polypeptides which further degdrade to amino acids. Gelatin nutrient media was used for liquification assay and media containing fish isolates were kept in an incubator and transferred to a refrigerator on daily basis for 15-20 minutes. Gelatin did not solidify even at low temperature, which means fish isolates were degrading the gelatin. The main objective of this test was to diffrentiate different bacteria like gram positive, rod shape bacteria, pathogenic and non-pathogenic bacteria and also identify the bacteria which were producing the gelatinase [28], and extracellular enzymes used for digestion of gelatin. This test was also helpful in the identification of *Bacillus*, *Pseudomonas*, and *clostridium* bacteria. The amino acids produced as a result of degradation by gelatinases of bacteria could be used for their metabolic pathway [28].

Moreover, beads were also formed by using the commercial gelatin and were compared with the beads of extracted gelatin and it was observed that the beads of extracted gelatin were more stable as compared to commercial gelatin. As concentration of antibiotics increased, the zone formation around beads also increased, it means that beads can be used as drug carrier [36]. Beads can be used for the sustained release of drugs, vaccines, antibiotics, and hormones. Using gelatin beads as drug delivery system is a recent advancement and is gaining popularity because gelatin is easily available, degraded, and companionable and cheap [36, 37].

FTIR spectra was performed for gelatin beads to identify the different functional groups present in gelatin and bonds which maintain the structural stability of structure. Characterization of beads by documenting the spectra at 400–4,000 cm−1 range was observed using flourier-transform infrared spectroscopy (FTIR) (Bruker). FTIR spectra for both commercial and extracted gelatin beads was compared. FTIR spectroscopy was actually done to observe the physical structure of substance and to observe the changes in commercial and extracted gelatin beads. [27] given that, many properties of gelatin described its applications therefore, FTIR analysis helped to identify the possible functional groups or moieties responsible for the structural and functional properties of the gelatin extracted from the fish scales. Amide-1 bands have been reported to represents C=O stretching/hydrogen bonding couple with COO, amide-II represents bending vibration of N-H groups, and stretching vibrations of C-N

groups. Peaks of the gelatin observed at 3433 cm-1 attributed to the presence of hydrogen bond water and amide-A, 1630 cm-1 peaks corresponds to the occurrence of amide-I, at 1565 cm-1 is indicating amide-II, band at 1240 cm-1 indicates the amide-III, peaks ranges from 1460 cm-1 to 1380 cm-1 were attributed to the symmetric, and asymmetric bending vibrations of methyl group.

Alginate beads have been reported to be used along with sowing for slow release of inoculum with the advantage that they are not easily degradadable [38]. It could be said the beads injected with the inoculum can be used as carrier but the beads coated with the inoculum degraded micobe may engulf the beads. Gelatin beads as carrier of inoculum can be used in agriculture field for growth of plants. Plant growth experiments were also performed to check the efficacy of beads as inoculum carrier. It was observed that seed germination in pots that contained seeds with inoculated beads showed high germination (23 %) as compared to the control or seeds which were provided with only inoculum without beads. Similar response was recorded in case of shoot length and moisture content 46 %, 81 %, and 85 %, respectively. A number of roots, shoots and fresh weight were 56 %, 12 %, and 50 %, respectively. These parameters were greater in seeds with only inoculum than control seeds and seeds with inoculated beads. The dry weight was same in case of both inoculated beads provided to the seeds and seeds with only inoculum. A slow release from the micro-beads has been reported ranging from 10^4-10^6 CFU g^{-1} depending on the different parameters. The wet and dry inoculants enhanced the development of wheat and tomato seedlings growing in unfertile soil, and biodegraded within 15 days in moist soil [38].

Improvement in growth was recorded in inoculated plants and better moisture content in seeds showed that shoots were dark green as compared to the seeds with only inoculum or non inoculated seeds. In plant-microbe interaction both plant and microbe receive benefits from each other [39]. Considering the above considerations, the present study highlights the future success stories expected from using fish-based gelatin as a drug delivery system and inoculum carrier of plant growth-promoting bacteria in the field of the agriculture industry.

5. Conclusion

It was concluded from the current study that fish scales are an excellent source of gelatin and the yield of gelatin increased as the number of scales increased. Microbial inoculum (fish isolates) of SY and SCC5 also enhanced gelatin yield by greater than 50%. The extracted gelatin was used for beads formation and comapared with the beads of commercial gelatin. It was noticed that beads of extracted gelatin were more stable. The peaks of FTIR spectra also showed the stability of extracted gelatin beads. Beads were coated and injected with antibiotics to check their efficacy as carriers of drugs. It was concluded that beads could be used as drug carriers or in a drug delivery system. Moreover, gelatin beads were also inoculated and coated with fish pathogens and used in plant-microbe interaction. It was found that they can also be used as carriers of microbes to improve the growth of plants. Using indigeneous microbial isolates used in current study, extraction of gelatin from fish scales eliminates pollution from the environment and is a cheap source of gelatin. Its beads can be used as carriers in the agriculture and pharmaceutical industry.

Conflicts of Interest

Authors declare there is no conflict of interest.

References

1. Jarwar AA. A status overview of fisheries and aquaculture development in Pakistan with context to other Asian countries. *Aquaculture Asia Magazine*, 2008.
2. Laghari MY. Aquaculture in Pakistan: Challenges and opportunities. *Int J Fish Aquatic Stud*. 2018;6(2):56-59.
3. Hussain J, Rabbani I, Aslam S, Ahmad H. An overview of poultry industry in Pakistan. *World's Poult Sci J*. 2015;71(4):689-700.
4. Ranjan A. The China-Pakistan economic corridor: Options before India. *Institute of Chinese Studies*. 2015;10(1):1-25.
5. Mariod AA, Fadul H. Gelatin, source, extraction and industrial applications. *Acta Sci Pol Technol Aliment*. 2013;12(2):135-147.
6. Hamidi-Asl E, Dardenne F, Blust R. De Wael K. An improved electrochemical aptasensor for chloramphenicol detection based on aptamer incorporated gelatine. *Sensors*. 2015;15(4):7605-7618.
7. Shyni K, Hema G, Ninan G, Mathew S, Joshy C, Lakshmanan P. Isolation and characterization of gelatin from the skins of skipjack tuna (*Katsuwonus pelamis*), dog shark (*Scoliodon sorrakowah*), and rohu (*Labeo rohita*). *Food Hydrocoll*. 2014;39:68-76. https://doi.org/10.1016/j.foodhyd.2013.12.008
8. Okuyama K. Miyama K. Mizuno K. Bächinger HP. Crystal structure of (Gly-Pro-Hyp) 9: Implications for the collagen molecular model. *Biopolymers*. 2012;97(8):607-616. https://doi.org/10.1002/bip.22048
9. Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem*. 2009;78: 929-958. https://doi.org/10.1146/annurev.biochem.77.032207.120833
10. Haug IJ, Draget KI, Smidsrød O. Physical and rheological properties of fish gelatin compared to mammalian gelatin. *Food Hydrocoll*. 2004;18(2):203-213. https://doi.org/10.1016/S0268-005X(03)00065-1
11. Rbii K. *Formation of high molecular weight aggregates in gelatin and behavior in aqueous solution* [dissertation]. Institut National Polytechnique de Toulouse; 2010.
12. Jones RT. *Gelatin: Manufacture and physico-chemical properties*. London, UK, Pharmaceutical Press; 2004.
13. Duconseille A, Astruc T, Quintana N, Meersman F, Sante-Lhoutellier V. Gelatin structure and composition linked to hard capsule dissolution: A review. *Food Hydrocoll*. 2015;43:360-376. https://doi.org/10.1016/j.foodhyd.2014.06.006
14. Basu S, Bose C, Ojha N, et al. Evolution of bacterial and fungal growth media. *Bioinformation*. 2015;11(4):182-184.
15. Hassan EM, Fatmi AA, Chidambaram N. Enteric composition for the manufacture of soft. Google Patents. https://patents.google.com/patent/EP1545475A1/en, 2014.
16. Pogue BW, Patterson MS. Review of tissue simulating phantoms for optical spectroscopy, imaging and dosimetry. *J Biomed Opt*. 2006;11(4):e041102. https://doi.org/10.1117/1.2335429

17. James C. The book of alternative photographic processes. Boston, USA, Cengage Learning; 2015.
18. Ramos M, Valdes A, Beltran A, Garrigós M. Gelatin-based films and coatings for food packaging applications. *Coatings*. 2016;6(4):e41. https://doi.org/10.3390/coatings6040041
19. Ul Rehman W, Majeed A, Mehra R, et al. *Gelatin: A comprehensive report covering its indispensable aspects*. Nova Science Publishers, Inc.;2016.
20. Nik Aisyah N, Nurul H, Azhar M, Fazilah A. Poultry as an alternative source of gelatin. *Health Environ J*. 2014;5(1):37-49.
21. Eriksson A, Burcharth J, Rosenberg J. Animal derived products may conflict with religious patients' beliefs. *BMC Med Ethics*. 2013;14(1):e48. https://doi.org/10.1186/1472-6939-14-48
22. Zakaria S, Bakar NHA. Extraction and characterization of gelatin from Black tilapia (Oreochromis niloticus) scales and bones. Paper presented at the Kota Kinabalu (MY): Paper presented at: International Conference On Advances in Science, Engineering, Technology And Natural Resources; August 27-28, 2015, Kota Kinabalu, Malaysia. https://iicbe.org/upload/3199C0815040.pdf
23. Lin CC, Chiou TK, Sung WC. Characteristics of gelatin from giant grouper (Epinephelus Lanceolatus) skin. *Int J Food Prop*. 2015;18(11):2339-2348. https://doi.org/10.1080/10942912.2014.980947
24. Zhang QT, Tu ZC, Xiao H, et al. Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate. *Food Biopro Processing*. 2014;92(1):30-37. https://doi.org/10.1016/j.fbp.2013.07.006
25. Wangtueai S, Noomhorm A. Processing optimization and characterization of gelatin from lizardfish (Saurida spp.) scales. *LWT-Food Sci Technol*. 2009;42(4):825-834. https://doi.org/10.1016/j.lwt.2008.11.014
26. López-Velázquez JC, Rodríguez-Rodríguez R, Espinosa-Andrews H, et al. Gelatin–chitosan–PVA hydrogels and their application in agriculture. *J Chem Technol Biotechnol*. 2019;94(11):3495-3504. https://doi.org/10.1002/jctb.5961
27. Das M, Suguna P, Prasad K, Vijaylakshmi J, Renuka M. Extraction and characterization of gelatin: a functional biopolymer. *Int J Pharm Sci*. 2017;9(1):239-242.
28. dela Cruz TEE, Torres JMO. Gelatin hydrolysis test protocol. American Society for Microbiology; 2012. https://link.springer.com/content/pdf/10.1007/s13213-019-01533-z.pdf
29. Iwamoto S, Nakagawa K, Sugiura S, Nakajima M. Preparation of gelatin microbeads with a narrow size distribution using microchannel emulsification. *AAPS Pharm Sci Tech*. 2002;3(3):72-76. https://doi.org/10.1208/pt030325
30. Nazir K, Yongtong M, Kalhoro MA, Memon KH, Mohsin M, Kartika S. A preliminary study on fisheries economy of Pakistan: Plan of actions for fisheries management in Pakistan. *Can J Basic Appl Sci*. 2015;3(01):7-17.
31. DeRuiter, J. (2005). Amides and related functional groups. *Principles of Drug Action, 1*, 1-16.

32. Herpandi H, Huda N, Adzitey F. Fish bone and scale as a potential source of halal gelatin. *J Fish Aquatic Sci*. 2011;6(4):379-389.
33. Baziwane D, He Q. Gelatin: The paramount food additive. *Food Rev Int.* 2003;19(4):423-435. https://doi.org/10.1081/FRI-120025483
34. Bichukale A, Koli J, Sonavne A, Vishwasrao V, Pujari K, Shingare P. Functional properties of gelatin extracted from poultry skin and bone waste. *Int J Pure App Biosci*. 2018;6(4):87-101. http://dx.doi.org/10.18782/2320-7051.6768
35. Sahoo N, Sahoo RK, Biswas N, Guha A, Kuotsu K. Recent advancement of gelatin nanoparticles in drug and vaccine delivery. *Int J Biol Macromol.* 2015;81(1):317-331. https://doi.org/10.1016/j.ijbiomac.2015.08.006
36. Su K, Wang C. Recent advances in the use of gelatin in biomedical research. *Biotechnol Letters*. 2015;37(11):2139-2145. https://doi.org/10.1007/s10529-015-1907-0
37. Foox M, Zilberman M. Drug delivery from gelatin-based systems. *Expert Opin Drug Deliv*. 2015;12(9):1547-1563. https://doi.org/10.1517/17425247.2015.1037272
38. Bashan Y. Alginate beads as synthetic inoculant carriers for slow release of bacteria that affect plant growth. *Appl Environ Microbiol.* 1986;51(5):1089-1098. https://doi.org/10.1128/aem.51.5.1089-1098.1986
39. Schirawski J, Perlin MH. Plant–microbe interaction 2017—the good, the bad and the diverse. *Intl J Molecul Sci.* 2018;19(5):e1374. https://doi.org/10.3390/ijms19051374