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RESEARCH PAPER

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Utilization of Fish Scales for Microbe-mediated Gelatin Extraction: A New Biotechnology and Microbiology Avenue

Rabia Anwar Rao[1], Aisha Waheed Qurashi[1], Iram Liaqat[2,*], Mawra Gohar[1], Urooj Zafar[3], Muhammad Amjad Khan[5], Saraj Bahadur[4,*]

[1]Lahore Garrison University, Department of Biology, Sector C phase 6 DHA Lahore, Pakistan.
[2]Government College University, Department of Zoology, Microbiology Lab, Lahore, Pakistan.
[3]Univeristy of Karachi, Department of Microbiology, Karachi, Pakistan.
[4]College of Forestry Hainan University Haikou, 570228, China.
[5]Hainan University Haikou, College of Ecology and Environment, 570228, China.

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Corresponding Author
Tel.: +923417824964
+8613111981002
E-mail: iramliaq@hotmail.com
sirajbahadur14@gmail.com

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Abstract

Waste materials are of major concern around the globe. This study aimed to utilize the scales of fish (*Labeo rohita*) in microbe-mediated gelatin extraction. Three bacterial isolates were isolated from the fish scales and one bacterial isolate FSW3 showing maximum gelatin yield (61.4 %) was subjected to further analysis. The bacterial culture FSW3 (identified as *Staphylococcus* spp.) with enhanced production of extracted gelatin was set for physiochemical testing, FTIR analysis, UV-Vis spectroscopy, and stereomicroscopic analysis. The extracted gelatin was successfully utilized in various applications including plant growth promotion under salt stress, anti-adhesion films, firmness of yogurt, biodegradable plastic, and anti-microbial food coatings. Results showed that 0.01 mM gelatin promoted plant growth promotion under salt stress. FSW3 extracted gelatin decreased the 2-fold biofilm-forming ability of *E. coli,* when exposed to the gelatin-based anti-adhesion films coated on a glass slide and polystyrene sheet. The gelatin showed promising findings as anti-microbial food coatings for tomatoes preservation after 21 days and helpful in the firmness of yogurt. Another advantage of gelatin was casting it into biodegradable plastic. The current study provides a promising approach for fish scales microbe-mediated gelatin extraction and uses it to explore new avenues in biotechnology and microbiology.

Introduction

Fish products are an important source of protein containing 15 – 20 % of protein (Fagbenro et al., 2005; Usman et al., 2022). Different methods are developed to convert the wastes into useful products. In the fish industry, almost 50 % or more materials (containing gelatin) are not used as food and are responsible for creating almost 32 million tons of waste (Arvanitoyannis et al., 2008). Gelatin is hydrolyzed form of a protein and obtained by hydrolysis of collagen present in fish skin waste and a source of protein (Shankar et al., 2016; Ali pal et al., 2020).

Main objective of present study is to highlight the significance of indigenous microbes involved in the extraction of gelatin from waste material like aquaculture and fish farming industry as a promising approach for gelatin provision. Religious or ethical sentiments of diverse communities and some historical diseases of cows (Wangtueai & Noomhorm, 2009) led to the possible offering of this approach.

Gelatin is present in different parts of animals and contains few amino acids. The denaturation/melting temperature of gelatin gel is above 30°C whereas the gelatin denaturation temperature is above 40°C (Hayashi & Oh, 1983). Gelatin contains many residues of

glycine, proline, and 4 -hydroxyproline residues (Jamilah & Harvinder, 2002). The structure of gelatin contains multi-standard polypeptide chains of proline, hydroxyproline, serine, etc. (Wilson et al., 2018). Gelatin contains higher protein content and no fat content (Chaplin, 2015). According to the methods of gelatin processing, it is divided into two types: (i) type A gelatin: Isoelectric point exists at pH 8 – 9, performed using acid treatment methods (ii) type B gelatin whose isoelectric pH 4-5 obtained from alkali treatment (Shankar et al., 2016). i Edible coatings and films based on gelatin can be prepared, leading to the development of new strategies for the cost-effective and recyclable packaging of food (Merina et al., 2017). The functional properties of these films can be enhanced by adding different substances like cross-linkers and plasticizers. The shelf life of foods can be increased with the addition of antimicrobial and antioxidants to these films (Alfaro et al., 2014). The gelatin obtained from fish scales can be used in plant growth promotion (Wilson et al., 2018) as salt tolerance, helpful in replacement of conventional anti-adhesion films (Liu et al., 2022), can be used to develop biodegradable plastics by using different non-harmful chemicals with it, can be used in a cosmetic product, medicine, foods, etc.

The current study describes a very promising microbe mediated approach for gelatin extraction from fish scales and potential uses of extracted gelatin in different industrial field of applied microbiology. Our research describes a sustainable use of fish scales (a major pollution sources in aquaculture industry) as a cheaper and halal source of food by converting waste into value added products.

Materials and Methods

Isolation of Bacterial Cultures

The scales of healthy Labeo *rohita* (Rahu) fish were collected from local market of Lahore, Pakistan. Scales were then transported under sterile conditions to Biology laboratory, Lahore Garrison University. These samples were washed using sterile water thoroughly to clean followed by drying in sterile glass container. The scales were packed in sterile container and stored at 4° C till use.The collected samples of one gram of fish scales (*Labeo rohita*) were serially diluted (10 fold) and 100 µL from 10^{-2},10^{-4} and 10^{-6} dilution was spreaded on sterile Nutrient Agar plates (Cappuccino and Sherman, 2002). The plates were then placed into the incubator at 37° C for 24 hours. The resultant bacterial isolates were then purified onto separate nutrient agar plates. Five different type of bacterial isolates were isolated from fish scales labelled as FSW1, FSO2, FSW3, FST4 and FSC5. These bacterial isolates were identified on the basis of their morphological, microscopic characterization and biochemical profile. The obtained isolates (n=5) were subjected to gelatin hydrolysis.

Biochemical Profiling and Morphological Characterization of Bacterial Isolates

Bacterial cultures were morphologically characterized by spore staining (Cappuccino and Sherman, 2002) and Gram staining. Biochemical profiling was performed in the basis of results of the Methyl red test, Voges prausker test, catalase test, and citrate utilization test.

Preparation of Bacterial Cultures for Extraction Process

Bacterial cultures were grown in nutrient broth for 24 hours and centrifuged (Hermle Labort Technik) at 10,000 rpm for 15 minutes. The supernatant was discarded and pellets were collected for later use.

Extraction Process

Microbe-mediated extraction of gelatin was done by using fish scales by modifying (introduction of bacterial pallets in each step) the alkali treatment method designed by Das et al. (2017).

The scales were dried, measured, and stirred using a 5 % sodium chloride (NaCl) solution along with bacterial pallets in the ratio of 1:10 for about 30 minutes. This step was repeated twice. Then the scales were treated twice with 0.4 % alkali solution sodium hydroxide (NaOH) in the ratio of 1:10 for 30 minutes, in a digital linear shaking incubator. In the next step, scales were treated with 10 % hydrogen peroxide (H_2O_2) in 1:4 weight by volume to remove lipids from the scales. This step was preceded three times for 30 minutes, in a shaking incubator at 25°C temperature and 120 rpm (optimised). Then was followed to the next step was demineralization using 0.5 N EDTA (ethylene diamine tetra acetic acid) along with 3 ml of bacterial isolates for 16 hours in a shaking incubator in the ratio of 1:4. Then the scales were recovered through a sieve and washed with distilled water. In the next step, scales were soaked in 0.05 M acetic acid in the ratio of 1:4 for three hours. In the final step, water was added in the ratio of 1:3, and the extract was kept in a hot air oven at 60°C for 24 hours. The next day, the dried extract was placed at room temperature and ground to powder. Gelatin extraction was done in 4 sets including a control (chemical extraction) and with bacterial culture. Bacterial cultures were cultivated in sterile broth at pH 7, temperature 37 °C and cell densities adjusted at 0.5 at 600 nm. Percentage yield obtained with each culture was compared with control. Extraction of gelatin was further increased up to 65.14 % when growth optimization of bacterial culture (FSW3) was ensured.

Physiochemical Testing

Physiochemical properties such as yield, pH, ash content, moisture content, proline content, and protein

content were determined using stereomicroscopy, FTIR analysis, UV-Vis spectroscopy (Specord 200 plus, Germany), and stereomicroscopy (MEIJI). ing,

Effect of Gelatin Concentration on Plant's Growth Under Salt Stress

The previously described method i.e., Afrasayab et al.2010 erefollowed, Petri plates with filter paper were autoclaved (Hirayama) and dried. Seeds of *Triticum aestivum* were sterilized with 0.1% $HgCl_2$ solution followed by repeated washing to remove all the impurities of $HgCl_2$. The 100mM salt solution was prepared to create salt stress. Different concentrations of gelatin were also prepared to optimize the requisite concentration of gelatin for plant growth. The different concentrations of gelatin used in milli molar were 0.001, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1. Each plate contained 5 seeds per plate. Each plate was provided with 2.5 mL (1 M) salt solution, 3 mL gelatin of respective concentration, and 2.5 mL distilled water. It was kept in dark after covering until seeds starts to germinate then shifted to light after 3 days.

Biodegradable Plastics from Fish Scale Gelatin

To make biodegradable plastic from fish scales, a beaker was filled with a 27 % extracted gelatin solution and 2.5 mL of 100 % glycerine. and toThe solution was boiled until the mixture gets foamy. Then the mixture was allowed to cool in molds. And kept at ambient temperature for at least one week then after one-week results were noted. The endpoint is plastic formation in the shape of molds (Caroline et al., 2012).

The Firmness of the Yogurt Test

The experiments were performed to check the efficacy of gelatin utilization to enhance yogurt consistency. For this experiment, 3 test tubes were taken. Filled with 10 mL of milk and boiled. Then test tube A was inoculated (at 37°C temperature) with 1 mL starter culture inoculum and test tube B was inoculated with 1 mL inoculum. Then 100 microersliter solution of 1% commercially available gelatin was added to test tube B. In the test tube, C, 100 microersliter solution of 1% extracted gelatin was added and then it was incubated at 37 °C for 24 hours, and results were noted (Raphaelides & Gioldasi, 2005).

Preparation of Gelatin-based Anti-microbial Food Coatings

Gelatin-based anti-microbial food coatings were prepared by the method of Thaker et al., 2017. Following raw materials were used for this purpose as per procedure

1. Extracted gelatin
2. Commercially available Chitosan
3. Garlic crude and lime juice extract

Preparation of Garlic Crude and Lime Juice Extract

Garlic was firstly peeled, washed, and ground to get the extract. The lime extract was also collected by squeezing the lemon after cutting with a sterile knife. Both extracts were passed through a fine sterilized cotton cloth and filtrates were collected in small vials for further use.

Preparation of the Coating Solutions

Powder of 10 g extracted gelatin was completely dissolved in sterile 60 mL distilled water. Chitosan solution was prepared in 1 % acetic acid by adding 9 g chitosan in 100 mL 1 % acetic acid at 55° C for 30 minutes to get a homogenous blend. Different concentrations of garlic and lime extracts were prepared in test tubes as:

i. Garlic extract 3 ml with 7 mL lime extract.
ii. Garlic extract 4 ml with 6 mL lime extract.
iii. Garlic extract 5 ml with 5 mL lime extract.
iv. Lime extract 3 ml with 7 mL garlic extract.
v. Lime extract 4 mL with 6 mL garlic extract.

These concentrations were added to the chitosan-gelatin blend and mixed well to get the final volume of 100 mL. The anti-microbial activity of the above preparations was evaluated on fresh tomatoes (Thaker et al., 2017) to check their effects shelf life of tomatoes.

Efficacy of Gelatin-based Anti-microbial Food Coating Solution on Fresh Tomatoes

Fresh tomatoes (same size and weight) were selected and placed under 6 different conditions. Bacteria isolated from rotten fruits were also used in this study. Each tomato was dipped in its respective antimicrobial solution, followed by the introduction of bacterial isolates on surfaces, and placed in a refrigerator for 21 days to check the anti-microbial effect of the preparations (Sucharitha et al., 2018). After 21 days, the results were observed and noted. Different treatment of coating solution was prepared as follow:

Sample A: Washed with sterile distilled water.

Sample B: Washed with sterile distilled water and treated with inoculum.

Sample C: Treated with gelatin-chitosan blend with 3 mL garlic extract and 7 mL lime extract with inoculum.

Sample D: Treated with gelatin-chitosan blend with 4 mL garlic extract and 6 mL lime extract with inoculum.

Sample E: Treated with gelatin-chitosan blend with 5 mL garlic extract and 5 mL lime extract with inoculum.

Sample F: Treated with gelatin-chitosan blend with 7 mL garlic extract and 3 mL lime extract with inoculum.

Sample G: Treated with gelatin-chitosan blend with 6 mL garlic extract and 4 mL lime extract with inoculum.

Physiological Analysis of Coated Tomatoes

The tomato's weight loss and decay percentage (index of quality) were analyzed for storage and -post-treatment following the previous method (Patel and Goyal., 2015). Decay percentage was calculated (Saavedra et al., 2016). Briefly, the decay of tomatoes was observed on daily basis and calculated in percentage.

$$\text{Percentage decay} = \frac{\text{Decayed fruits}}{\text{Total fruits}} \times 100$$

Compositional and Bio-chemical Analysis of Coated Tomatoes

Compositional and biochemical analysis of coated tomatoes was done including pulp formation, titratable acidity (TA), reducing sugar content, and total phenolic content.

Biofilm Forming Ability by the bacteria Obtained from Fish Scales

Biofilm formation was done by following the method of Liaqat et al., (2008); Qurashi and Sabri, (2012). L-broth was prepared and a loop full of each bacterial inoculum was added in separate test tubes and incubated for 24 hours at 37 ◦C. Then bacterial growth OD was recorded at 600 nm and adjusted at 0.5 nm and incubated at 37 ◦C under nonshaking conditions. Then after 6 days, OD at 600 nm was observed for planktonic cells. Empty test tubes were obtained by discarding planktonic cells and 10 mL of 0.1% crystal violet solution was poured into empty tubes and kept at room temperature for 20 minutes. Then crystal violet solution was discarded and 10 mL of 70 % ethanol was added to dissolve attached stained cells adhered cells and left at room temperature for 20 minutes then the OD of biofilm was taken at 570 nm.

To make it clear a statement is added in methodology that both these isolates didn't hydrolysed gelatin (FSO2) or showed very weak/negligible hydrolysis. But biofilm efficacy was determined because these microbes were associated with fish scales, that's why biofilm potential was determined for the both isolates as well

Preparation of Gelatin Film for Anti-adhesion

Gelatin films were prepared following the method of Horii et al. (2018). Gelatin solution (4.5%) was prepared in sterile distilled water. The solution was cast in plastic plates and allowed to dry for two days on a clean bench. Films obtained after drying were thermally cross-linked (Memmert, Germany) at 140◦ C for 3.5 hours. The films were stored in a clean dried container for further use.

Anti-biofilm Efficacy of Gelatin-based Anti-adhesion Film

Gelatin-based films were coated on sterile glass slides and polystyrene sheets which were then dipped into the flask containing L-broth (Qurashi and Sabri, 2012).

i. Setup A: Without Inoculum (*E. coli)* + glass slides with gelatin coating.
ii. Set up B: Inoculum (*E. coli*) + glass slides with gelatin coating.
iii. Setup C: Inoculum *E. coli*) + glass slides without coating.
iv. Sample D: Without Inoculum (*E. coli*) + polystyrene sheet with coating.
v. Sample E: inoculum (*E. coli*) + polystyrene sheet with coating
vi. Sample F: Inoculum (*E. coli*) + polystyrene sheet without coating.

E. coli culture were inoculated in above setups and incubated for 24 hours at 37◦ C. Then culture OD was adjusted to 0.5 at 600 nm and incubated again at 37◦C under nonshaking conditions for 5-6 days (108-144 hours). After 144 hours, OD (600 nm) was observed for planktonic cells. On the other hand, Crystal violet solution (0.1 %) was added to each flask and kept at room temperature for 20 minutes. The crystal violet solution was discarded and 10 mL of 70 % ethanol was added to dissolve attached stained cells and left at room temperature for 20 minutes. The OD of biofilm was taken at 570 nm as biofilm of bacterial cultures was expressed as normalized biofilm (OD 570/600 nm). The glass slides and polystyrene sheet were stained with 0.1 % crystal violet solution and observed under a microscope (Liaqat et al., 2010; 2014).

Results

Isolation of Bacterial Cultures

Five different type of bacterial isolates were isolated from fish scales and named as FSW1, FSO2, FSW3, FST4 and FSC5. The obtained isolates (n=5) from fish scales were subjected to gelatin hydrolysis. Among all isolates, only 1 isolate i.e. FSO2 showed negative result (media remains solidified) while remaining four isolates FSW1, FSW3, FST4 and FSC5 were positive for gelatin hydrolysis (Figure 1B). Isolate FST4 isolate showed very low hydrolysis. These bacterial isolates were identified on the basis of their morphological, microscopic characterization and biochemical profile. Morphology showed that out of 5 isolates, 2 (FSW3 and FSC5) were gram positive while 3 (FSW1, FSO2 and FST4) were observed as gram negative. Out of two gram positive bacterial isolates, 1 (FSW3) was cocci while other 1 (FSC5) was rods. The gram negative bacterial

isolates were further cocci (FSW1, FSO2) and rods 1(FST4), respectively.

Identification of Bacterial Isolates

The bacterial isolates were further processed for their identification using standard microbiological techniques. Bacterial isolates showed respective resemblance to *Staphylococcus* spp (FSW1 and FSW3)., *Bacillus* spp. (FST4) And *Pseudomonas* spp. (FSC5) using standard microbiological techniques (spore staining, Gram staining, methyl red test, Voges prausker test, catalase test, and citrate utilization test).

The isolated bacterial cultures were then used in the extraction process as shown in Figure 1.

Physiochemical Testing

Table 1 summarizes the yield of gelatin, proline, protein extraction, and percentage degradation of selected isolates showing gelatin hydrolysis. The maximum yield of gelatin was obtained with FSW3 isolate was 57.33 % and its yield was increased up to 61.4 % after growth optimization (Table 1). Table 2 shows, that extracted gelatin has a pH of 4 while commercial gelatin has a pH of 5. Ash and moisture contents were 1.4 % and 8.8 %, respectively as shown in figure 2. FTIR and UV-Vis spectroscopy analysis of FSW3 xextracted gelatin was shown in Figure. 3.

Figure 1. Schematic diagram of microbe mediate gelatin extraction.

Table 1. Yield of Gelatin, proline and protein with microbes mediated extraction

Bacterial strains	Yield of gelatin (%)	Proline Concentration (µg/g)	Protein content of Scales	Protein content of Scales + Inoculum	Percentage degradation
Control	38.26	120 ± 1.414	11 g	45.12 g	30 %
FSW1 (*Staphylococcus* spp.)	54.08	80 ± 1	11 g	33.07g	20 %
FSW3 (*Staphylococcus* spp.)	57.33	45 ± 1	11 g	39.27g	25 %
FSC5 (*Pseudomonas* spp.)	53.08	28 ± 1	11 g	43.13 g	29 %
FSW3 (growth optimization)			61.4		

Table 2. Physiochemical properties of the FSW3 extracted gelatin

Physiochemical properties	Commercial gelatin	Microbe mediated extracted gelatin
pH content	5	4
Moisture content	10.2%	8.8%
Ash content	1.09%	1.4%

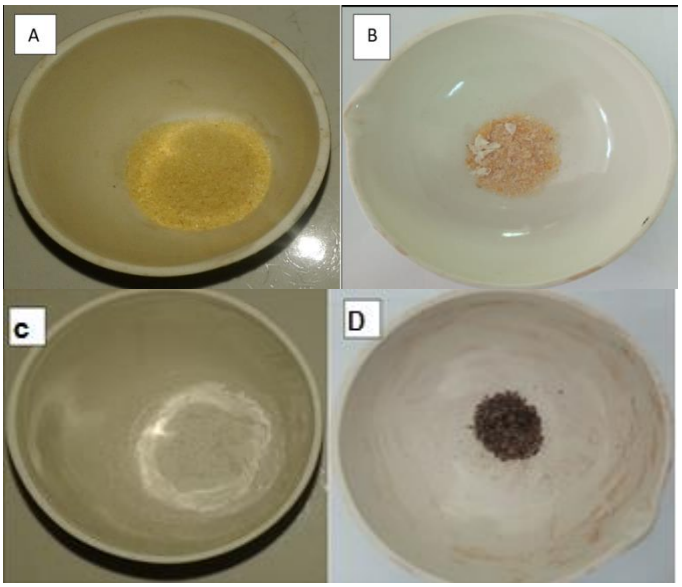

Figure 2. Morphological and biochemical profile of gelatin extracted by microbe FSW3. (A). Commercial gelatin, (B) Microbe mediated extracted Gelatin (C) Ash contents of commercial gelatin, (D) Ash contents of commercial gelatin.

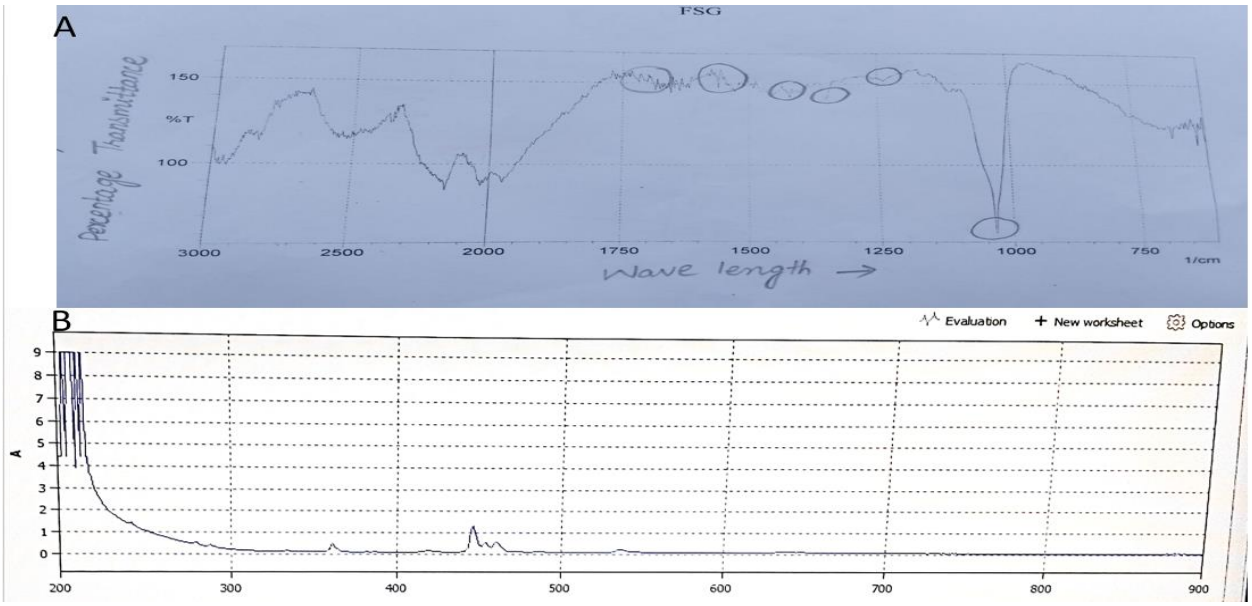

Figure 3. Characterization of FSW3 extracted gelatin via (A) FTIR analysis (B) UV-Vis spectroscopy of FSW3 extracted gelatin.

Plant Growth Promotion Under Salt Stress

The gelatin concentration was optimized and results were observed that are shown in Table 3. The maximum plant growth was observed by using the concentration of 0.01 mM. Increased gelatin content promotes fungal growth that restricts the growth of plants.

Biodegradable Plastic from Fish Scale Gelatin

After one week, biodegradable plastic was obtained. The plastic produced was thick, soft, and can be denatured easily while thin plastic films were also seen on the sides. The image of plastics is shown in Figure 4A.

Firming Yogurt Thickness Test

The gelatin property to cause thickness in yogurt was checked and compared with commercial, extracted gelatin, and without gelatin. The thickest yogurt was produced from the sample containing extracted gelatin. Results are shown in Figure 4B.

Efficacy of Gelatin-based Anti-microbial Food Coating Solution on Fresh Tomatoes

Gelatin-based anti-microbial food packaging solution (10 mL) was used to enhance the shelf life of tomatoes. The following results were observed

Physiological Studies

Decay Percentage

During storage, the rotting of tomatoes was predominant in non-treated samples. Decay means to decompose by the action of microbes. The decay percentage was calculated. The decay percentage in different samples is shown in Table 4 and Figure 4. The decay percentage was calculate. The decay percentage in different samples is shown in Table 4 and Figure 4. Sample D starts deacy at day 2; Sample A, B, and E starts to decay from day 3 while sample C, F, and G starts to decay from day 4. The uncoated tomato was decayed completely on day 13. Results showed that the composition applied to samples C, F, and G was effective in extending the shelf-life of tomatoes. Results showed that sample G was better than the rest of the others to use in the future.

Weight Loss

Weight loss was minimum in sample E while maximum in samples F and G as shown in Figure 5A.

Titratable Acidity (TA)

Figure 5B showed that the amount of acid present in sample C was highest on day 21 while it is lowest in sample F. Sample E shows less amount of acid as compared to sample C.

Table 3. Effect of FSW3 extracted gelatin on weight loss of plants

Concentrations of gelatin (mM)	Fresh weight	Dry weight of plant	Root length of plant (cm)	Shoot length of plant
0.001	0.28±0.063	0.098±0.017	9.38±2.088	8.15±2.856
0.0015	0.234±0.063	0.074±0.005	6.7±2.344	8.68±3.270
0.005	0.218±0.052	1.24±0.0068	7.2±1.834	9.98±1.882
0.01	0.39±0.019	0.19±0.0215	18.75±1.250	15.22±1.111
0.02	0.266±0.02	0.06 ±0.006	13.8±0.817	13.84±0.844
0.03	1.14±0.02	0.088±0.008	5.5±0.744	10.84±2.270
0.04	0.302±0.37	0.056±0.007	10.6±0.714	13.4±0.933
0.05	0.336±0.077	0.08±0.011	12.9±3.028	11.54±3.090
0.06	0.328±0.050	1.862±0.206	10.52±1.412	12.02±1.831
0.07	0.352±0.020	0.1014±0.009	15.56±0.923	15.2±0.740
0.08	0.162±0.159	0.068±0.012	1.82±0.751	5.32±3.1571
0.09	0.12±0.044	0.086±0.012	2.68±1.850	4.6±3.026
0.1	0.302±0.041	0.072±0.010	10.75±2.230	12.42±2.459

The different concentrations of gelatin used in milli molar were 0.001, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1.

Table 4. Comparison of % decay between control and treated sample

Samples	Day 1	Day 2	Day 3	Day 6	Day 7	Day 8	Day 13	Day 16	Day 20
A	0	0	1-24	25- 49	50- 74	50- 74	75 or more	75 or more	75 or more
B	0	0	1-24	1-24	25- 49	5 or more	75 or more	75 or more	7 or more
C	0	0	0	1-24	25- 49	5- 49	50- 74	50- 74	75 or more
D	0	1-24	25- 4	50- 74	75 or more	75 or more	75 or more	75 or more	5 or more
E	0	0	1-24	1-24	25- 49	25- 49	25- 4	50- 74	75 or more
F	0	0	0	1-24	25- 49	25- 49	50- 74	0- 74	5 or more
G	0	0	0	1-24	25- 49	25- 49	5 or more	75 or more	75 or more

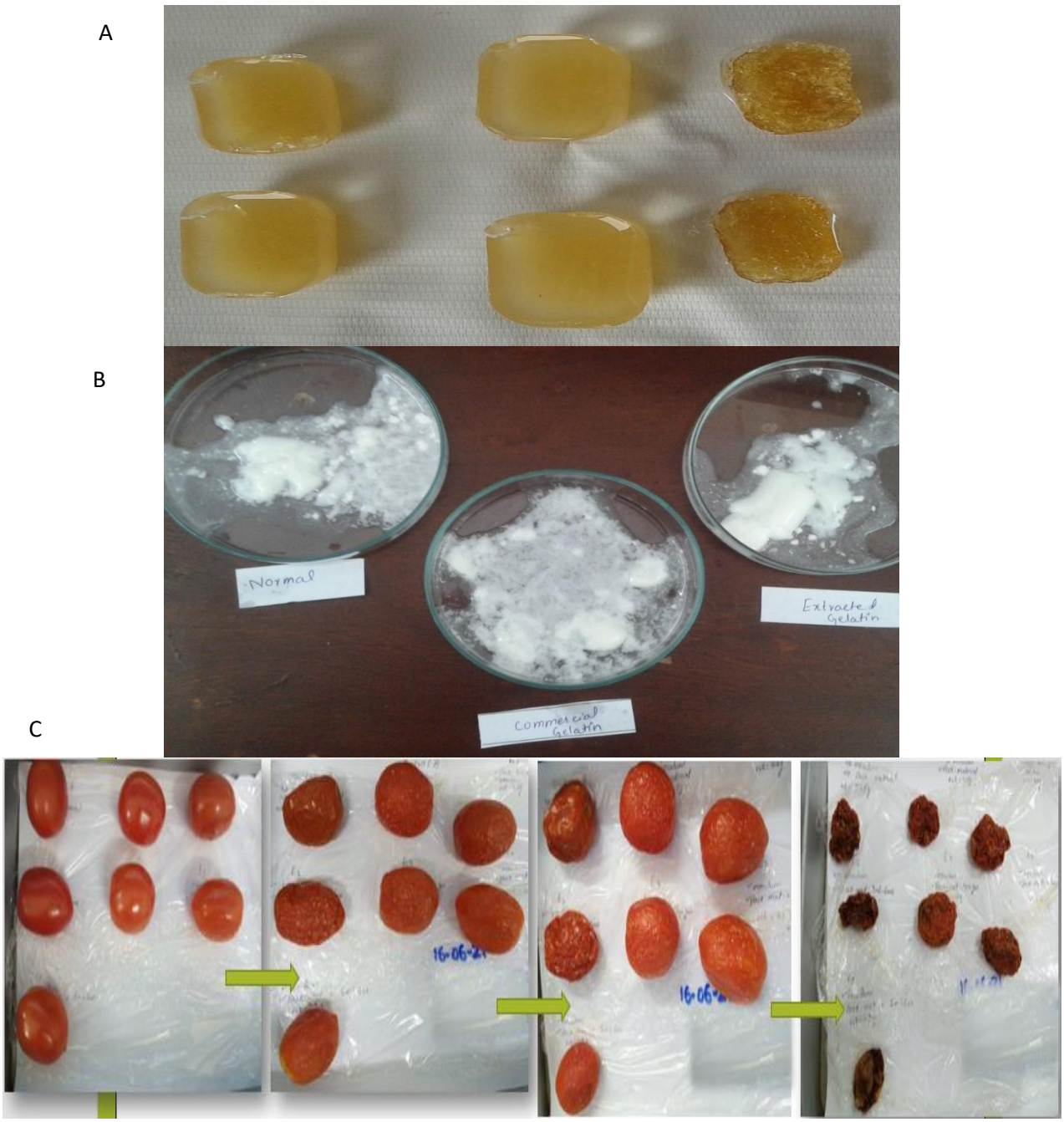

Figure 4. FSW3 extracted gelatin use in (A) synthesis of biodegradable plastic, (B) yogurt thickness (c) anti-microbial food coating for tomatoes packaging.

Reducing Sugar Content

Glucose and fructose are the main sugars present in tomatoes as reported previously. Sample D and G contain the highest reducing sugar contents as compared to fresh tomato labeled as control sample F.T (Fresh Tomato) shown in figure 5C. Sample E shows the lowest sugar content on 21st day.

Total Phenolic Content

During the ripening of tomatoes, a decrease in phenolic content causes increases fruit softness. Sample C and D showed low phenolic content as compared to samples F.T, A, B, E, F, G.

Biofilm Formation from the Bacteria Obtained from Fish Scales

Biofilm formation was performed by using the three bacteria isolated from fish scales. Clear biofilm was produced by FSW1, FSO2, FSW3, and FST4 while biofilm produced by FSW1 was not much better. Results of optical density of broth after 3 days and of ethanol is shown in table 1.6 and 1.7. The biofilm ring formation is shown in Figure 6. Biofilm was visible as a purple color ring along the test tube linings showing attached biofilm cells that retained the dye (Figure 6A). The highest biofilm formation was recorded in isolates FSW3 as compared to the rest of the isolates (Figure 6B).

Gelatin Based Anti-adhesion Film

Gelatin-based film was formed and shown in figure 7A. It was ecoated on a polystyrene sheet and glass slide and showed the reduction of biofilm formation on the polystyrene sheets coated with gelatin-based films (Figures 7B and C).

Discussion

Consumption of livestock increases, and waste products also get accumulated in the environment. Efforts should be oriented to use waste materials in the form of useful products to reduce pollution. The present study is aimed to utilize fish waste products into value addition that is acceptable due to being halal i.e gelatin. collected sample in addition to bacterial cultures isolated. A review of microscopic, cultural characteristics and biochemical profiles reported that isolated bacterial cultures might belong to *Pseudomonas* spp., *Staphylococcus* spp., and *Bacillus* spp. he previous studies performed in Thailand concluded that *Bacillus cereus* is involved in collagen hydrolysis (Pal & Suresh, 2016). Microbe-mediated extraction was done by optimizing the growth of microbes to 0.5 at 600 nm which increases the yield up to 61.4 %. From previous literature reported in 2019, it was proved that gelatin production was increased up to 80 % by enzymatic hydrolysis followed by hydrothermal pretreatment (Zhang et al., 2019). The pH of the extracted gelatin was 4 whereas its value is 4 in the previous studies (Matmaroh et al., 2011). The moisture

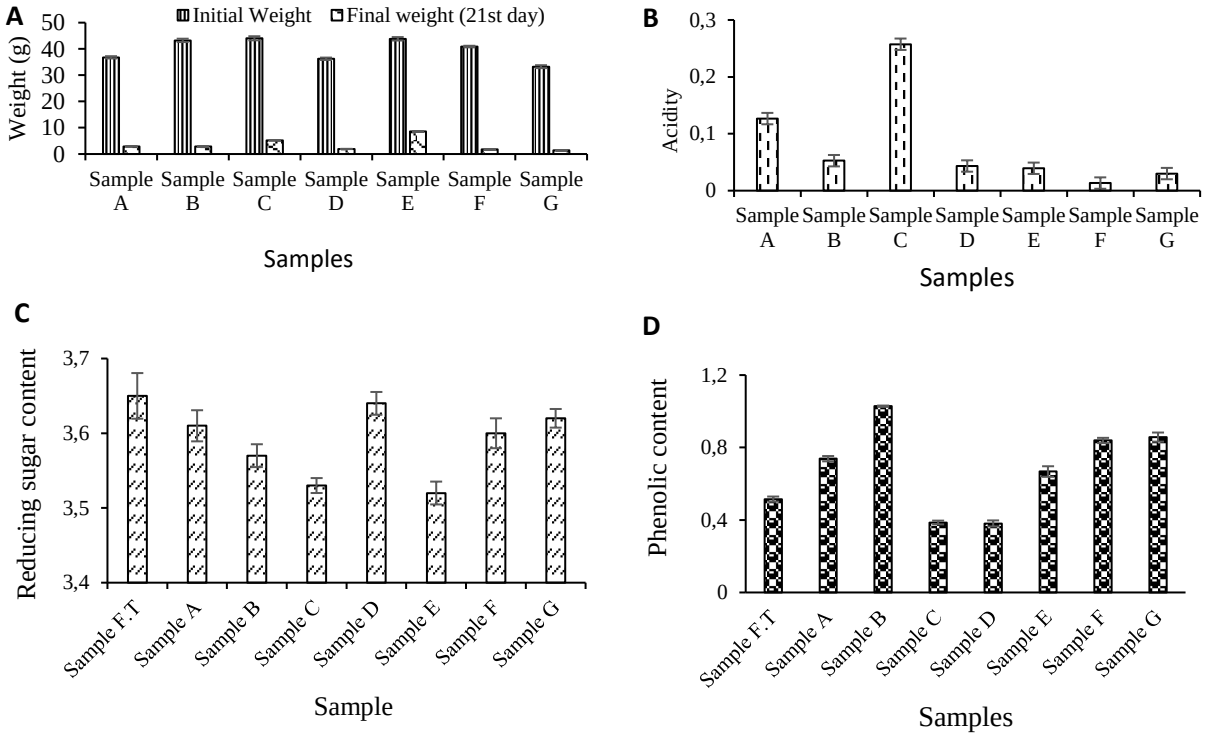

Figure 5. Physiological Studies of FSW3 extracted gelatin coated tomatoes (A) Comparison between initial and final weights of samples at 21st day, (B) Titratable acidity of samples at 21st day, (C) Reducing sugar content of samples at 21st day, (D) Total phenolic content of samples at 21st day.

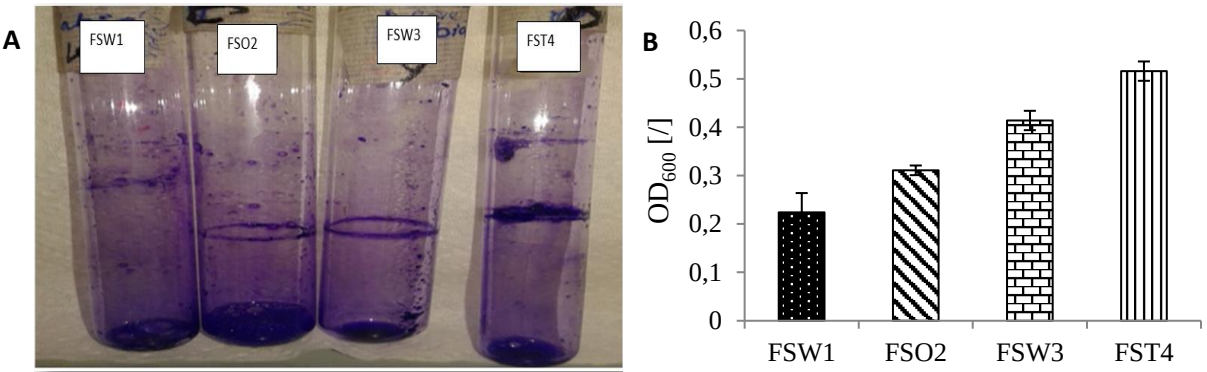

Figure 6. Biofilm formation assay (A) Biofilm ring formation in test tubes (B) Biofilm quantification.

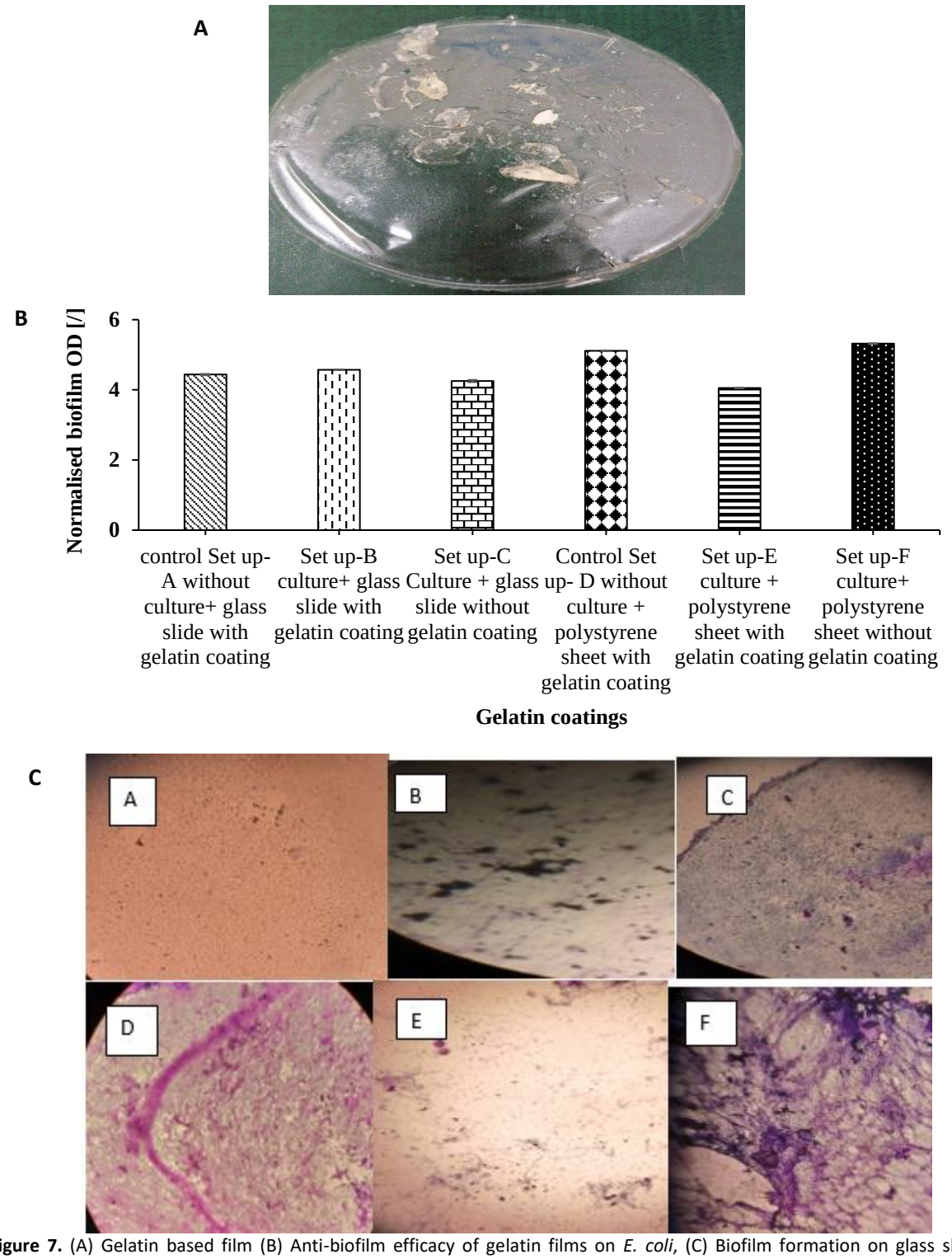

Figure 7. (A) Gelatin based film (B) Anti-biofilm efficacy of gelatin films on *E. coli*, (C) Biofilm formation on glass slides and polystyrene sheets.

content of extracted gelatin was (8.8 %) lower than the moisture content of the gelatin (15 %) as reported earlier (Karim & Bhat, 2009). Ash content was lower (1.4 %) than the values reported in previous studies i.e. 2.6 % (Zhou *et al.,* 2006). Proline and protein analysis of extracted gelatin showed control sample yields the highest proline and protein content than the gelatin obtained from cultures. Previous studies in India reported that 90 % protein content was present in gelatin extracted from fish scales (Merina et al., 2017).

FTIR spectrum shows the functional groups and secondary structures (Muyonga et al., 2004). In a previous study, it was reported that different vibrational modes of peptide bonds can be represented as an amide bond. Hydrogen bonding coupled with COO can be represented by the amide I (1630cm^{-1}) band, bending vibrations of N-H groups and the stretching vibrations by C-N groups can be shown as amide II (1565 cm^{-1}) band. Vibrations in the plane of N-H and C-N bound amide can be shown as amide III (1240 cm^{-1}) band (Merina et al.,

2017). Differences in spectral analysis of extracted protein were because of differences in polypeptide chain conformation. It was proved in previous studies that amide I conformation plays an important role to study the nature and extent of conformational changes of proteins. Fish scale gelatin has a slightly higher value for amide I band which shows loss of molecular order because of thermal un-coupling of the intermolecular cross-link as reported in previous studies (Ahmad et al., 2011). Muyonga et al. (2004) proved that low temperatures give rise to lower amide I band.

UV-vis. Spectrophotometer was used to describe the absorption of chromophore groups at 210-240 nm which indicates the presence of characteristic peptide bond fragments (Hermanto & Fatimah, 2013). Spectrum values were the same proved from the past and previous studies.

The gelatin extracted from fish scales in this study was used in making biodegradable plastics by using glycerin and compared with the plastic obtained from commercial gelatin. Results showed that plastic obtained from fish scale gelatin was better than produced from commercial gelatin. Gelatin-based film was formed and coated on a polystyrene sheet and glass slide and showed the reduction of biofilm formation on the polystyrene sheets coated with gelatin-based films. Thin films are shown at the corners of the plastic. Over the past few decades, observations show that a remarkable increase in use of natural polymer-based films and coatings had occurred in the packaging of the food industry that helps in the protection of food from contamination, lowering its deterioration, extending its shelf-life and maintaining its quality and safety (Malhotra et al., 2015). Past and present studies revealed that the presence of gelatin-based anti-adhesion films helps in controlling bacterial growth. Another study conducted in Japan showed that gelatin-derived films were sufficient in cytocompatibility and they have a property of biodegradability that makes them better to use in surgery (Mizuta et al., 2021).

Low concentrations of gelatin were found to be effective in thickning of yogurt as well as plant growth promotion under salt stress, however, at higher concentration of gelatin results in retardation in seedling growth due to fungal contamination. From previous studies, it was proved that gelatin films are better to use than conventional films because they do not have any cytotoxic effects (Horii et al., 2018; Lu et al., 2022). Arab et al., 2022 also reported the efficacy of gelatin in yogurt thickness and firmness. Future implications of present study seems to be in biological fertilisers or soil formulations.

Previous studies concluded that gelatin-chitosan coatings being edible can increase the shelf life of Indian salmon fillets along with garlic and lime extracts (Thaker et al., 2017). Results showed that weight loss was minimum in sample E as compared to the rest of the samples which proved that equal concentrations of garlic and lime extract helped reduce the weight loss. A study conducted in Spain proved that gelatin coatings along with cellulose nanocrystals help to reduce the loss of acid present in strawberries (Fakhouri et al., 2014).

The highest amount of total reducing content was present in D and G on the 21st day while the lowest value for sample E was observed for reducing content. The highest phenolic content was observed in sample B and while Sample C and D shows the lowest value. Research conducted in 2014 showed that gelatin and zein coatings helped maintaining the sugar and phenolic contents in mango (Gol & Rao, 2014). A compressive study performed in Thailand helps to reveal that Shellac and gelatin-based composite films help to maintain sugar content and phenolic content in banana fruits (Soradech et al., 2017).The benefits of coating film on food can help to overcome food insecurity by preventing the coated samples from loss in weight, and acid content, reducing sugar content, delaying the decay incidence of food, and loss of phenolic content in food. However, further biosafety concerns must be considered before processing the coatings on food items as lot of research is needed in this context before reaching any final application.

Conclusions

In the present study, among three bacterial strains isolated from fish scales, one isolate FSW3 (*Staphylococcus* spp.) showed significantly increased gelatin yield. Physiochemical properties and characterization of FSW3 extracted gelatin showed promising use from a biotechnology perspective. Anti-microbial coating prepared from extracted gelatin helped increase post-harvest life of tomatoes in combination with chitosan, lime, and garlic extract. Microbe-mediated gelatin resulted in promoted plant's growth under salt stress, enhanced firmness to yogurt, effective against biofilm formation, and synthesis of biodegradable plastics. The study provides a very promising approach for fish scales microbe mediated gelatin extraction and uses it to explore new avenues in biotechnology and microbiology.

Ethical Statement

Studies involving animal subjects Generated Statement: No animal data is included in this study.

Studies involving human subjects Generated Statement: No human studies are presented in this manuscript.

Inclusion of identifiable human data Generated Statement: No potentially identifiable human images or data being presented in this study.

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Author Contribution

Conceptualization, Rao, Rabia A.; Qurashi AW, Liaqat I, Gohar M, Bahadur S.; methodology, Rao, Rabia A.; Qurashi AW, Zafar U, Gohar M.; software, Qurashi AW.; validation, Rao, Rabia A.; Qurashi AW, Liaqat I, Gohar M; formal analysis; Liaqat I, Qurashi AW, Gohar M. Khan MA.; resources, writing—review and editing, Rao, Rabia A.; Liaqat I, Zafar U, Qurashi AW, Bahadur S. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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