Using Gelatin-Iron Oxide Nanoplexes as binder and Preservative for Minced Beef Meat

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Abstract- Meat and meat products are rich source of proteins, which serve as a good food for microbial growth. Microbial contamination leads to food spoilage. There are different techniques that have been used for preservation of meat. Colloidal iron (iron nanoparticles) has showed tremendous potential in latest reports as an antibacterial agent due to its regulated size, low toxicity, and strong magnetic nature. Gelatin act as a binding and stabilizing agent. It alters the properties of the nanocomposite and ensuring the long-term durability of the nanoparticles by preventing particle clumping. This method is simple and "green" as it produces no toxic by-products.

Total (n=15) food samples were gathered from bakeries and grocery shops. Food pathogens were isolated and characterized using Gramme staining and routine biochemical testing. Iron oxide nanoparticles were synthesized using Gelatin as stabilizing agent. Characterization of Gelatin-Iron oxide nanoparticles were performed by UV, FTIR, XRD and SEM techniques. The inhibiting effect of Gelatin-iron oxide nanoparticles was investigated using the broth micro-dilution process. The preservative effect of Gelatin-Iron oxide nanoparticles was assessed on 10g minced beef meat, coated with three different concentrations (62.5 µg/mL, 31.2µg/mL and 15.6µg/mL). The meat was stored at 4℃ and 25℃ for 16 days for microbial analysis.

The results demonstrated that, Out of (n=15) food samples, total estimate of positive samples for *E. coli* was 4 from both bakery and grocery stores, while 3 samples were contaminated with *S.aureus* and 2 sample indicated the presence of *P. aeruginosa*. Gelatin-Iron oxide nanoplexes were found to be very efficacious with a lowest minimum inhibitory concentration (MIC) of 62.5 µg/mL, 31.2µg/mL and 15.6µg/mL against *P. aeruginosa*, *E. coli* and *S.aureus* respectively. The Total Viable Count (TVC) in minced meat specimens was substantially elevated ($p<0.05$) in the control group throughout the storage days. While TVC in treated samples of meat was under the accepted range of $5*10^5$ according to ICMSF, up to 8 days of storage at 4℃. Similarly, the TVC in meat stored at 25℃, still below this limit up to 6 days. So, it can be concluded that Gelatin-Iron oxide nanoparticles in lower concentration 62.5µg/mL shows maximum potential to sustain the integrity of minced meat in contrast with other treated specimens. As a result, we suggested minimizing the use of harmful additives for meat preservation.

Index Terms- Gelatin, Iron-Oxide Nanoplexes, Preservative, Beef Meat

I. INTRODUCTION

The food market is a huge consumer of Gelatin. Gelatin act as a binder for food products. It can be incorporated into dairy products to stabilize whey and thereby hamper secretion of aqueous whey production from yoghurts, curds, and cream cheese. It is also implemented in meat and aspics as an adhesion and/or coating agent. The meat processing business uses Gelatin in their products for a variety of purposes, like ensuring optimum texture and taste. (Haug, I. J., & Draget, K. I. 2009). Gelatin is a protein-rich, fat-free, free from cholesterol and calorie-free substance. (Bagal-Kestwal *et al.*, 2019). Gelatin is used to substitute a portion of the fats in reduced-fat butter and allowing the product to stick together and lower its calorific content while maintaining viscosity, texture, and flavor (Gómez-Guillén *et al.*, 2011). Gelatin has a significant advantage in the field of medicine due to its solubility and ease of digestion by humans. It is mostly employed for its film-forming, integrating and adhesive capabilities, as well as its hot water-soluble properties (BagalKestwal *et al.*, 2019). It is an important component in the manufacture of both soft and hard-core Gelatin capsules. Gelatin is also utilized as a tablet binder and as a coating substance to make ingestion easier or to cover undesirable tastes. Gelatin is a protein, so it works as a nutrient for microbes. According to the literature, microorganisms hydrolyzed the complex Gelatin and decomposed it into its constituent amino acids, that bacteria use in their biological metabolic activities (Sharma et al., 2006). It was also found that supplementing with peptone and Gelatin resulted in increased biomass output, possibly because they can both be utilized as nitrogen and carbon source (de Silva *et al.*, 2001). Gelatin appears to be a viable supply of chemicals required for the development of microorganisms like streptococci, pneumococci, dysentery bacilli, Brucella, and others that do not thrive on conventional synthetic media. (De Paula *et al.*, 2018). As we mentioned above Gelatin is a source of nutrient for microbial development, therefore we

complexed it with iron oxide nanoparticles to impart its antimicrobial properties. Colloidal iron (iron NPs) has showed immense potential as an antimicrobial in current research because of their regulated size and low toxicity (Akhter *et al.,* 2019). Iron oxide nanoparticles are used to inhibit the growth of a variety of foodborne pathogens, including *E. coli*, *S. aureus*, and *P. aeruginosa* (Azam *et al.,* 2012; Tran *et al.,* 2010). Iron oxide nanoparticles feasibly effective of creating O_2 oxidants capable of eradicating microbes by damaging proteins and DNA yet inflicting no harm to non-microbial tissues and in certain cases boosting osteoblast formation, so serving a dual function (Tran *et al.,* 2010).

II. Materials and Methods

Sampling size and area

In this research total (n=15) samples of various food items which included, 8 samples from bakery origin i.e., bread (n=1), cakes (n=2), pastries (n=2), biscuits with coconut filling (n=1), rasmalai (syrup filled rolls) (n=1), biscuits with chocolate chunks (n=1), and 7 grocery shop samples, including meat (n=1), potato (n=2), cooked rice (n=1), mixed samosa (rissole) (n=1), and tomato (n=2) were collected from District Kohat. The initial screening was performed in Microbiology and synthesis of Gelatin-Iron Oxide Nanoplexes were done in Pharmacy department at Kohat University of Science and Technology, Khyber Pakhtunkhwa, Pakistan. All the specimens were swiftly shipped to Microbiology Laboratory of Kohat University of Science and Technology.

Bacterial tests isolates and biochemical Analysis

The spread plate method was applied to isolate the food-borne bacteria from bakery and grocery food items. However, different Biochemical tests were performed as by Wang *et al.* (2015) and El-Gendy *et al.* (2021).

Preparation of Gelatin-Iron oxide nanoplexes and Characterization of nanoparticles

Chemical precipitation method was used for the preparation of Gelatin-Iron oxide nanoplexes as describe by Selimovic *et al.* (2022) with some modifications. Characterization of Gelatin-Fe_3O_4 nanoparticles were done by Ultraviolet spectrophotometry (UV), Scanning electron microscopy (SEM), X-ray diffraction (XRD), Fourier transform Infrared spectroscopy (FTIR) techniques (Jamzad & Bidkorpeh, 2020). For the Characterization of Gelatin-Fe_3O_4 nanoparticles, the sample was proceeded to the central research laboratory University of Peshawar, Pakistan.

Antibacterial Activity Assay and Determination of minimum inhibitory concentration (MIC)

The antibacterial activity of Iron oxide nanoparticles was conducted via an agar-well diffusion assay as previously reported by Moges, A., & Goud, V. V. (2022). MIC was determined by broth microdilution method as reported by Weerakkody *et al.* 2010; Brown-Elliott *et al.,* 2012; Brook *et al.,* 2013; Balouiri *et al.,* 2016 and Wiegand *et al.,* 2008; ISO-20776-1, 2019). The all food pathogens test isolates were treated in the same way.

Meat sample coating and storage Microbiological analysis of meat

Coating and storage of beef meat sample was followed according to Hussain *et al.,* (2020). Microbiological analysis was performed by pour plate count technique explained by Wang *et al.* (2015).

Statistical analysis

The experiment was intended with Gelatin-Iron oxide nanoparticles treatments and storage time frame as fixed parameters and replicates as random factors. The data were presented as mean±SD and analyzed using Statistix version 09 software. All the parameters were carried out in triplicates. The p value <0.05 was taken to be statistically significant.

III. RESULTS

Isolated strains of foodborne pathogens Identification of foodborne bacteria

The current study found that out of (n=15) samples of food, the total number of positive samples obtained from bakeries and grocery shops were 9. Though 5 samples were positive from bakery-based items containing (n=2 *E. coli*, n=2 *S. aurous* and n=1 *P. aeruginosa*) while 4 samples were positive from grocery items namely (n=2 *E. coli*, n=1 *S. aurous* and n=1 *P. aeruginosa*). The *E. coli* and *P. aeruginosa* were appeared in pink color, which illustrated Gram negative bacteria under microscopic examination while *S. aurous* was appeared in blue color which indicated Gram positive bacteria. *E. coli* was the most common pathogen found in bakery and supermarket goods, followed by S. aurous and P. aeruginosa. This was verified further by culturing on differential and selective medium, along with biochemical test characterization (table 1). *S. aurous* and *E. coli* were found in each of the positive samples, which is very alarming and indicates unclean manufacturing processes. Although *Pseudomonas spp*. is a common bacterium that can be noticed anywhere, its presence in food items are thought to be inappropriate.

Table 1. Results of Biochemical tests used against isolated food borne microorganisms.

Bacteria	Gram Staining	Catalyse	Oxidase	Citrate	Coagulase	Indole	Methyl red
E. coli	-	+	-	-	-	+	+
S. aureus	+	+	-	+	+	-	+
P. aeruginosa	-	+	+	+	-	-	-

Iron oxide nanoparticle synthesis and characterization

The animal base Gelatin may act as either a reducing and a capping agent to form a strong coating on metallic iron oxide nanoparticles and the incorporation of ammonia causes the color to shift from darkish orange to brownish black. The color change gave the confirmation of the synthesis of Gelatin- Fe_3O_4 nanoparticles. It

indicated that Gelatin is an effective capping and reducing agent while ammonia has the ability to synthesize Fe_3O_4 nanoparticle.

UV analysis was used to provide further verification. The sample was sent to the University of Peshawar, Pakistan for characterization of Gelatin-Fe3O4 nanoparticles.

UV spectrophotometry (UV)

The obtained blackish magnetic Fe_3O_4 nanoplexes were confirmed by Shimadzu UV-1800 double bean spectrophotometer, through the appearance of characteristic band around a wavelength of 231 nm, shown in **(Fig.1)**

Scanning electron microscopy (SEM)

The surface characteristics of Gelatin-Fe3O4 nanoparticles has been assessed using the SEM analysis, as shown in **Fig. 2.** SEM results clearly showed the presence of synthesized nanoplexes. The Fe_3O_4 nanoplexes were cubic in shape. Because of aggregation of nanoparticles, only few individual particles were observed.

X-ray diffraction (XRD)

XRD pattern has been broadly used in nanoparticle study to illustrate crystal structure and size of nanoparticles. **Fig. 3** shows the XRD spectrum of Gelatin- Fe_3O_4 nanoparticles. The XRD diffractogram of Gelatin based iron oxide nanoplexes showed clearly crystalline nature of sample and displayed numerous unique peaks at (220), (311), (400) and (440). The size of nanoparticles was determined by Scherrer equation,

$$D\ (\text{Size nm}) = K\lambda/\beta\cos\theta$$

Where the wavelength is 1.540598, peak position (2 theta) is 33.63768, FWHM value is 3.55544 and D (nm) value is 23.34222656 nm, so the overall size is ~23nm. The XRD results was obtained from QUI.

Fourier transform Infrared spectroscopy

FTIR spectrum shows the functional group of Gelatin and Iron Oxide at different wavelengths. These functional groups include, N-H Stretching at 3120 cm^{-1}, the CH_3 stretching at 3002 cm^{1}, C=O group at 2070 cm^{-1} and characteristic Fe-O bond bending at 578 cm^{-1} of Fe_3O_4, as shown in **Fig.4**. The FTIR scanning was captured via Subtech Ascii (range 4000 cm^{-1} - 400 cm^{-1}) from central research laboratory, University of Peshawar, Pakistan.

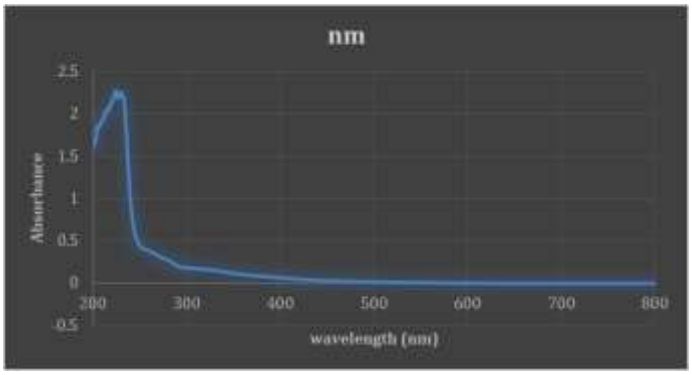


Figure 1: UV evaluation of Gelatin-Fe3O4 Nps. The graph depicts the typical band at a wavelength of approximately 231 nm.

The Shimadzu Uv-1800 double bean spectrophotometer was used for the UV analysis.

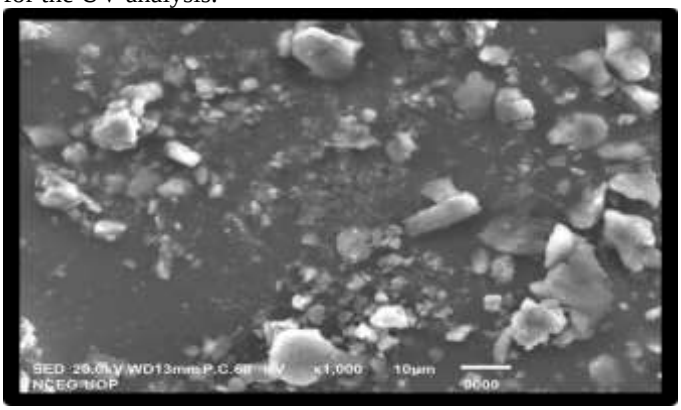


Figure 2: SEM picture shows Gelatin-Fe3O4 nanoparticles. It exhibits the crystalline nature of Gelatin-Iron Oxide nanoplexes. The picture of SEM was obtained via JEOL SEM JSM 5910.

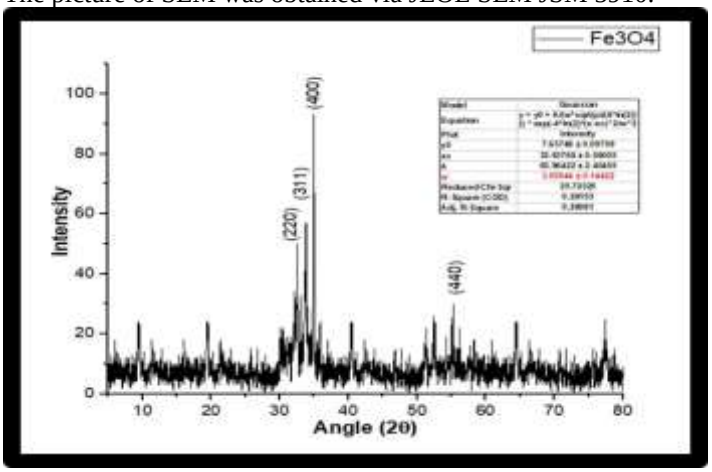


Figure 3: XRD analysis of Gelatin based Fe_3O_4 nanoparticles. The Scherrer equation was used to calculate the size of nanoparticles, which was 23nm. The XRD results was taken from Quaid e Azam university, Islamabad, Pakistan.

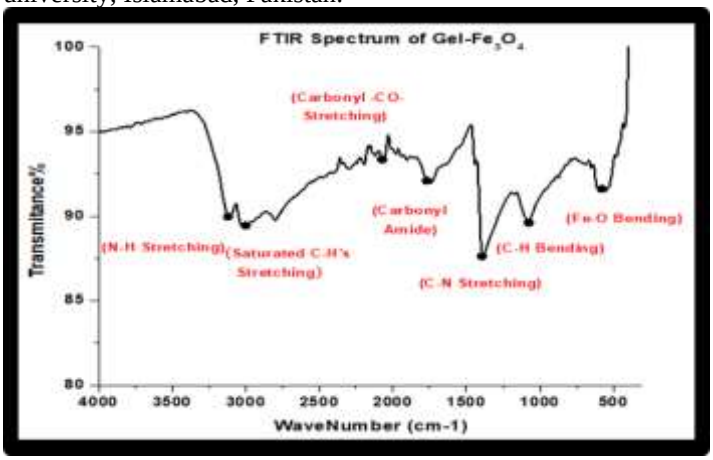


Figure 4: FTIR analysis of Gelatin-Fe_3O_4 nanoparticles. It shows the functional group of Gelatin and Iron Oxide at different wavelengths. The FTIR scanning was captured on Subtech Ascii (range 4000cm-1 - 400 cm-1) from central research laboratory, University of Peshawar, Pakistan.

Antibacterial Activity Assay

Initially, we checked the inhibitory activity of iron oxide nanoparticles without coating with Gelatin to confirm, whether iron oxide nanoplexes have antimicrobial properties or not. Iron oxide nanoplexes were found to be active against all the test isolates of food-borne pathogens. *E. coli* was found to be most sensitive to iron oxide nanoparticles with zone of inhibition of 19 mm, 17.5 mm and 16mm at the concentration of 300µg/mL, 250 µg/mL and 200µg/mL respectively, followed by *S. aureus* which was recorded at 15 mm,13 mm and 12 mm at the concentration of 300µg/mL, 250 µg/mL and 200µg/mL respectively. While *P. aeruginosa* was found to be partially sensitive to iron oxide nanoparticles and zone of inhibition was recorded at 12.5 mm, 11mm and 10 mm at the concentration of 300µg/mL, 250 µg/mL and 200µg/mL respectively, shown in **Fig.4**. The antimicrobial activity of iron oxide nanoplexes is due to ions in iron oxide, they bind to membrane proteins (thiol groups) and generate oxidative stress, resulting in protein degradation and membrane impermeability. All of this finally results in microbial death.

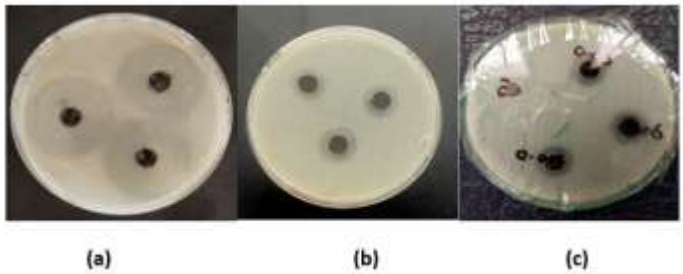


Figure 5: Iron oxide nanoparticles had a suppressive impact against (a) *E. coli*, (b) *S. aureus*, and (c) *P. aeruginosa*. The bar graph below depicts this activity.

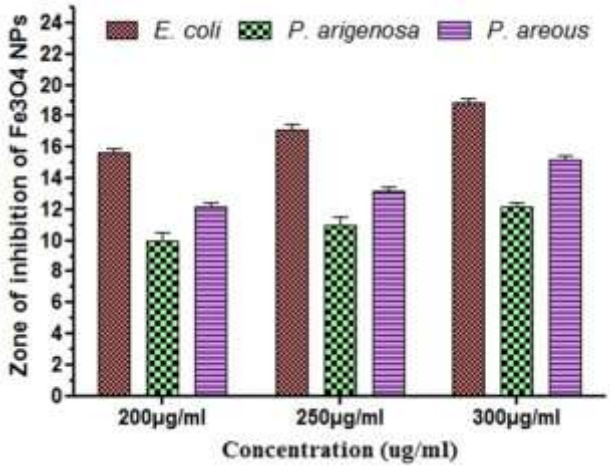


Figure 6: Bar graph of zone of inhibition of *E. coli*, *S. aureus* and *P. aeruginosa.*

MIC of Gelatin-Iron Oxide nanoparticles against isolated specimens of foodborne pathogens

Following the incubation period, 25l of resazurin dye was poured into each microtiter plate well, from left to right, up to well number 12. The samples were then incubated for 2 to 4 hours to examine color shift. After incubation, rows with no shade change (blue resazurin color remained unaltered) were reported as beyond the MIC value. The dye resazurin served as an indicator. The living (viable) cells looked pink, whereas the deceased cells seemed blue. Figures below show the MIC for *E. coli, S. aureus* and *P. aeruginosa.*

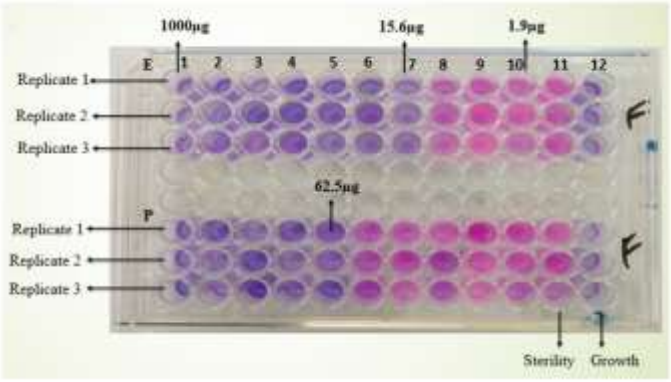


Figure 7: Determination of MIC against isolated strains of foodborne pathogens. The initial concentration was 1000µl, and the final concentration was 1.9µl of Gelatin-Iron oxide nanoparticles against *E. coli* and *P. aeruginosa*. Following the incubation period, 25µl of resazurin dye was introduced to each well. The row no.11 served as sterility or negative control whereas, row no. 12 was acted as growth or positive control. The negative control, row number 11, converted the natural color of resazurin (blue or purple) to the reduced state (red-colorless), whereas raw number 12 indicated living cells with no color alteration. Because there were no color changes in row 7, the concentration of gelatin-based iron oxide nanoparticles was used as the MIC value against *E. coli*, while the amount of Gelatin-Iron oxide in this well was 15.6g/mL. The MIC of *P. aeruginosa* was demonstrated in the following experiment, where row no. 5 did not change any color; hence, in this row the concentration of Gelatin-Iron oxide nanoparticles was chosen as the MIC value against *P. aeruginosa*, and the concentration of Gelatin-Iron oxide nanoparticles in the well was 62.5g/mL. This experiment was carried out in three replicates.

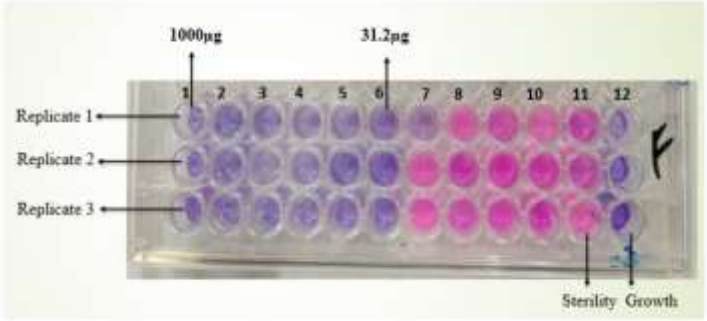
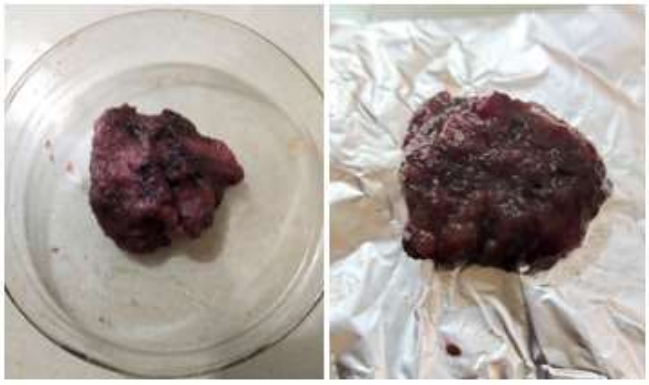


Figure 8: MIC of Gelatin-based iron oxide nanoplexes against *S. aureus* strain. The concentration of Gelatin-Iron oxide nanoplexes was used as the MIC value against *S. aureus* in row 6, since there was no color change. In this well, the concentration of gelatin-based iron oxide is 31.2 g/mL. The procedure was carried out in triplets.

Preservation of Gelatin-Iron oxide nanoparticles on minced beef meat



(A) (B)

Figure 9: A and B exhibit beef steak that has been coated with Gelatin-Fe3O4 nanoparticles.

Beef meat Preservation by coating with Gelatin-Fe_3O_4 nanoplexes at refrigerator condition

The results of coating beef meat with Gelatin-Iron oxide nanoparticles stored at 4℃ and 25℃ for 16 days were described in **Table 2** and **Table 3**, respectively. Initially, there were no significant differences between the control and treated samples. After few days, the total viable count of the control samples increased quickly than the samples treated with Gelatin-Fe_3O_4 nanoparticle. The microbial growth in control had a drastically increased from day 4 to 16 and reached the limit $1.7*10^6$ CFU/g and $2.2*10^7$, respectively, which exceed the permissible limit, by International Commission on Microbiological Specifications for Foods (M. Alizadeh-Sani., 2020, E.Z. Panagou 2014). Whereas all other treatments samples were still below this limit and retarded the microbial growth up to 8 days of storage at 4℃. Similarly, with the concentration of 62.5 mg, 31.2 mg and 15.6 mg Gelatin-Fe_3O_4 nanoparticles of meat, retarded the growth up to 6 days at 25℃. The results demonstrated that the incorporation of Gelatin-Fe3O4 nanoparticles suppressed TVC in minced beef meat.

Hence, Gelatin-Fe_3O_4 nanoparticles can be used to enhance the shelf life of minced beef meat up to 8 days at 4℃ and up to 6 days at 25℃. It is also observed that all treatments can decrease the microbial growth in comparison with control sample. Overall, as the storage days increased, the microbial load also increased, and control group had showed higher significant difference ($p<0.05$) than that of other treated samples. The obtained result was confirmed via one way ANOVA.

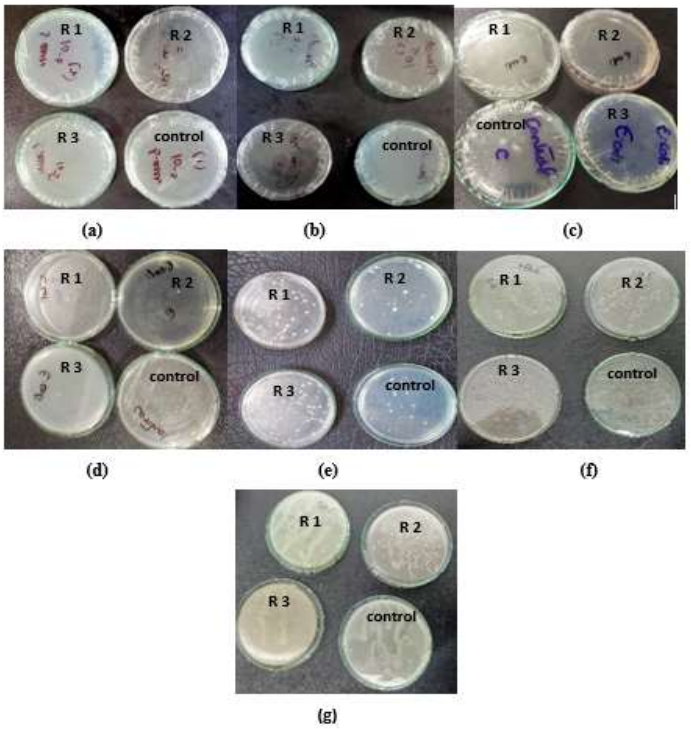


Figure 10: Microbial analysis of meat sample, tested at different days in a refrigerated condition (4ºC). (a) At day 0, there was no growth in both treated and control. (b) At day 2, same result was observed like day before. (c) At day 4, there was no growth in treated sample of iron oxide nanoplexes but few colonies were observed in control sample. (d) At day 6, treated samples were observed with no growth while control was heavily contaminated. (e) At day 8, few microbial colonies were observed in treatment samples while in control sample the microbial load reached to $9.2*10^6$, which is far beyond from accepted limit. (f) At day 12, the microbial load increased in both treated samples while in control samples, exceeded from permissible range. (g) At day 16, the microbial load reached to unacceptible limit which was not significant ($p<0.05$), according to statistical analysis. The colonies were manually counted by colony counter in the lab of pharmacy department.

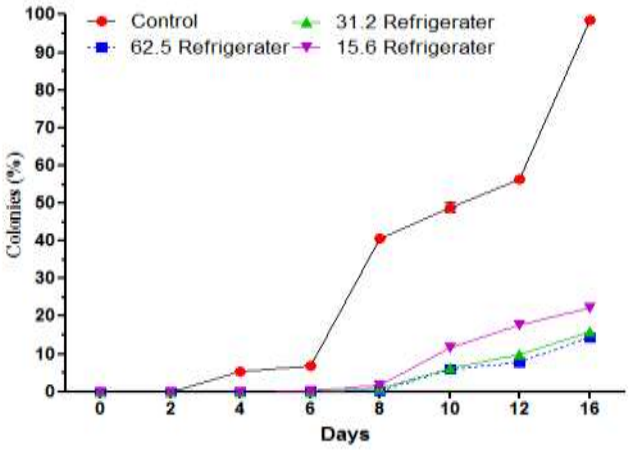


Figure 11: Effect of different concentrations of Gelatin-based iron

oxide nanoplexes on the ground beef meat stored at 4°C in percent colonies.

Table 2: Mean and standard deviation of meat sample coated with three different concentrations of Fe_3O_4 nanoplexes along with control group at 4°C.

Days	Control	Refrigerator			P values
		62.5	31.2	15.6 mg/ml	
0	0±0	0±0	0±0	0±0	NA
2	0±0	0±0	0±0	0±0	NA
4	12±3	0±0	0±0	0±0	0.2495
6	15.33±2.08	0±0	0±0	0.33±0.58	0.2625
8	91.67±1.53	0.67±0.58	2±2	3.67±0.58	0.2921
10	110.67±5.03	13.33±1.53	14.33±0.58	26.33±1.53	0.1419
12	127.67±4.51	17.67±1.53	22±2	40±1	0.1176
16	222.67±3.06	32.67±1.15	36±1	50±1	0.1453

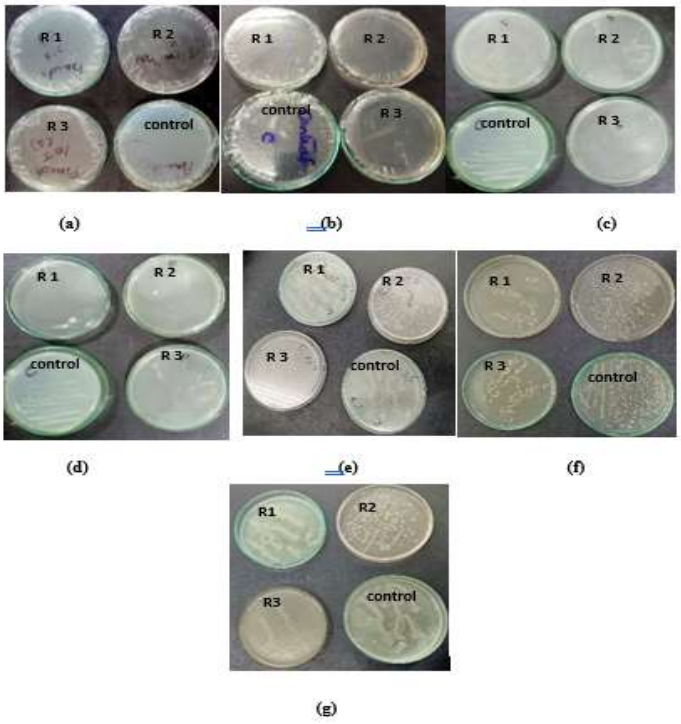


Figure 12: Microbial analysis of meat sample, tested at different days under room temperature (25°C). (a) At day 0, there was no growth in both treated and control. (b) At day 2, there was no growth in treated samples while in control group, few colonies were observed. (c) At day 4, there was no growth seen in treated samples of iron oxide nanoplexes but growth rate was increased in control sample. (d) At day 6, treated samples were encountered with few microbial colonies while control was heavily contaminated, reached to $1.05*10^7$ CFU/g. (e) At day 8, microbial load were gradually increased in treatment samples while in control sample the microbial load reached to $1.16*10^7$ CFU/g, which is far beyond from accepted limit. (f) At day 12, the microbial load increased in both treated samples while in control samples, axceeded from permissible range. (g) At day 16, the microbial load reached to unacceptible limit which was not significant ($p<0.05$), according to statistical analysis. The colonies were manually counted by colony counter in the lab of pharmacy department.

Table 3: Mean and standard deviation of meat sample coated with three different concentrations of Fe_3O_4 nanoplexes along with control group at 25°C.

Days	Control	Room Temperature			P values
		62.5	31.2	15.6 mg/ml	
0	0±0	0±0	0±0	0±0	NA
2	9±3.61	0±0	0±0	0±0	0.1487
4	36.33±1.53	1.67±1.53	3±1	4.33±0.58	0.1548
6	104.67±1.53	6.67±0.58	8.33±0.58	9±1	0.2078
8	113±3	13±1	21.67±0.58	28.67±1.53	0.1145
10	217±1	27.33±1.53	31.67±1.53	40.33±1.53	0.1486
12	380.67±5.51	41.67±1.53	48±1	52.67±1.53	0.1749
16	421.33±3.51	55±1	59±1	64.67±1.53	0.1619

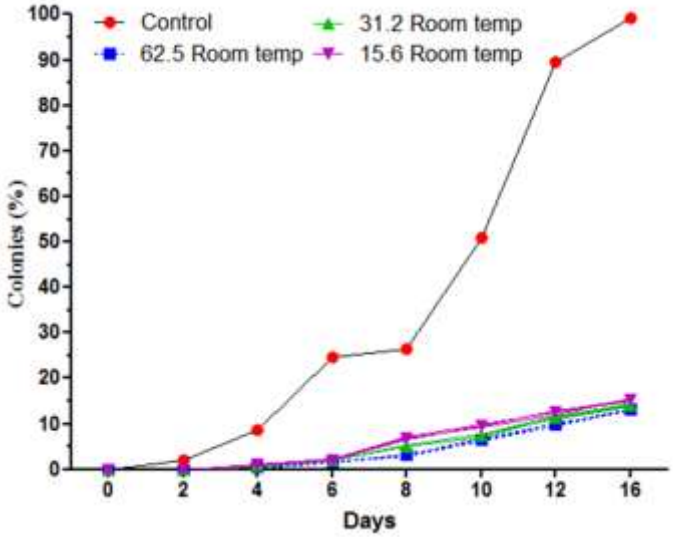


Figure 13: Effect of different concentrations of Gelatin-based iron oxide nanoplexes on the ground beef meat stored at 25°C in percent colonies.

IV. DISCUSSION

In this investigation, a total of 15 food samples were subjected to processing, and three pathogen genera (*E. coli*, *P. aeruginosa*, and *S. aureus*) were obtained. *E. coli* grew the fastest, followed by *S. aurous* and then *P. aeruginosa*. Based on previous research on nanomaterials, *E. coli* found the most prevalent in food samples (Abdalhai *et al.*, 2015). *Staphylococcus spp*. was found in the majority of the breads and cakes in the four samples. Humans require vital nutrients from bakery items. According to research, bread products account for a significant share of energy consumption. (Agte *et al.*, 2002; Bartrina et al., 2004; Vanelli *et al.*, 2005). Hence, contamination of food with *S. aurous* offers evidence on the unsanitary processing conditions.

The detrimental consequences of bare IONPs have highlighted by (Mahmoudi M *et al.*, 2011, Mahmoudi *et al.*, 2010). Its sustainability in the environment is improved and it is protected from degradation when incorporated with Gelatin.

The broth microdilution technique found the MICs of Gelatin-Iron Oxide against *E. coli*, *P. aeruginosa*, and *S. aureus* to be 62.5g, 15.6g, and 31.2g, respectively. It indicates that even at low concentrations Gelatin-based iron oxide showed best inhibitory activity.

The UV analysis of Gelatin based Fe_3O_4 Nps showed the characteristic band at a wavelength around ~231 nm. The XRD spectra of Gelatin based iron oxide nanoplexes displayed

numerous unique peaks at (220), (311), (400), (422), (511) and (440). FTIR examination of IONPs demonstrated a variety of functional groups, including the OH stretching of the COOH groups and the CH3 stretching of the Fe_3O_4 C=O group. Our outcomes coincide with those of Patil RM *et al.* (2014). While using Scherrer's formula, the crystalline size of the IONPs was 23 nm; our results correspond with (Ansari et al., 2017).

At 4ºC, the meat covered with Gelatine-Fe_3O_4 nanoplexes performed well for up to 8 days, whereas at 25ºC, the meat treated with Gelatine-Fe_3O_4 nanoplexes performed effectively for up to 6 days.

As time increase, the TVC of the control samples increased quickly than the samples treated with Gelatin-Fe_3O_4 nanoplexes. The microbial growth in control had a tremendous increased from day 4 to 16 and reached the limit $1.7*10^6$ CFU/g and $2.2*10^7$, respectively, which exceed the permissible limit, by International Commission on Microbiological Specifications for Foods (M. Alizadeh-Sani., 2020, E.Z. Panagou 2014). Whereas all other treatments samples still below this limit and retarded the microbial growth up to 8 days of storage, with concentration of 62.5 mg, 31.2 mg and 15.6 mg. The antibacterial mechanism of Fe_3O_4 NPs is assumed to be come in connect with the formation of reactive oxygen species, which are capable of interacting with microbial cell wall and cell membrane breakdown, as well as genetic material destruction. It has been observed that Fe_3O_4 at nanometer with a smaller size has antibacterial action on both Gram positive and Gram negative bacteria with an enhanced degree of inhibitory zone (Tran *et al*., 2010). In previous study by Kang *et al*., (2020) noticed that Iron oxide was more efficient in maintaining meat quality. The reduction in the TVC during storage might be due to their reduced size.

V. CONCLUSIONS

The broth dilution approach demonstrated the most antimicrobial efficacy. The synthetic Iron oxide NPs treated with Gelatin shown increased antimicrobial properties against food-borne pathogens such as *E. coli*, *S. aureus*, and *P. aeruginosa*. The antibacterial capabilities of Fe_3O_4-NPs are attributed to their unique qualities, which include their weight and wide surface area.

The meat covered with Gelatin-based Fe_3O_4 nanoplexes performed best at 4ºC for up to 8 days, while the treated meat performed well at 25ºC for up to 6 days. Furthermore, bacterial colonies were significantly ($p<0.05$) reduced in the beef meat sample by using Gelatin-Iron Oxide nanoplexes at a concentration of 62.5 mg/10g.

It was demonstrated that the number of bacteria in minced beef meat lowered when exposed to Gelatin-Iron oxide nanoplexes.

Meat covered with nanoplexes has a longer lifespan and preservation period can be extended from two days to one week. Therefore, we advised avoiding the use of harmful additives in meat preservation. Further it increases incomes, saves lives and improves country's economic state.

However, detail study is necessary to investigate the toxicological effect on mammals and highlight critical components and their interaction in preservation process.

Declarations

Authors' contributions: FK performed the experiments, conducted statistical analysis, and wrote the draft manuscript. NA and SMH helped FK in statistical analysis and layout. WU and SAK analyzed the data and provided suggestions to improve the manuscript.

Conflict of Interest: The authors declare that they have no conflict of interest.

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