



## Eco-friendly antibacterial/antioxidant photoluminescent film based on gelatin/phycoyanin liposome/zero-valent nano iron/gold-iridium pigment

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### ABSTRACT

The aim of this study was to produce biodegradable photoluminescent film that has antibacterial and antioxidant properties suitable for active food packaging. Based on this, gelatin was used to prepare the active film. Phycocyanin nanoliposome, zero-valent iron nanoparticle and gold/iridium composite photoluminescent pigment were used to modify the chemical structure of gelatin film. The central composite design was used to investigate the effect of phycocyanin nanoliposome, iron nanoparticle and pigment in investigating transparency, color properties and antioxidant property of films. The antibacterial properties of the films were investigated against three strains of *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive) and *Bacillus cereus* (Gram positive). Scanning electron microscopy (SEM) images of pure gelatin film and gelatin/phycoyanin liposome/iron/photoluminescence pigment (Gel/NL/Fe/P) composite film were examined and compared. SEM images showed the smooth and coherent surface of the gelatin film, where liposome, iron and gold/iridium nanoparticles were visible on the film surface. Examining the transparency of the film and the color properties of the films confirmed the significant effect of nanoliposome on these properties. Phycocyanin strongly increased the antioxidant property of the film by about 40%. Iron nanoparticles increased the antioxidant property of gelatin film by about 20%. Liposome and zero valent iron significantly increased the antibacterial properties of the films. The antibacterial activity of the films was almost equal to that of *Staphylococcus aureus* and *Bacillus cereus* and more than *Escherichia coli*. Gel/NL/Fe/P films with the ability to produce green color in dark conditions, with antioxidant and antibacterial properties, can be used in active food packaging, and can also be used as sensors or smart kits.

### 1. Introduction

Biodegradable films are polymeric biomaterials that are obtained from natural and renewable sources and are easily decomposed after consumption under suitable conditions of humidity, temperature, and the presence of oxygen and by the metabolism of living organisms, and no toxic substances remains in the environment. Natural polymers have many advantages over synthetic polymers, especially that these polymers are non-toxic, biodegradable, and biocompatible. The tendency to use natural and renewable polymers in various fields is

increasing so that they can replace synthetic packaging [1-3]. Gelatin can form a network of physical connections in a hybrid system. When properly plasticized, it can produce strong and flexible films. The use of gelatin in the packaging of highly perishable food products such as meat and fish is due to low cost, availability, functional characteristics, mechanical and optical properties, structural resistance against microorganisms and sensory acceptability [4].

Phycocyanin, a blue pigment, is a light receiver with antioxidant properties, which gives blue color to many cyanobacteria, and that is why they are known as blue-

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green algae. Phycocyanin is a compound soluble in water and has antioxidant properties. Phycocyanin has many applications in the food industry, cosmetic-health industries, biotechnology, medicine, disease diagnosis and treatment. Most of the attention has been focused on the use of phycocyanin as a nutrient compound, especially in healthy foods where *Spirulina Platensis* is used as an active compound. Phycocyanin is also used for medicinal purposes due to its high antioxidant properties. This combination is able to replace chemotherapy drugs with severe side effects in the treatment of cancers [5 and 6].

Compared to liposomes, nanoliposomes provide more surface area due to their smaller particle size and increase solubility, improve bioavailability and controlled release, and more precise delivery of substances to target areas. They also have higher colloidal stability and create less turbidity. Also, recently, researchers have focused on the use of nanocomposites in the production of new types of active packaging with antimicrobial compounds. The size of nanoliposomes is of interest due to its effect on stability, the release capacity of the compounds enclosed in the core and the effect on the various properties of the production films containing them [7].

Today, the use of nanoparticles to improve the mechanical properties of biopolymers in the packaging industry has eliminated the limitation of using biodegradable films. One of the important developments in the field of packaging is the use of nano bio-composites, which disperse nano particles in a biopolymer matrix and lead to the strengthening and strengthening of biopolymers. The use of nano-filler compounds in protein films and the production of nanocomposites is one of the ways to improve the characteristics of biopolymer films. The use of these compounds improves the mechanical, thermal and barrier properties of the resulting nanocomposites compared to the primary polymer. Minerals, metals and metal oxides have attracted the attention of nanotechnology researchers due to their ability and resistance to harsh processing conditions. Because nanoparticles are a bridge between the bulk state of materials and the atomic or molecular state, they are of great interest [8 and 9]. Metal oxide nanoparticles have unique chemical and physical characteristics, high surface-to-volume ratio, which lead to many applications in making antioxidant coatings. Nanotechnology is known in many industries including packaging and food processing. It is used as a nano-scale material for food packaging. Due to the nanometer size of the particles that are dispersed in the polymer matrix, they show better thermal and physicochemical properties [10].

Sustained luminescence is the phenomenon in which a substance is excited by high-energy light irradiation, and

after the excitation stops, visible light is emitted from the excited electronic states of the substance for an assessable period of time from seconds to several hours will remain. In the process of luminescence, the electrons of the target substance, which are in the ground state or energy alignment under normal conditions, are excited to more energetic states by receiving energy from a specific source. In the return of the electrons of the material from the excited state to the ground state, the excitation energy is released as light. Normally, the excitation energy is higher than the emitted light energy due to the involvement of some intramolecular or atomic transitions, including thermal energy loss. Since the luminescence process does not require high temperatures and occurs at relatively low temperatures, the emitted light is also called cold light. In photoluminescence, which is one of the most widely used types of luminescence, excitation is carried out by light photons. Fluorescence and phosphorescence are types of photoluminescence. Photoluminescence pigments have very wide applications [11 and 12].

Sensors are a tool to control the events in the surrounding environment. These tools take physical, mechanical or chemical characteristics from the environment and turn them into measurable parameters. There are different types of sensors that can detect gases, liquids and even biological reactions [13-19]. One of the types of sensors are chemical oxygen sensors based on fluorescence [20]. Fluorescent-based oxygen sensors are one of the most advanced and reliable systems for measuring gases in the empty space of packaging products. Bioactive films with antioxidant and antibacterial properties, which at the same time have the ability to identify the environment intelligently, are an ideal system in food packaging [21].

In this study, an active film with appropriate antioxidant and antibacterial properties was prepared based on gelatin. Gelatin film was modified with phycocyanin nanoliposome, iron nanoparticles and photoluminescence pigment. The prepared film had photoluminescent properties. The film produced with the ability to produce green light in dark environments has the ability to be used in attractive packaging of food products. Also, this film is suitable for active packaging of food products sensitive to oxidative spoilage and microbial spoilage due to its antioxidant and antibacterial properties and can increase the shelf life of packaged food products.

## **2. Materials and methods**

### **2.1. Chemicals**

Gelatin, phycocyanin and gold/iridium composite pigment were purchased from Temad Kala Company

(Tehran, Iran). Zero-valent iron nanoparticles, glycerol monostearate, glycerol, calcium nitrate, saturated sodium chloride and other chemical compounds were prepared and used from Merck (Germany) and Aldrich (USA). The standard strains of bacteria of *Escherichia coli* (Gram negative) O157:H7 (ATCC 25922), *Staphylococcus aureus* (Gram positive) (ATCC 6538) and *Bacillus cereus* (Gram positive) (PTCC 1154) were obtained from the Scientific and Industrial Research Organization of Iran.

## 2.2. Gelatin/liposome/iron/pigment film preparation

Phycocyanin nanoliposome was prepared and used according to the method of Chung et al. (2018) [22]. To prepare gelatin control film, polymer solution including gelatin powder was prepared in 100 mL of distilled water. Then, the solution was heated for 45 minutes at 60 °C at a speed of 500 rpm by a magnetic heater while stirring to produce a uniform solution. At this stage, glycerol was added as a softener with a concentration of 50% by weight of gelatin and the gelatin solution was stirred for 30 minutes. In order to prepare films with phycocyanin nanoliposomes, zero-valent iron nanoparticles and green photoluminescence pigment, after mixing gelatin and glycerol solution, different percentages of phycocyanin nanoliposomes on three levels and zero-valent iron nanoparticles on three levels and green photoluminescence pigment at three levels were added to the solution (according to the Table 1). To remove air bubbles, the hot dough was centrifuged for 2 minutes and poured into a Teflon mold and dried at 35 °C for 24 hours and then separated. In order to make the moisture characteristics uniform, the films were kept in a desiccator containing a saturated solution of calcium nitrate for 48 hours at a temperature of 25 °C. Finally, the prepared films were kept in special zipped bags in a dark environment until the relevant tests were performed. Fig. 1 shows the prepared videos (20 films). The images of the films are displayed in two conditions of ambient light and darkness, and the images of the films in the dark conditions show the photoluminescence properties of the films by emitting green light.

## 2.3. Scanning electron microscopy (SEM)

Surface morphology, surface gaps and surface density of pure gelatin film and composite film of gelatin/phycocyanin liposome/iron/photoluminescent

pigment were investigated using scanning electron microscope under ambient conditions. To perform this test, the film was prepared in dimensions of 0.5×0.5 cm and coated with gold nanoparticles. A scanning electron microscope with a voltage of 10 kV was used for imaging with different magnifications.

## 2.4. Color of the film

The color characteristics of the films were tested based on Hunter Lab parameters, including L\* (lightness-darkness), a\* (red-green) and b\* (yellow-blue) at 5 different points by a colorimeter using a standard white screen.

## 2.5. Film transmittance

The transmittance (opacity) of the prepared film against visible light was evaluated using a spectrophotometer, the rectangular film strip was placed inside the spectrophotometer cell with four repetitions and the absorbance was checked at a wavelength of 600 nm. The opacity of the films was calculated using the following equation:

$$O = \text{Abs}_{600} / L$$

Abs is the amount of absorption, L is the thickness of the film in millimeters and O is the amount of turbidity.

## 2.6. Antioxidant property

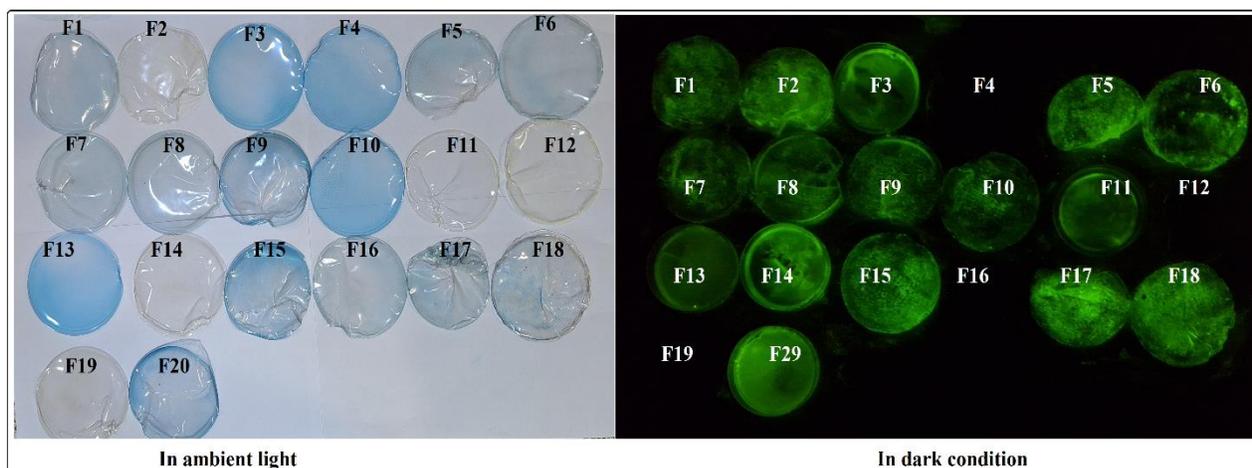
DPPH (2,2-diphenyl-1-picrylhydrazyl) radical quenching method was used to determine the antioxidant activity of the films. To determine the free radical inhibitory power, 0.025 g of the film was dissolved in distilled water (4 mL) and the mixture was shaken (5 minutes), 1 mL of 1 mM ethanol (DPPH) solution was mixed with it. The solution absorbance was recorded at 517 nm after 30 minutes of storage at room temperature. The percentage of antioxidant property was calculated as follows.

$$\text{Antioxidant (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}$$

Abs<sub>control</sub>: Absorbance of DPPH solution (1 mM)

Abs<sub>sample</sub>: Absorbance of film sample

In this test, 1 mM DPPH solution without the presence of film sample extract was considered as a control sample.



**Fig. 1.** Gelatin films prepared with photoluminescent properties by emitting green light in dark conditions

## 2.7. Antimicrobial tests

Disc diffusion method was used to investigate the antimicrobial effect on three strains of *Escherichia coli* (Gram negative) O157:H7 (ATCC 25922), *Staphylococcus aureus* (Gram positive) (ATCC 6538) and *Bacillus cereus* (Gram positive) (PTCC 1154). The films were cut into discs with a diameter of 4 mm with a punching machine. Mueller Hinton agar plate was prepared. The agar surface was inoculated with 0.1 ml of 106 CFU/mL of the bacteria. The films were placed on the surface of the prepared plate. The plates were incubated at 37 °C for 24 hours and growth inhibition zones were measured as a clear halo around the films with a digital caliper (mm).

## 2.8. Statistical analysis

The central composite design (in three levels) was used to investigate the effect of nanoliposome amount, iron amount and fluorescent pigment amount on film transparency, color properties, antioxidant properties and antibacterial properties of gelatin film (Table 1). Design Expert version 10 software was used to design the central composite statistical scheme, calculate mathematical models, regression coefficients and draw the corresponding curves. The probability level of 95% was used to check the models, analyze the data and check the difference of means.

## 3. Results and Discussion

### 3.1. Response surface method

Response surface methodology (RSM) is a set of mathematical methods that determine the relationship between one or more response variables with several independent variables (the subject of study).

**Table 1.** List of films prepared based on the central composite design

Film number	A: NL (g/100)	B: Fe (g/100)	C: P (g/100)
1	0.02	0	0.2
2	0	0.04	0.5
3	0.05	0	0.5
4	0.05	0	0
5	0.02	0.02	0.2
6	0.02	0.02	0.2
7	0.02	0.02	0.2
8	0.02	0.02	0.2
9	0.05	0.02	0.2
10	0.02	0.04	0.2
11	0.02	0.02	0.5
12	0	0	0
13	0	0	0.5
14	0.02	0.02	0.5
15	0	0.02	0.2
16	0.05	0.04	0
17	0.02	0.02	0.2
18	0.02	0.02	0.2
19	0	0.04	0
20	0.05	0.04	0.5

Although many researchers and scientists consider this method as a pseudo-model, this method is very practical and useful in multivariate analysis. In this study, the response surface method in the form of a central composite statistical design was used to investigate the effect of nanoliposome amount, iron amount, and fluorescent pigment amount on film transparency, color properties, antioxidant properties, and antibacterial properties of gelatin film. Table 2 shows the mathematical models, regression coefficients and adjusted regression coefficients between independent variables (amount of

**Table 2.** Model equations and regression coefficients for extraction efficiency of bioactive compounds

Response	Equation	R <sup>2</sup>	AdjR <sup>2</sup>
Transparency	77.07-5.60*A-1.07*B- 0.84*C+0.06*A*B+0.61*A*C+0.29*B*C+3.36*A <sup>2</sup> + 1.07 *B <sup>2</sup> - 1.44*C <sup>2</sup>	0.951	0.907
	77.81-5.55*A-0.78*B- 0.77*C+0.24*A*B+0.001*A*C+0.22*B*C+2.95*A <sup>2</sup> +1.17*B <sup>2</sup> - 1.80 *C <sup>2</sup>		
L	-5.19-4.07*A+0.91*B-1.06*C+0.32*A*B+0.11*A*C- 0.23*B*C+2.09*A <sup>2</sup> -1.0*B <sup>2</sup> +0.49*C <sup>2</sup>	0.972	0.946
a	-8.39-10.62*A+1.51*B+0.87*C+0.48*A*B-0.45*A*C- 1.23*B*C+9.93*A <sup>2</sup> -1.80*B <sup>2</sup> -3.65* C <sup>2</sup>	0.982	0.966
b	25.50+9.34*A+3.11*B+0.71*C-5.28*A*B-0.68*A*C- 0.67*B*C	0.929	0.866
Antioxidant	4.94+1.13*A+1.37*B+0.04*C- 0.85*A*B+0.04*A*C+0.04*B*C-0.53*A <sup>2</sup> -0.28*B <sup>2</sup> -0.33*C <sup>2</sup>	0.902	0.856
E. coli	5.74+1.27*A+1.50*B-0.01*C- 1.01*A*B+0.04*A*C+0.00*B*C-0.63*A <sup>2</sup> -0.55* B <sup>2</sup> -0.24*C <sup>2</sup>	0.936	0.878
S. aureus	5.43+1.25*A+1.48*B-0.01*C- 0.94*A*B+0.06*A*C+0.02*B*C-0.50*A <sup>2</sup> -0.28*B <sup>2</sup> -0.51*C <sup>2</sup>	0.929	0.865
B. cereus		0.983	0.974

nanoliposome, amount of iron and amount of fluorescent pigment) and dependent variables (film transparency, color properties, antioxidant properties and antibacterial properties).

### 3.2. Examination of surface morphology

Fig. 2 shows SEM images of pure gelatin film and gelatin/liposome/iron/photoluminescent pigment film. As it is clear, the pure gelatin film is a homogeneous and uniform film without pores. The smooth surface without cracks in this film indicates the structural strength of the film, which indicates the proper interactions between gelatin polymer chains. The smooth and gap-free structure has a very good effect on the physical and mechanical structure of the films, which can guarantee the practical use of these films. In the gelatin/liposome/iron/photoluminescent pigment film, the presence of liposome nanoparticles, iron nanoparticles and photoluminescent pigment nanoparticles (iron/iridium) on the surface of the film is quite evident. Particles are observed in the form of grains on the surface of the film almost uniformly. These particles, which have most of the antioxidant and antibacterial properties, considering that they are present on the surface of the film, they can show their antioxidant and antibacterial activity well, and the results of the investigation of these properties confirm the mentioned claim. Chen et al. (2022) have investigated the effect of nanoparticles on the surface morphology of gelatin film composited with chitosan.

They have confirmed the presence of nanoparticles on the surface of the film with SEM images, and the results of their research are in good agreement with the results of the present research [23].

### 3.3. Film transmittance

The turbidity and light transmission in biodegradable films are of great importance, because in addition to affecting the appearance of the packaging, it can also be effective in the optical protection of food products sensitive to photo-oxidative spoilage. In modified gelatin films that have photoluminescent properties and create a special effect in dark environments by emitting green light, the opacity and light transmission rate are affected by additives added to the film structure. Fig. 3 shows three-dimensional (3D) and perturbation curves of the effect of three factors of nanoliposome, iron and photoluminescent pigment on film transmittance. As it is seen, nanoliposome had the greatest effect on transmittance, and iron and pigment showed very little effect on transmittance. Due to the blue color of phycocyanin nanoliposome, this additive shows its effect by changing the color of the film from colorless to blue. Considering that the photoluminescent pigment shows its light only in dark conditions and is colorless in ambient light conditions, and since the transmittance of the film was measured in ambient light conditions, this pigment has very little effect on the transmittance of film at ambient light conditions.

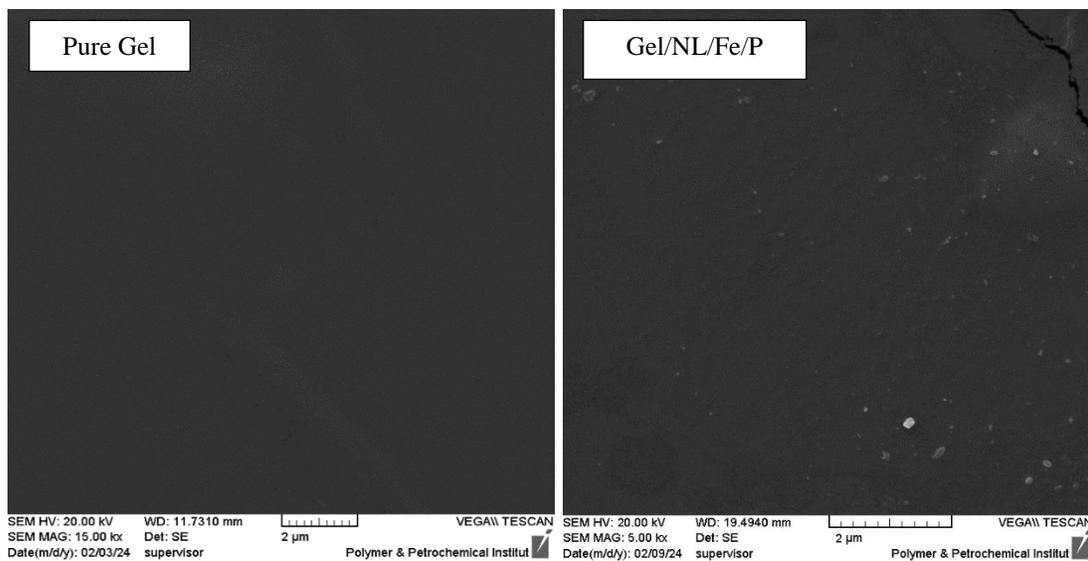


Fig. 2. SEM images of pure gelatin film and gelatin/liposome/iron/photoluminescent pigment film

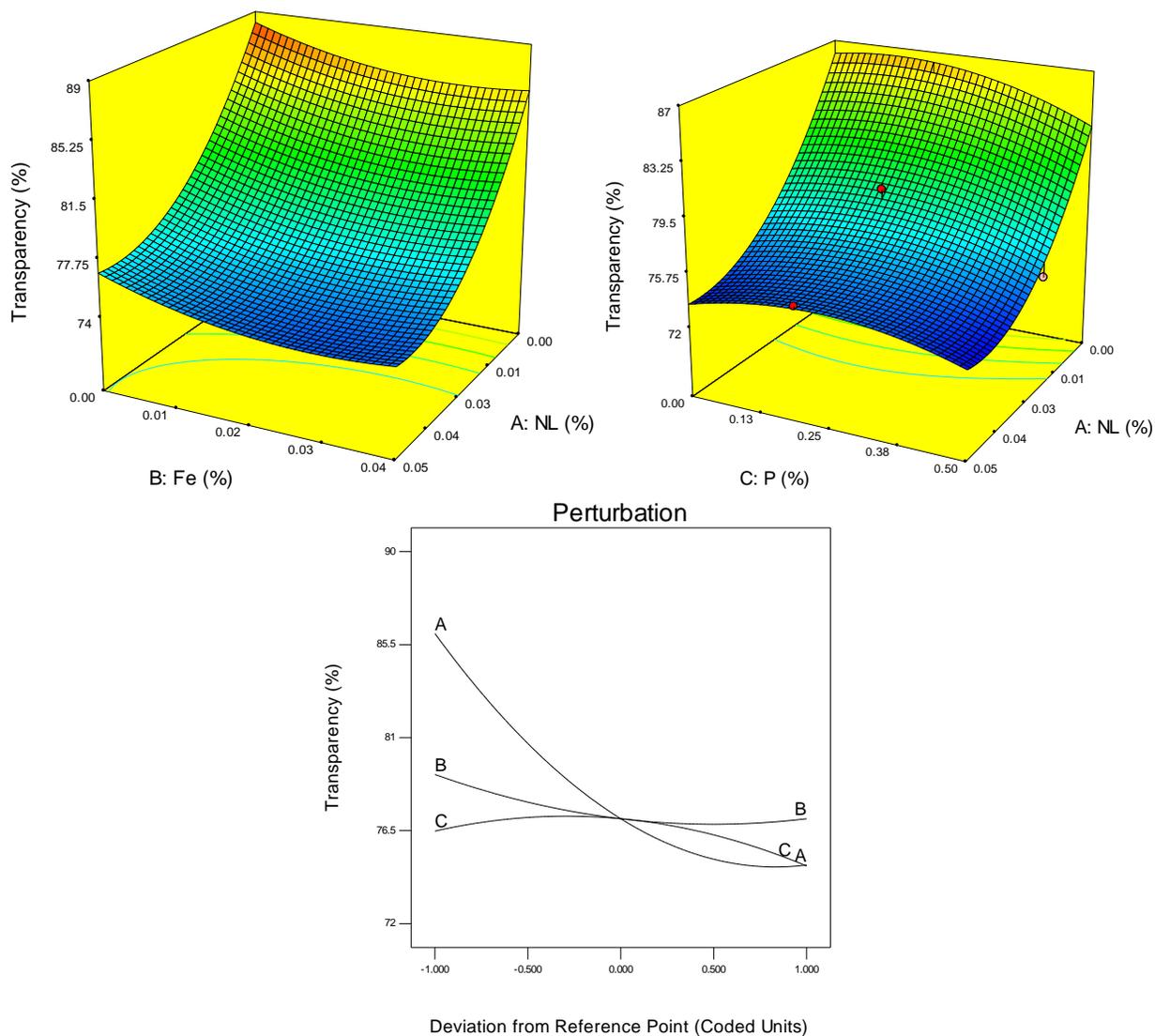
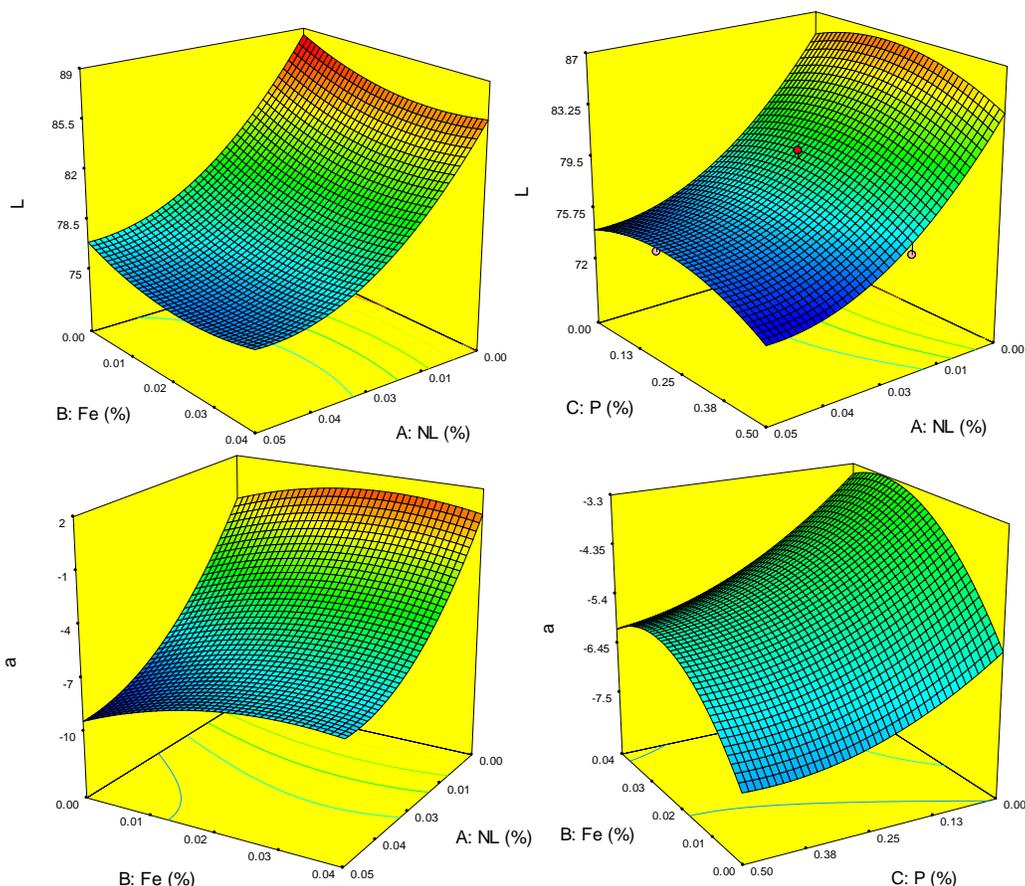


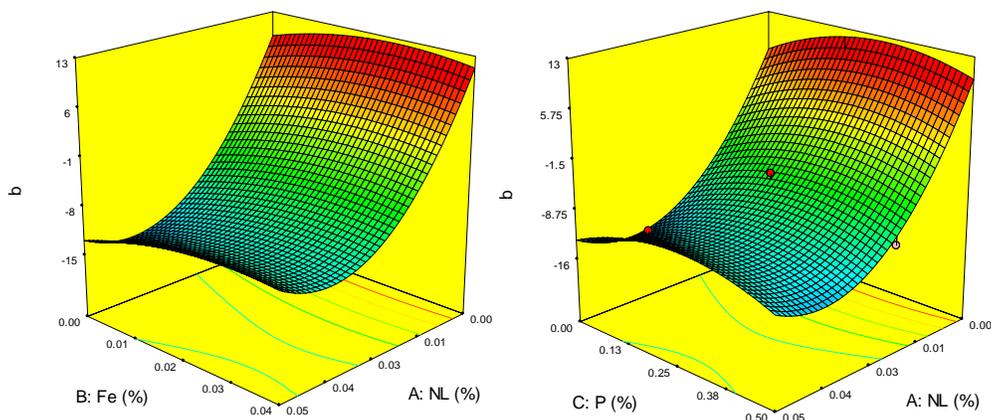
Fig. 3. 3D and perturbation curves of the effect of nanoliposome, iron and photoluminescent pigment on film transparency

### 3.4. Color properties

The color characteristics of films are very important in the appearance of packaging and customer attraction. Food packaging has made many advances in design and appearance in recent years. In this study, due to the fact that the prepared films have photoluminescent properties and in dark conditions, it will produce a special effect by producing green color, it will create a very good performance in the appearance design of the packaging. Based on this, the color properties of the film including lightness (L), green-red (a) and blue-yellow (b) of the film have been investigated. Fig. 4 shows the three-dimensional curves of the effect of nanoliposome, iron and photoluminescent pigment on the color properties of the film. According to the results of all three factors, nanoliposome, iron and photoluminescent pigment have reduced the transparency of the film. Considering that phycocyanin has a blue color and strongly changes the color of the film, the change of film transparency due to this factor is more than other factors. Iron nanoparticles and photoluminescent pigment showed little effect on film lightness. Green-red factor (a) and blue-yellow factor (b) were also strongly influenced by phycocyanin nanoliposome. According to the blue color of phycocyanin, this additive has moved a and b factors

towards more negative numbers, and whatever factor b moves towards negative numbers, it indicates a greater intensity of blue color in the film, which is completely consistent with the actual appearance of the film. Iron nanoparticles have also pushed factors a and b to more positive numbers, which was expected due to the yellow-orange nature of this pigment, because positive numbers in factor b indicate more yellowness of the film. Considering that the color analysis of the films was in ambient light conditions and considering that the photoluminescence pigment does not cause many color changes in ambient light conditions, this pigment did not cause a noticeable change in the color characteristics. According to Fig. 1, gelatin films containing photoluminescent pigments do not show color changes in ambient light conditions, while these films strongly luminesce in dark conditions and show green color, which is clearly evident in Fig. 1. This photoluminescence characteristic of films, whose photoluminescence intensity depends on the amount of photoluminescence pigment and the amount of other additives, can be used in the design of photoluminescence color sensors and kits for the analysis of gases, volatile substances and aromatic substances in food products.





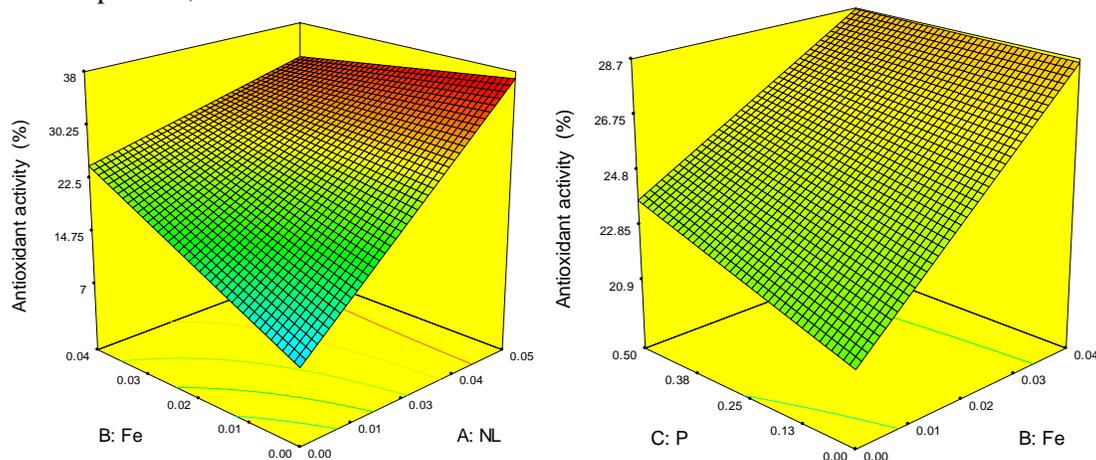
**Fig. 4.** 3D curves of the effect of nanoliposome, iron and photoluminescent pigment on the color properties

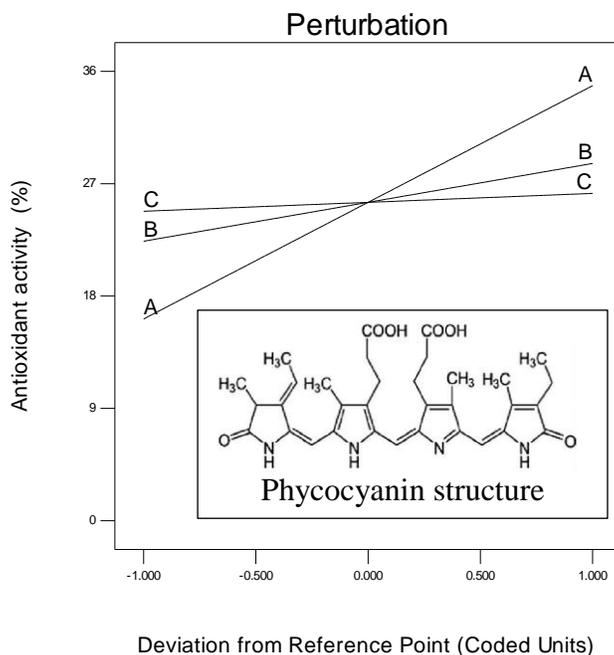
### 3.5. Antioxidant property

Oxidation of food products leads to the destruction of their chemical structure and spoilage and reduces the storage period and shelf life. One of the recent developments in the food packaging industry is related to the production of active films, which, having antioxidant properties, can increase the shelf life of packaged food products by controlling oxidative spoilage. Fig. 5 shows 3D and perturbation curves of the effect of three factors of nanoliposome, iron and photoluminescent pigment on the antioxidant property of the film. According to the curves, it was found that the photoluminescence pigment has no significant effect on the antioxidant property, but the zero-valent iron nanoparticles increased the antioxidant property by 10 to 20%. Zero-valent iron is a nanoparticle with electron donating ability that can be effective in controlling and suppressing DPPH radicals. The greatest effect on the antioxidant property is related to phycocyanin nanoliposome, so that this additive has

increased the antioxidant property by 20 to 40%. According to what is reported in the sources, phycocyanin is an extremely strong antioxidant and inhibits free radicals and prevents the oxidation of fats, as well as having strong anti-inflammatory properties. According to the chemical structure of phycocyanin, this substance can easily inhibit free radicals and shows antioxidant capacity.

There are many studies that have investigated the antioxidant properties of phycocyanin in different conditions and have proven it. These studies have also investigated the use of phycocyanin in composites with other chemicals and confirmed its antioxidant properties [24 and 25]. Sahin et al. (2022) have controlled the antioxidant property of this pigment by encapsulating phycocyanin and reported the controlled release and long-term preservation of its antioxidant property. The results of similar research conducted on the antioxidant property of phycocyanin nanoliposomes confirm the results of the present research [26].





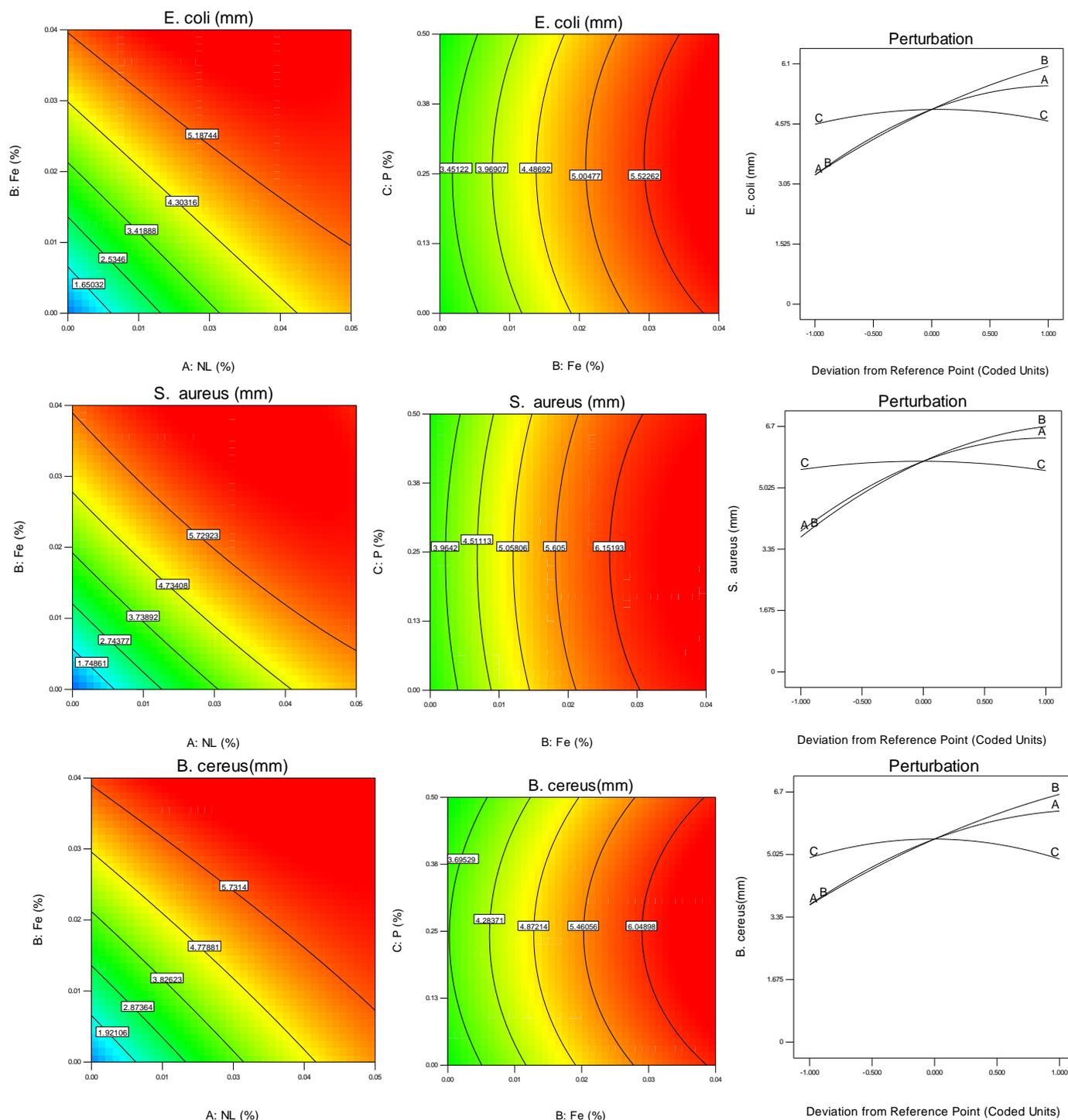
**Fig. 5.** 3D and perturbation curves of the effect of nanoliposome, iron and photoluminescent pigment on the antioxidant property

### 3.6. Antibacterial property

An antibacterial agent is a compound that kills or stops the growth of microorganisms. Antibacterial compounds are used to eliminate bacterial contamination. The toxicity of these compounds for humans and other animals is very small. Of course, long-term use of antibacterial compounds reduces the number of microbial flora in the body, which may have negative effects on health. The use of antibacterial materials in the structure of packaging films is a new way to inhibit the growth of bacteria in food products and prevent microbial spoilage and ultimately increase the shelf life of food products. According to what has been reported in previous researches, the antibacterial effect of antibacterial films against Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) is usually higher than Gram-negative bacteria (*Escherichia coli*). The cell wall structure of Gram-positive and Gram-negative bacteria is different, which makes the resistance of these bacteria different to antibacterial compounds. Gram-negative bacteria have a more complex cell wall than Gram-positive bacteria. Antibacterial agents have less ability to penetrate into the cells of Gram-negative bacteria because the physical and chemical structure of Gram-negative bacteria is more complex, and therefore antibacterial agents have less ability to inactivate them. In Gram-positive bacteria, antibacterial agents easily penetrate the bacterial wall and make the bacteria inactive because these bacteria have a simpler wall and have less physical and chemical resistance against bacteria. Gram-negative cell

cover is a multi-layered and very complex structure. The cytoplasmic membrane, which is called the inner membrane in Gram-negative bacteria, is covered by a flat sheet of peptidoglycan. An external capsule or S layer may also be present. The space between the inner and outer membrane is called periplasmic space. Compared to Gram-positive bacteria, Gram-negative bacteria are more resistant to antibiotics due to their impermeable wall. Fig. 6 shows the contour plot and perturbation curves of the effect of the three factors of nanoliposome, iron and photoluminescent pigment on the antibacterial property (two Gram-positive bacteria of *Bacillus aureus* and *Staphylococcus aureus* and one Gram-negative bacterium *Escherichia coli*) (halo of non-growth of bacteria) of the films. According to the obtained results, the photoluminescent pigment (gold/iridium) had very little antibacterial effect on all three types of Gram-positive and Gram-negative bacteria. While nanoliposome and zero valent iron nanoparticles have significantly increased the antibacterial properties of the film against all three types of bacteria. The effect of nanoliposome and zero-valent iron nanoparticles on both types of Gram-positive bacteria (*Bacillus aureus* and *Staphylococcus aureus*) was almost the same, but the effect of these additives on gram-negative bacteria (*Escherichia coli*) was less, which the reason for this phenomenon is mentioned above. In fact, liposomal nanoparticles and zero-valent iron by binding to the bacterial cell wall have disturbed the biological conditions of the bacteria and inhibited their growth.

There are many studies that have reported the antibacterial capacity iron nanoparticles, which confirm the results of properties of nanoliposomes, phycocyanin and zero-valent iron nanoparticles, which confirm the results of the current research [27-30].



**Fig. 6.** Contour plot and perturbation curves of the effect of nanoliposome, iron and photoluminescent pigment on the antibacterial property

#### 4. Conclusion

In this study, phycocyanin nanoliposome, zero-valent iron nanoparticle and gold/iridium composite

photoluminescent pigment were used to modify the chemical structure of gelatin film. According to the results, the pure gelatin film was a homogeneous and

uniform film without pores. In the gelatin/liposome/iron/photoluminescent pigment film, the presence of liposome nanoparticles, iron nanoparticles and photoluminescent pigment nanoparticles (iron/iridium) on the surface of the film was clearly evident. Nanoliposome had the greatest effect on turbidity, and iron and pigment showed very little effect on film turbidity. With regard to the blue color of phycocyanin, this additive moved factor a and b towards more negative numbers, and whatever factor b moves towards negative numbers indicates a greater intensity of blue color in the film, which was completely consistent with the film's appearance. Photoluminescence pigment did not cause a noticeable change in the color characteristics. Gelatin films containing photoluminescent pigments did not show color changes under ambient light conditions, while these films strongly luminesced and showed green color in dark conditions. The photoluminescence pigment had no significant effect on the antioxidant property, but the zero-valent iron nanoparticles increased the antioxidant property by 10-20%. Phycocyanin nanoliposome had the greatest effect on the antioxidant property, so that this additive increased the antioxidant property by 20 to 40%. Photoluminescent pigment (gold/iridium) had very little antibacterial effect on all three types of Gram-positive and Gram-negative bacteria. While nanoliposome and zero valent iron nanoparticles significantly increased the antibacterial properties of the film against all three types of bacteria. The effect of nanoliposome and zero-valent iron nanoparticles on both types of Gram-positive bacteria was almost the same, but the effect of these additives on gram-negative bacteria was less. The photoluminescence feature of films, whose photoluminescence intensity depends on the amount of photoluminescence pigment and the amount of other additives, can be used in the design of color photoluminescence kits and sensors for the analysis of gases, volatile substances and aromatic substances in food products. Gelatin film modified with phycocyanin, zero-valent iron nanoparticle and photoluminescent pigment, having suitable antioxidant and antibacterial properties, can be used in active packaging of foods exposed to oxidative and microbial spoilage.

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