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Development of Active PCL/β-Glucan Films Enriched with Natural Extracts and Gold Nanoparticles for Packaging of Cold Plasma-Treated Fish Paste

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Abstract

Growing concerns about conventional plastic packaging due to its non-degradability, risk of transferring toxic compounds to food, and environmental pollution have necessitated the development of environmentally friendly alternatives such as edible and biodegradable films. This study aimed to develop an active packaging system based on a polycaprolactone/beta-glucan (PCL-BG) nanocomposite film modified with oak fruit pair extract (OFPE), apricot kernel extract (AKE), and gold nanoparticles (AuNPs) and study its suitability for packaging fish paste treated with cold plasma. The evaluation of the film's antibacterial properties showed that the active compounds had a strong inhibitory effect, with the largest inhibition zones of 27.78 mm against *Bacillus cereus* and 21.38 mm against *Escherichia coli* observed in the film prepared with the highest extract concentration. Thermal analysis (DSC) showed a significant improvement in thermal stability, with the melting temperature of the final composite film increasing to 81.6 °C. Microbiological and chemical evaluations over 30 days of storage showed that the combined treatment significantly ($p < 0.05$) reduced microbial growth (Total Viable Count), reduced the production of volatile nitrogen compounds (TVB-N), controlled lipid oxidation (TBA), and maintained pH stability and sensory acceptance. Regression models for all quality parameters showed high accuracy and predictive power ($R^2 > 99.27\%$). This study clearly demonstrates the effectiveness of the combined strategy of cold plasma and active packaging in maintaining the quality and increasing the shelf life of fish paste.

Keywords: Active packaging, Antibacterial, Biodegradable film, Cold plasma

Introduction

Conventional plastic packaging has raised significant concerns due to its non-degradability, the transfer process of potentially toxic compounds to food, and its role in environmental pollution. These challenges have driven the development of environmentally friendly alternatives, including edible and biodegradable films (Dadkhah, Pirsa, Javadi, & Mohtarami, 2024; Eslami, Pirsa, Mohtarami, & Bener, 2025; Forouzan, Pirsa, & Alirezalu, 2024; Pirsa, Bener, & Şen, 2024). In this context, polycaprolactone (PCL), a biodegradable aliphatic polyester, has gained attention for its favorable mechanical properties and processability. However, the standalone application of PCL faces limitations, particularly the need to enhance its functional properties. To address these limitations, combining PCL with natural polymers such as beta-glucan (BG) presents a promising solution. BG is a polysaccharide known for its

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film-forming ability and high biocompatibility. Furthermore, incorporating natural active ingredients like oak fruit pair extract (OFPE) and apricot kernel extract (AKE) which are rich in phenolic and antioxidant compounds, respectively along with antimicrobial gold nanoparticles (AuNPs), can facilitate the development of advanced composite films with both antioxidant and antimicrobial capabilities. Such nanocomposites are highly valuable for minimizing the growth of spoilage microorganisms during food processing and storage, thereby extending shelf life and improving food safety. Gold, being the least reactive metal, possesses very stable chemical properties. It is non-toxic, exhibits good biocompatibility, and is multivalent, allowing it to bind different types of ligands (Gupta *et al*., 2016).

The extremely large specific surface area of AuNPs provides abundant sites for binding to target bacteria. AuNPs have shown antibacterial activity against both Gram-positive and Gram-negative bacteria (Slavin, Asnis, Häfeli, & Bach, 2017). Compared to conventional antibiotics, AuNPs do not easily induce drug resistance because they target a variety of molecules (DNA and protein). Therefore, it becomes difficult for bacteria to develop a system that can defend against all damages (Zhao *et al*., 2018). The preparation and antibacterial properties of AuNPs have been extensively studied, but no comprehensive summary has been published to date. Recently, non-thermal discharge plasma (Cold plasma) has attracted much attention as a means to kill microorganisms due to its high efficiency and environmental compatibility (Barba, Koubaa, do Prado-Silva, Orlien, & de Souza Sant'Ana, 2017). Furthermore, cold plasma treatment can be conducted at room temperature and atmospheric pressure, making it a safe and energy-efficient method. Various plasma sources are available, including dielectric barrier discharge (DBD), corona discharge, and microwave discharge (Misra *et al*., 2018; Bahmanpour, Asefi, Alizadeh, & Pirsa, 2024). The inactivation of bacteria during plasma discharge is primarily attributed to two main factors: the strong electric field generated within a specific range and the diverse active species produced by the plasma. While plasma technology has been widely utilized in the biomedical field for over a decade, its application has recently expanded into the food industry. For instance, it has been employed for disinfecting food industry wastewater (Patange *et al*., 2018). Specifically, DBD plasma has proven effective in eliminating bacteria and yeast in apple juice (Liao *et al*., 2018).

Fish paste products, commonly known as fish cakes. According to the Korean Food Standards Codex, is characterized as a processed seafood product comprising salt-soluble proteins extracted from fish flesh. Fish muscle is mechanically separated from the bone, thoroughly washed with water, and blended with cryoprotectants to produce a hydrated protein concentrate known as surimi. This Japanese term, which translates to "washed fish mince," refers to the refined fish myofibrillar protein obtained through this process. It is a refined fish myofibrillar protein that is produced through multiple step-by-step processes including heading, gutting, filleting, deboning (mincing), washing, dewatering, mixing with cryoprotectants, freezing, and metal detection for HACCP. Myofibrillar proteins make it an excellent ingredient for the production of food products. It has excellent gelling properties and forms strong, elastic gels when heated. Textural properties such as gel strength are the main determinants of the price and quality of surimi. The quality of surimi and its gelling properties are mainly influenced by both intrinsic factors (effect of fish species, seasonality, sexual maturity and freshness or hardness) and extrinsic factors (harvest, handling, water characteristics, processing time and temperature, solubilization of myofibrillar proteins during processing) (Nishinari & Ooizumi, 2020; Panpipat, Thongkam, Boonmalee, Çavdar, & Chaijan, 2023).

The aim of this study was to develop an antibacterial polycaprolactone/beta-glucan film modified with hydroalcoholic oak fruit pair

extract, apricot kernel extract, and gold nanoparticles for active packaging of cold plasma-treated fish paste.

Materials and Methods

Chemicals

All chemicals used in this study, including polycaprolactone and beta-glucan, were obtained from international and domestic suppliers. The polymer base of the film (polycaprolactone and beta-glucan) was purchased from the official representative of Sigma-Aldrich in Iran. Plant materials, including oak and apricot kernels, were purchased directly from the local market in Urmia and prepared for the extraction process. Also, microbial culture media and other laboratory chemicals were obtained from Merck, Germany and Sigma-Aldrich, USA. The bacterial strains tested (*Bacillus cereus* and *Escherichia coli*) were also obtained from the reputable collection of the Scientific and Industrial Research Organization of Iran.

Preparation of Plant Extracts

Two distinctive extraction methods were selected to optimally address the distinct chemical profiles of each plant material. The Soxhlet method was chosen for oak fruit pair due to its high efficiency in extracting non-polar to semi-polar compounds (e.g., fixed oils, waxes) from its dense and oily tissue. In contrast, the maceration method at room temperature was selected for apricot kernels to gently extract more polar and potentially heat-sensitive target compounds, such as amygdalin, while minimizing their degradation.

Extraction of Oak Fruit Pair Extract (OFPE)

The extraction of oak fruit pair extract was performed using the Soxhlet method with 86% ethanol as the solvent. In this process, 200 g of oak fruit pair powder was mixed with 500 ml of ethanol and kept in the dark for 48 hours with intermittent stirring (20 minutes per day). The resulting solution was then filtered using Whatman filter paper and concentrated using a rotary evaporator at 50 °C under vacuum conditions. The concentrated extract was stored in sterile Petri dishes in an incubator at 40 °C for final evaporation of the residual solvent. The final product was stored in the dark at refrigerator until use (AOAC, 2005; Zhang *et al*., 2020).

Extraction of Apricot Kernel Extract (AKE)

After separating and drying apricot kernels in an oven, the obtained powder was subjected to maceration extraction using 70% ethanol for 72 hours. Specifically, 10 g of the powder was dissolved in 100 ml of ethanol and stirred simultaneously on a magnetic stirrer at medium speed and room temperature. Following the extraction period, the mixture was centrifuged, and the supernatant was filtered through Whatman No. 1 filter paper. The filtered supernatant was then concentrated under vacuum using a rotary evaporator at 45 °C. The final extract was stored in a freezer after complete evaporation of the residual solvent at 45 °C until use (Zhang *et al*., 2020).

Preparation of Composite Film

The nanocomposite film was prepared by first preparing a polymer base solution with equal proportions of polycaprolactone (PCL) and beta-glucan. Specifically, beta-glucan powder (2 g) was dissolved in 50 ml of distilled water and heated on a magnetic stirrer at 80 °C for 2 hours. Simultaneously, a PCL solution was prepared by dissolving 2 g of the polymer in 10 ml of chloroform and stirring for 30 minutes. The PCL solution was then added dropwise to the beta-glucan solution, followed by the addition of Tween 80 as emulsifier (1% w/w of solids). After 30 minutes of stirring, oak fruit pair extract and apricot kernel extract (0.5% w/w each) were incorporated into the formulation, and the mixture was homogenized at high speed for 2 minutes using a homogenizer (model D9I12, Heidolf, Germany). Subsequently, gold nanoparticles (0.03% w/w) were added, and the mixture was magnetically stirred for 10 minutes before being subjected to ultrasonication for 10 minutes. Glycerol (30% w/w of dry matter) was

then introduced as a plasticizer, and stirring continued for 15 minutes at room temperature. Finally, 25 ml of the prepared solution was cast onto 8 cm plates and dried in an oven at 38 °C. The resulting films were carefully peeled off and stored in light-proof bags at room temperature (Pirsa *et al*., 2024).

In addition to the final composite film (PCL/BG/OFPE/AKE/AuNPs), several film formulations with varying concentrations of OFPE, AKE, and AuNPs were prepared based on the experimental design (See Table 1-B and Table 2-A) to investigate their individual and combined effects. These included the base film (PCL/BG) and films containing individual additives (PCL/BG/OFPE, PCL/BG/AKE, PCL/BG/AuNPs) for comparative analysis in DSC and antibacterial tests. The concentration ranges for the additives were selected based on preliminary experiments and literature review. The extract concentrations (0.25-0.75% w/w) were chosen to ensure significant antimicrobial efficacy while maintaining the film's structural integrity and avoiding negative effects on its physical properties. The concentration of AuNPs (0.015-0.045% w/w) was selected due to the fact that low loadings are sufficient to enhance antibacterial activity and thermal stability without causing aggregation or compromising the film matrix.

DSC Examination of Films

In order to determine the DSC (Differential Scanning Calorimetry) of the samples, initially about 0.03 g of each film was placed in an aluminum pan in a package and was carried out at a rate of 10 °C per minute and at the temperature range of 0 to 500 °C for 45 minutes in the presence of helium gas by a differential scanning calorimetry device (10-PT Linsies, Germany).

Antimicrobial Activity of Films and Extracts

The antimicrobial activity of nanocomposite films containing oak fruit pair extract, apricot kernel extract, and gold nanoparticles was evaluated against two bacterial strains - *E. coli* O157:H7 (ATCC 25922) and *B. cereus* (PTCC 1154) - using the disk diffusion method. Film samples were cut into 6-mm diameter discs using a sterile punch and aseptically placed on Mueller-Hinton agar plates. The agar surface had been previously inoculated with 0.1 mL of bacterial inoculum containing 10^5 -10^6 CFU/mL. After incubation at 37 °C for 24 hours, the inhibition zones appearing as clear halos around the film discs were measured in millimeters using a digital caliper.

The antibacterial properties of the extracts were studied using the agar well diffusion method on two strains of bacteria, *E. coli* O157:H7 (ATCC 25922) and *B. cereus* (PTCC 1154). Briefly, the surface of Mueller Hinton agar plates was first inoculated uniformly (by swabbing) with 0.1 ml of inoculum containing 10^6 CFU/ml of the tested bacteria. Then, wells (5 mm in diameter) were created in the inoculated agar using a sterile cork borer. Subsequently, 30 µl of the pure extract was poured into each well. The plates were incubated at 37 °C for 24 hours. Then, the zone of inhibition was measured in millimeters as a clear halo around the wells with a digital caliper (Balouiri, Sadiki, & Ibnsouda, 2016).

Preparation of Cold Plasma Treated Fish Paste

Common carp was freshly obtained from the fish market (Urmia, Iran). It was transferred to the processing laboratory after being stored on ice. The surimi preparation process was carried out manually in the laboratory according to the method of Jeon, Myung, & Min, (2022). After beheading and abdominal emptying, common carp was filleted and washed manually, then deboned. The meat was ground using a meat grinder equipped with a plate with 3 mm diameter holes. After that, the minced meat was washed with cold water at a temperature below 10 °C for three times with a ratio of 4:1 water to fish meat, twice with distilled water and then once with a 0.3% brine solution, each time for 15 minutes. After each washing, the fish meat was poured into a clean cloth to remove excess water. Then, 4% sugar, 4% sorbitol, and 0.3% sodium tripolyphosphate were added to the

prepared surimi and mixed in a meat mixer (Mulinex, 1000w, Made in France). Finally, the prepared fish paste was treated using a Dielectric Barrier Discharge (DBD) plasma device (Diener electronic, Jettingen, Germany). Argon gas (99.997% purity) was used as the feed gas to generate the cold plasma. This gas was chosen due to its inert nature, food-grade safety, efficiency in generating stable plasma, and effectiveness in microbial inactivation while minimizing potential adverse effects on food quality. The prepared fish paste sample was placed on the base inside the device, then argon gas was injected into the device at a constant rate of 6 liters per minute to produce plasma, and the samples were treated under vacuum conditions with a pressure of 600 mTorr and a power of 20-30 watts. The sample was exposed to cold plasma treatment with argon gas for 6 minutes and packaged for subsequent tests (Jeon *et al*., 2022).

Packaging of Fish Paste with Nanocomposite Film

After preparing the cold plasma treated fish paste, the packaging film prepared as mentioned above was used for antimicrobial packaging and preservation. For this purpose, 50 g of cold plasma treated fish paste and 50 g of fish paste treated with conventional heating method were packed in edible film and conventional packaging in the same dimensions and kept at refrigerator for one month (Fig. 2). Sa mples were taken on day 1, 15, 30and the following tests were performed.

Packaged Fish Paste Tests

pH Measurement

One g of fish paste samples was mixed with 9 ml of distilled water (in Falcon tubes) and homogenized with a homogenizer (Ultra Turrax T25, IKA, Germany) at 13,500 rpm for 30 seconds. Then, the pH of the fish paste samples was measured at room temperature using a digital pH meter (Hanna HI9025c, Portugal) (Zhang *et al.*, 2020).

Determination of Total Volatile Nitrogenous Bases (TVB-N)

This test was performed according to the AOAC method (2002) and its amount was calculated in milligrams per hundred grams of fish paste according to the following equation:

$$TVB - N = \frac{M \times 1.4}{N} \times 100$$

where M and N are the amount of sulfuric acid consumed and the weight of the sample, respectively.

Measurement of Thiobarbituric Acid (TBA)

Measurement of Thiobarbituric Acid is based on the spectrophotometric values (HACH, DR/2000, USA) of the pink complex resulting from the reaction of one malonaldehyde molecule obtained from distillation with two thiobarbituric acid molecules added to the distilled solution, and its amount is expressed in milligrams of malonaldehyde per kilogram of sample according to the following equation (Rahman, Al-Amiri, & Al-Riziq, 2021).

$$TBA = \frac{A_s - A_b}{50} \times 200$$

Total Bacterial Count (TVC)

Tryptic soy agar (TSA) medium was used for total bacterial count after preparing serial dilutions, surface culture was performed on the plates and the samples were incubated at 32 °C for 48 hours and then the colonies were counted (AOAC, 2005).

Sensory Evaluation

Sensory evaluation of the samples was carried out according to the 5-point hedonic test by 10 panelists. This study was approved by the Research Ethics Committee of Urmia University. For this purpose, the samples were coded and evaluated in terms of sensory characteristics (overall acceptance). A score of 5 indicated the highest quality of the sensory characteristic and a score of 1 indicated the lowest quality for the sensory characteristic (Lawless & Heymann, 2010).

Statistical Analysis

In this study, response surface methodology and a D-Optimal statistical design were employed for two key objectives:

1. To formulate and screen antibacterial films with different compositions of OFPE, AKE, and AuNPs (as shown in Table 1-B) and modelling their antibacterial responses (Inhibition Zone).
2. To investigate the effect of cold plasma treatment and active packaging on the quality parameters of fish paste during storage (as shown in Table 1-A).

This design was selected due to its high efficiency in providing reliable regression models with the minimum number of required experimental runs, which was particularly advantageous given the practical constraints of the study. For the first objective (film formulation), the factors were the concentrations of OFPE, AKE, and AuNPs.

Table 1-A. List of treatments performed on fish paste based on the D-Optimal design. (CP: Cold Plasma; Active film: PCL-BG-OFPE-AKE-AuNPs)

Run	Factor A: Time (Day)	Factor B: CP*	Factor C: Active film**
1	30	Unused	Used
2	15	Used	Unused
3	1	Unused	Used
4	1	Used	Unused
5	15	Used	Used
6	15	Unused	Unused
7	30	Unused	Unused
8	15	Unused	Used
9	30	Used	Unused
10	1	Unused	Unused
11	30	Used	Used
12	1	Used	Used

*CP: Cld Plasma

**Active film: Polycaprolactone/Beta-Glucan nanocomposite film containing Oak Fruit Pair Extract, Apricot Kernel Extract, and Gold Nanoparticles (PCL-BG-OFPE-AKE-AuNPs)

Table 1-B. List of selected films for testing antibacterial properties. (OFPE: Oak Fruit Pair Extract; AKE: Apricot Kernel Extract; AuNPs: Gold Nanoparticles)

Film Code	OFPE (%)	AKE (%)	AuNPs (%)
F1	0.75	0.25	0.045
F2	0.75	0.75	0.045
F3	0.25	0.25	0.045
F4	0.5	0.5	0.015

For the second objective (fish paste quality), the type of cold plasma treatment (treated and untreated) and the type of packaging (conventional packaging and active packaging) were considered as non-numeric factors, and storage time was included as a covariate. The final design for the application study consisted of 12 experimental runs or treatments (Table 1-A). All analyses were performed independently on each experimental unit. A 95% probability level was used to examine the significance of the means and the models. Design Expert software (version 10) was used for modeling, generating response surface curves, and comparing means.

Results and Discussion

DSC

Fig. 1 presents the DSC curves of the polycaprolactone-beta-glucan composite films. The thermal properties and phase transitions derived from these curves are as follows. The PCL/BG base film exhibited a glass transition (Tg) between 50-60 °C, associated with the amorphous regions of polycaprolactone. A distinct melting temperature (Tm) was observed at approximately 56-58°C, corresponding to the crystalline PCL domains. The measured enthalpy of fusion was 887.5 J/g, indicating a relatively high degree of crystallinity in the base film. Incorporation of apricot kernel extract (PCL/BG/AKE) resulted in a slight reduction in melting temperature to 53-55°C and decreased enthalpy of fusion compared to the base film. This might be due to the interactions between AKE components and the polymer matrix that reduce crystallinity. Similarly, the film containing oak fruit pair extract (PCL/BG/OFPE) showed noticeable alterations in the melting peak and a shift in glass transition toward lower temperatures, likely due to strong interactions between phenolic compounds in the extract and polymer chains. The addition of gold nanoparticles (PCL/BG/Au) significantly enhanced thermal properties, increasing the melting temperature to approximately 64.6 °C and elevating the enthalpy of fusion. These improvements indicate that gold nanoparticles function as nucleating agents, promoting crystallinity and thermal stability. The final composite film (PCL/BG/OFPE/AKE/Au) demonstrated the highest melting temperature at 81.6°C among all samples, forming a complex thermal structure with multiple phase transitions and enhanced thermal stability compared to the base film.

These findings collectively demonstrate that component interactions substantially influence the thermal properties of composite films. While gold nanoparticles enhance thermal stability through nucleation, plant extracts modify crystal structure via polymer chain interactions, ultimately affecting film performance in packaging applications. According to Ma, Shi, Su, & Wang, (2020), pure polycaprolactone of similar molecular weight (Mw ~50,000) exhibits a melting temperature of approximately 63.5°C and fusion enthalpy of about 94.5 J/g. In contrast, our PCL/BG base film showed reduced values for both parameters (Tm: 56-58°C; ΔHm: 887.5 J/g). This reduction primarily stems from the dilution effect and physical interactions with β-glucan, which disrupt PCL crystal ordering. Although molecular weight remained unchanged in our study, the observed decrease in Tm and ΔHm parallels the effects reported by Ma *et al.* for molecular weight reduction. This similarity suggests that the additives inhibit complete polymer chain crystallization, resulting in a crystal structure resembling that of lower molecular weight PCL. Conversely, gold nanoparticles increased both Tm and ΔHm, contrasting with the general trend described in the reference study. This opposing effect clearly indicates that nanoparticles reinforce PCL crystal structure through a distinct mechanism, likely acting as nucleation sites to enhance thermal stability. The comparison demonstrates that while our additives did not chemically degrade the polymer, they significantly influence crystalline microstructure and thermal properties through physical interactions (Ma *et al.*, 2020).

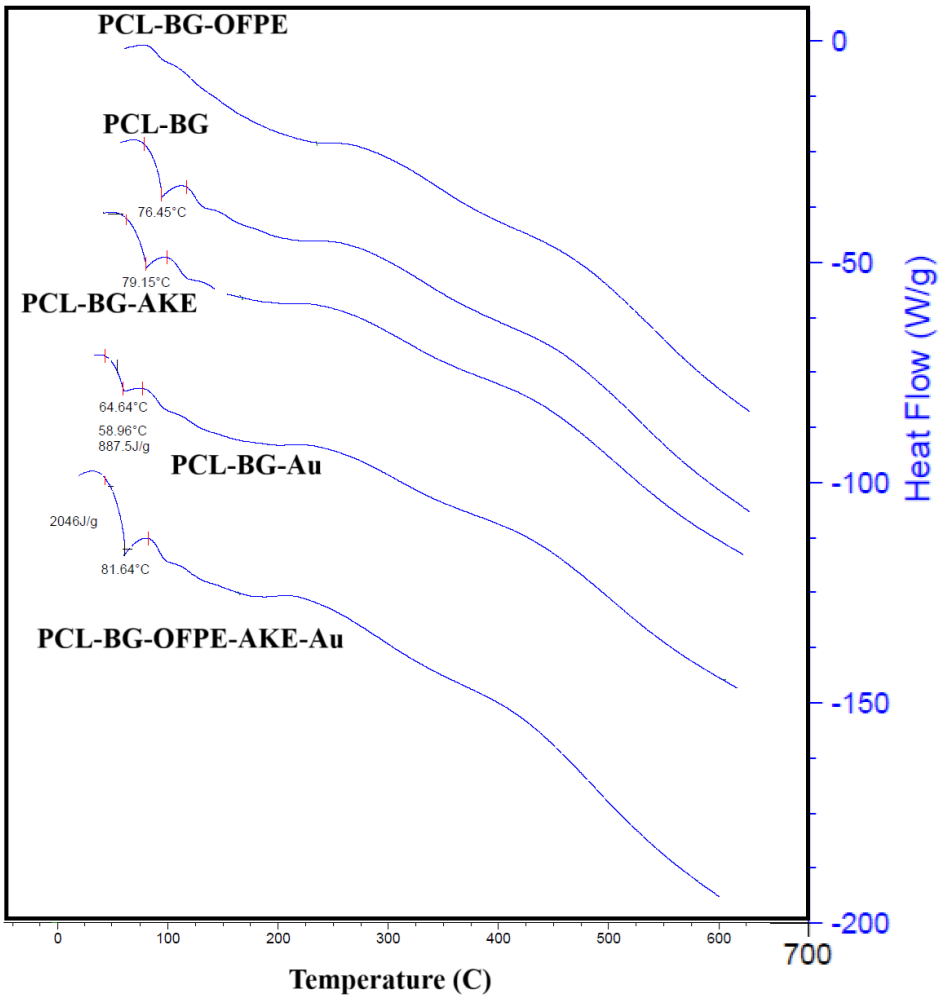

Fig. 1. DSC curves for polycaprolactone-beta-glucan based composite films

Antimicrobial Activity of Composite Films and Extracts

Fig. 2-B and Tables 2-A and 2-B show the antibacterial properties of composite films, incorporated with extracts and gold nanoparticles. Based on the results presented in Tables 2-A and 2-B, the antibacterial activity of composite films and their components against Gram-positive (*Bacillus cereus*) and Gram-negative (*Escherichia coli*) bacteria was investigated. As can be seen in Table 2-A, all composite films containing polycaprolactone/beta-glucan, oak fruit pair extract, apricot kernel extract and gold nanoparticles had inhibitory effects against both bacteria. The film containing the highest concentration of both extracts produced the largest zone of inhibition (27.78 mm for *Bacillus cereus* and 21.38 mm for *Escherichia coli*). This result indicates a strong synergistic effect between the active compounds in the film.

In general, all films produced a larger inhibition zone against *Bacillus cereus* (a Gram-positive bacterium) compared to *Escherichia coli* (a Gram-negative bacterium). This could be due to the complex structure of the cell wall of Gram-negative bacteria, which acts as a physical barrier to the penetration of antibacterial compounds. Comparison of the samples shows that increasing the concentration of gold nanoparticles from 0.015% to 0.045% (holding other factors constant) leads to a significant increase in the diameter of the inhibition zone, indicating the important role of gold nanoparticles in enhancing the antibacterial properties of the

film. Based on the data in Table 2-B, each of the compounds alone also has significant antibacterial activity. Oak fruit pair extract at a concentration of 0.75% produced a larger zone of inhibition (20.41 mm) against *Bacillus cereus* compared to *Escherichia coli* (15.65 mm). This effect is probably due to the presence of phenolic and flavonoid compounds with strong antibacterial properties in this extract. Apricot kernel extract at the same concentration also showed a greater inhibitory effect on *Bacillus cereus* (18.08 mm) than *Escherichia coli* (13.28 mm), which could be related to compounds such as amygdalin and its derivatives. Gold nanoparticles at a concentration of 0.045% showed a more balanced antibacterial effect against both bacteria (19.24 mm for *Bacillus cereus* and 18.31 mm for *Escherichia coli*). The mechanism of action of gold nanoparticles is probably through the production of reactive oxygen species, cell wall destruction, and disruption of intracellular processes in bacteria. The results of this section clearly show that the prepared composite films have a favorable and strong antibacterial effect against both Gram-positive and Gram-negative bacteria. The synergistic effect between the different components present in the film (plant extracts and gold nanoparticles) has led to a significant enhancement of the antibacterial properties. These films have a high potential for use in active packaging of food products, especially sensitive products such as fish paste. The generally larger inhibition zones observed against *Bacillus cereus* (Gram-positive) compared to *Escherichia coli* (Gram-negative) can be attributed to the fundamental structural differences in their cell walls. Gram-positive bacteria possess a thick, porous peptidoglycan layer that is more accessible to the antibacterial compounds (e.g., phenolics from extracts) released from the film. In contrast, Gram-negative bacteria have a complex outer membrane rich in lipopolysaccharides, which acts as a formidable hydrophobic barrier, initially restricting the penetration of these antimicrobial agents. The mechanism of cold plasma, involving reactive species that cause lipid peroxidation, is particularly effective at disrupting this outer membrane in Gram-negative bacteria. The more balanced effect of AuNPs against both types suggest their mode of action, potentially involving physical damage and oxidative stress, is less hindered by the structural differences in the cell envelope.

According to the results of the present study and the study of Holešová *et al.* (2021), the antibacterial performance of polycaprolactone (PCL) films containing nanoparticles is compared as follows. In the present study, the PCL/BG nanocomposite film containing plant extracts and gold nanoparticles produced a growth inhibition zone of 27.78 mm against *Bacillus cereus* and 21.38 mm against *Escherichia coli*, while in the study of Holešová *et al.*, the PCL film containing nano-clays functionalized with silver ions reported a growth inhibition zone of 12.5 mm against *Staphylococcus aureus* and 10.5 mm against *Escherichia coli*. In the present study, the antibacterial mechanism of action was a combination of multiple factors including the release of phenolic and flavonoid compounds from plant extracts, the synergistic effect of gold nanoparticles in the production of reactive oxygen species and disruption of the bacterial cell membrane, while in the reference study, the mechanism of action was mainly based on the release of silver ions from nano-clay and disruption of bacterial cell function. In the present study, the synergistic effect between different compounds led to a significant increase in antibacterial efficacy. The nanocomposite film produced in the present study had higher antibacterial efficacy than the films in Holešová's study. This superiority could be due to the following factors: the simultaneous use of multiple antibacterial mechanisms, the synergistic effect between different compounds and the higher potential of plant compounds and gold nanoparticles in inhibiting bacterial growth. Both studies showed that the modification of PCL films with nanoparticles and active compounds can lead to the development of active packaging with

effective antibacterial properties. However, the use of natural compounds in the present study could have additional advantages in terms of safety and environmental friendliness (Holešová *et al.*, 2021).

Both the present study and the research conducted by Cesur, Köroğlu, & Yalçın, (2018) independently validate that incorporating natural compounds and nanomaterials to enhance the antibacterial properties of polycaprolactone films. In the study by Cesur and colleagues, the inclusion of nano minerals and chitosan into the PCL matrix demonstrated significant antibacterial efficacy against both Gram-positive and Gram-negative bacteria. This result is consistent with our findings, where the combination of plant extracts and gold nanoparticles in the PCL/BG film resulted in strong bacterial inhibition. Both studies demonstrate that modifying the structure of PCL with active natural materials is an effective strategy for the development of antimicrobial packaging. Although the additives used are different, the results of both studies clearly emphasize the scientific principle that the formation of a nanocomposite network in a polymer matrix can significantly enhance the antibacterial efficacy of packaging films, confirming the potential of this strategy for application in the food industry (Cesur *et al.*, 2018).

Table 2-A- Inhibition zone of composite films against *Bacillus cereus* and *Escherichia coli*

Film Type	OFPE Concentration (%)	AKE Concentration (%)	AuNPs Concentration (%)	*B. cereus* (G+) (mm)	*E. coli* (G-) (mm)
PCL-BG-OFPE-AKE-Au	0.75	0.25	0.045	23.92±0.48*	20.25±0.51
PCL-BG-OFPE-AKE-Au	0.75	0.75	0.045	27.78±0.54	21.38±.37
PCL-BG-OFPE-AKE-Au	0.25	0.25	0.045	13.61±0.35	11.18±0.47
PCL-BG-OFPE-AKE-Au	0.5	0.5	0.015	16.40±0.44	12.58±.027

*The data were the average of three replicates.

Table 2-B- Inhibition zone of extracts and nanoparticles against *Bacillus cereus* and *Escherichia coli* bacteria

Compound	Concentration (%)	*B. cereus* (G+) (mm)	*E. coli* (G-) (mm)
OFPE	0.75	20.41±0.28*	15.65±.044
AKE	0.75	18.08±.029	13.28±0.55
AuNPs	0.045	19.24±.037	18.31±0.42

*The data were the average of three replicates.

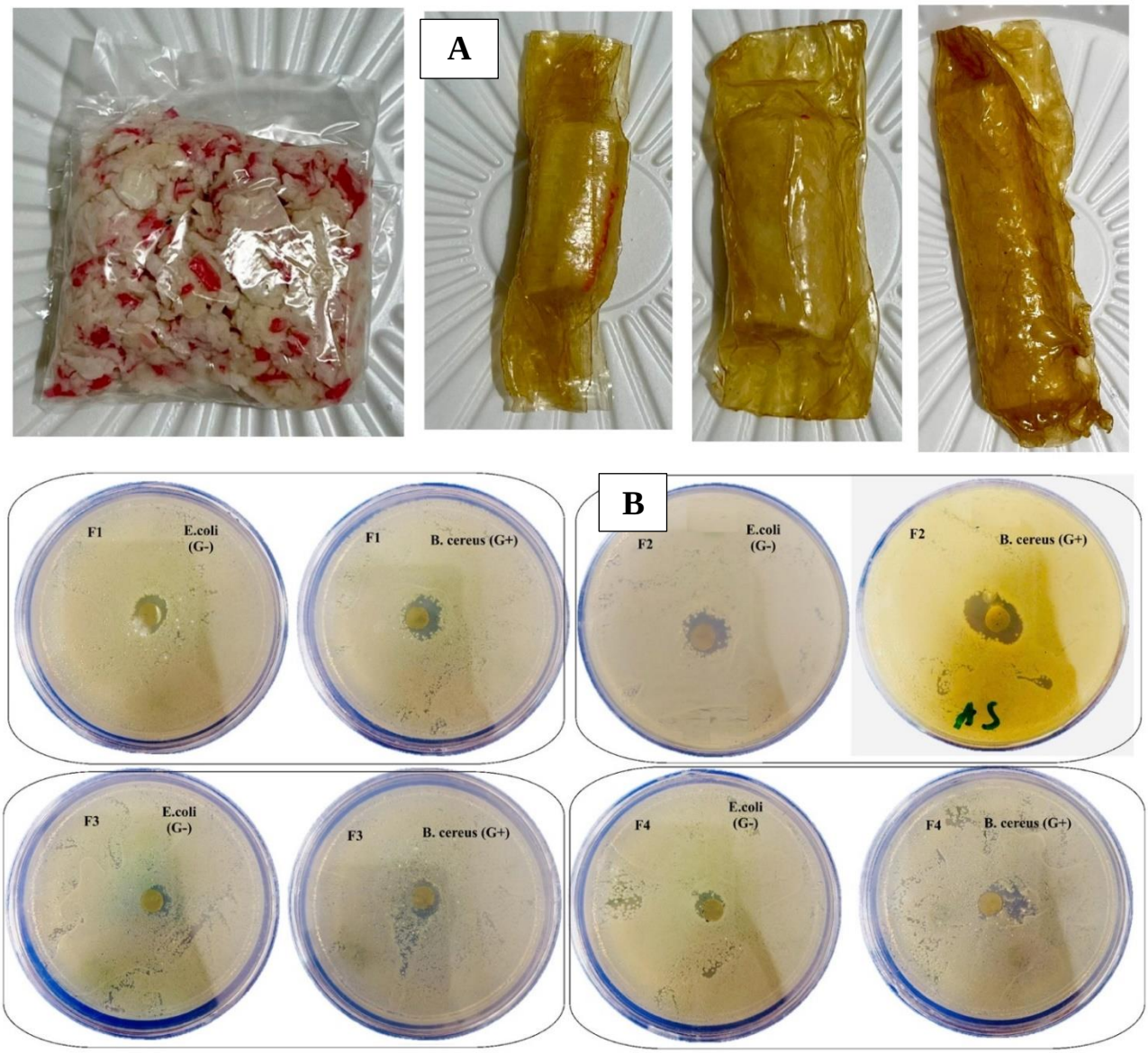

Fig. 2. Packaging of fish paste treated with head plasma with active composite films (A) and antibacterial properties of composite films (B)

pH of Fish Paste

Fig. 3 shows the three-dimensional diagram and interaction curve of the effect of storage time, cold plasma treatment and active film packaging on the pH of fish paste. Changes in the pH of fish paste during the storage period were observed based on the results obtained from the analysis of variance (ANOVA) of the quadratic response surface model. The presented model was statistically significant (p-value < 0.0001) and was able to explain 99.75% of the response changes. The values of R^2 (0.9975), adjusted R^2 (0.9932) and predicted R^2 (0.9692) indicate the favorable fit and high predictive power of the model. Among the studied factors, the storage time (A) was identified as the most effective factor on pH changes (p-value > 0.0001). The effects of cold plasma treatment (B) and active film packaging (C) were also significant at 99.96% and 99.72% confidence levels, respectively. Among the interaction effects, the interaction between time and cold plasma treatment (AB) and time and packaging type (AC) significantly affected pH,

while the interaction between cold plasma treatment and active film (BC) was not significant. The quadratic effect of time (A^2) was also significant, indicating a nonlinear trend in pH changes over time. The final regression equation was obtained as follows:

$pH= 6.57 + 0.33*A + 0.080*B + 0.048*C + 0.066*A*B + 0.059*A*C - 0.00917*B*C + 0.083*A^2$

The response surface and plots clearly showed the relationship between the independent variables and pH. With the increase in storage time, the pH of fish paste increased significantly. However, it was observed that the combined application of cold plasma treatment and active film packaging effectively prevented the sharp increase in pH - which is generally caused by bacterial spoilage and the production of alkaline compounds - and slowed down the spoilage process. Based on these findings, it can be concluded that the designed model has a high efficiency for predicting pH changes and the use of antibacterial active film in combination with cold plasma treatment is an effective strategy to maintain pH stability and shelf life of fish paste during storage.

Both the current investigation and the earlier work by Remya *et al.* (2016) provide independent confirmation of the positive effect of active packaging on maintaining pH stability in fish products. In Remya's study, the use of chitosan-containing film significantly prevented the increase in pH of barracuda fillets during refrigerated storage. This result is fully consistent with the findings of the present study, by which active packaging with polycaprolactone/beta-glucan film containing plant extracts and gold nanoparticles effectively inhibited the increase in pH of fish paste. Both studies indicate that active packaging plays a key role in maintaining pH stability and, consequently, extending the shelf life of fishery products by delaying the accumulation of alkaline compounds caused by microbial spoilage. This consistency of results confirms the effectiveness of active packaging strategy in controlling chemical indicators of spoilage in various fish products (Remya *et al.*, 2016).

TBA and TVB-N

Fig. 4 shows the three-dimensional diagram and interaction curve of the effect of storage time, cold plasma treatment and active film packaging on TBA and TVB-N of fish paste.

Changes in the thiobarbituric acid index of fish paste during the storage period were observed based on the results of the analysis of variance of the quadratic response surface model. The presented model was statistically significant at the 99.99% confidence level (p-value < 0.0001) and was able to explain 99.90% of the response changes. The R^2 values (0.999) and adjusted R^2 (0.997) indicate the optimal fit of the model. Storage time (A) was identified as the most effective factor in increasing the TBA index. This increase is mainly due to the intensification of lipid oxidation over time, which is accompanied by the formation of secondary oxidation products such as malondialdehyde (MDA). Cold plasma treatment (B) significantly reduced the TBA index, which is probably due to: inactivation of oxidative enzymes, reduction of the population of bacteria responsible for oxidation, and structural changes in fish tissue. Packaging with active film (C) showed the highest inhibitory effect in controlling oxidation, which can be attributed to the following: the presence of antioxidant compounds in plant extracts, the synergistic effect of gold nanoparticles, and the creation of a physical barrier against oxygen. The interaction effect of time and cold plasma treatment (AB) showed that the application of cold plasma can reduce the rate of oxidation caused by storage time. The interaction effect of time and active packaging (AC) showed the synergistic effect of these two factors in controlling oxidation in longer storage periods. The quadratic effect of time (A^2) indicates a nonlinear and intensifying pattern of oxidation in the final storage periods. The final regression equation was obtained as follows:

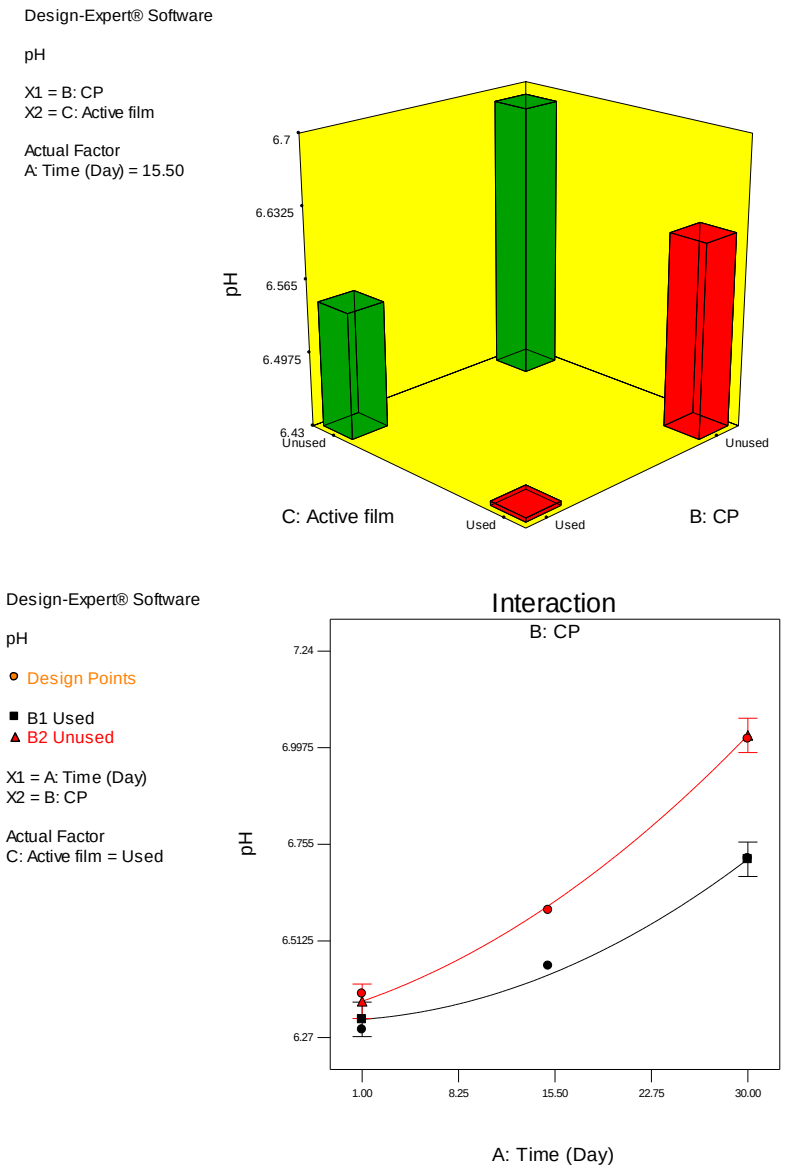

Fig. 3. Three-dimensional diagram and interaction curve of the effect of storage time, cold plasma treatment, and packaging with active film on the pH of fish paste

$$TBA = 0.95 + 0.91*A + 0.078*B + 0.18*C + 0.074*A*B + 0.19*A*C + 0.17*A^2$$

The response surface plots clearly showed that the simultaneous application of cold plasma treatment and active packaging could effectively prevent the increase of TBA index during the storage period.

The changes of the total volatile basic nitrogen index (TVB-N) of fish paste during the storage period were observed based on the results of the analysis of variance of the two-factor interaction (2FI) response surface model. The presented model was statistically significant at the 99.99% confidence level (p-value < 0.0001) and was able to explain 99.38% of the response variations. The values of R^2 (0.993) and adjusted R^2 (0.986) indicate the good fit of the model. Storage time (A) was identified as the most effective factor in increasing the TVB-N index. This increase is mainly due to the activity of endogenous proteolytic enzymes and the activity of spoilage microorganisms, which leads to the production of volatile nitrogen-containing compounds

such as ammonia, amines and trimethylamine. Cold plasma treatment (B) significantly reduced the TVB-N index, which might due to the inactivation of proteolytic enzymes and spoilage microorganisms by plasma active species and the reduction of the rate of protein degradation reactions. Packaging with active film (C) also showed a significant inhibitory effect on the formation of volatile nitrogen-containing compounds, which probably acts through the following mechanisms: antimicrobial activity of plant extracts presents in the film, reduction of oxygen permeability and creation of an unfavorable environment for the growth of TVB-N-producing bacteria, and absorption or neutralization of volatile nitrogen-containing compounds. The interaction effect of time and cold plasma treatment (AB) showed that the application of cold plasma could effectively reduce the rate of TVB-N accumulation during the storage time. The interaction effect of time and active packaging (AC) showed the synergistic effect of these two factors in controlling the production of volatile nitrogen compounds during longer storage periods. The interaction effect of cold plasma treatment and active film (BC) was not statistically significant, which probably related to the independent function of these two technologies in controlling different spoilage pathways. The final regression equation was obtained as follows:

TVB-N = 21.75 + 11.22*A + 2.41*B + 2.03*C + 2.51*A*B + 2.01*A*C - 0.12*B*C

The response surface and counter plots clearly showed that the simultaneous application of cold plasma treatment and active packaging can effectively prevent the increase of TVB-N index during the storage period.

This study demonstrates that the combination of cold plasma treatment and active packaging utilizing a film incorporated with antioxidant compounds represents an effective strategy for controlling lipid oxidation and extending the shelf life of fish paste. The synergistic effect of these two methods can simultaneously target different oxidation mechanisms and maintain the quality of the product during the storage period. Also, the combination of cold plasma treatment and active packaging with a film containing antimicrobial compounds can be an effective strategy to control the production of volatile nitrogen compounds and prevent growth of spoilage bacteriain fish paste. The synergistic effect of these two methods in controlling the TVB-N index indicates the high potential of this combined strategy in maintaining the quality and increasing the shelf life of fishery products.

Both the current investigation and the research conducted by Julyaningsih, Latief, & Dirpan, (2020) independently validate the efficacy of active packaging in regulating chemical indicators of fish spoilage. In Julyaningsih's study, the implementation of smart packaging incorporating chitosan-gelatin nanofibers substantially reduced thiobarbituric acid (TBA) values and total volatile basic nitrogen (TVB-N) levels in tuna fillets throughout the storage period. This finding is fully consistent with the results of the present study, in which active packaging with a polycaprolactone/beta-glucan nanocomposite film containing plant extracts and gold nanoparticles effectively prevented the increase in TBA and TVB-N indices in fish paste. Both studies indicate that active packaging plays a key role in maintaining the quality and increasing the shelf life of fishery products by inhibiting lipid oxidation, reducing TBA, and reducing TVB-N. This concordance of results confirms the effectiveness of active packaging strategy in simultaneously controlling oxidative and microbial spoilage in different fish products (Julyaningsih *et al.*, 2020).

Both the present study and Erkan's (2017) study independently confirm the role of active packaging in controlling chemical spoilage indices of fish. In Erkan's study, the use of active packaging significantly reduced the values of total volatile nitrogen (TVN) and thiobarbituric acid in traditional Turkish salted and dried fish (chiruz) during the storage period. This finding is fully consistent with the results of the present study, in which active

packaging with polycaprolactone/beta-glucan nanocomposite film containing plant extracts and gold nanoparticles effectively prevented the increase of TVB-N and TBA indices in fish paste. Both studies show that active packaging plays a key role in maintaining the quality and increasing the shelf life of fishery products, both dried fish and processed products such as fish paste, by inhibiting lipid oxidation, reducing TBA, and delaying the production of volatile nitrogen compounds. This concordance of results confirms the effectiveness of active packaging strategies in simultaneously controlling oxidative and microbial spoilage in different types of fish products (Erkan, 2017).

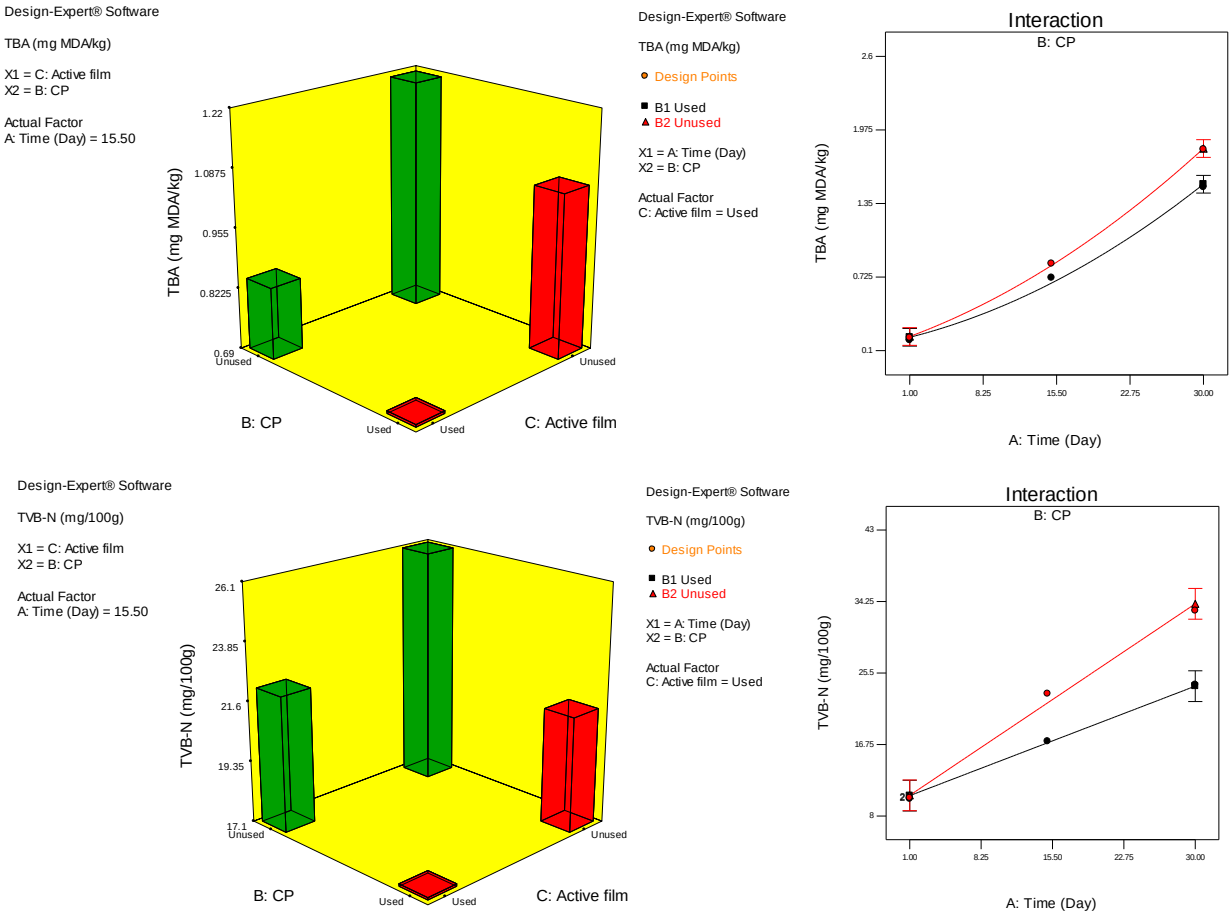

Fig. 4. 3D diagram and interaction curve of the effect of storage time, cold plasma treatment, and packaging with active film on TBA and TVB-N of fish paste

TVC

Fig. 5 shows the three-dimensional diagram and interaction curve of the effect of storage time, cold plasma treatment and active film packaging on the total microbial count of fish paste. The changes in the total microbial count of fish paste during the storage period were observed based on the results of the analysis of variance of the two-factor interactive response surface model (2FI). The presented model was statistically significant at the 99.99% confidence level (p-value < 0.0001) and was able to explain 99.27% of the response variations. The R^2 values (0.992) and adjusted R^2 (0.984) indicate the optimal fit of the model. Storage time (A) was identified as the most effective factor in increasing the microbial count. This natural increase is due to the proliferation and growth of microorganisms inherent to the food under storage conditions.

Cold plasma treatment (B) significantly reduced TVC, which can be attributed to the following mechanisms: direct destruction of the bacterial cell walls by plasma active species, destruction of bacterial DNA and cellular proteins, and oxidative damage to the cell membrane of microorganisms. Active film packaging (C) also showed a significant inhibitory effect on microbial growth, which works through the following: gradual release of antimicrobial compounds from plant extracts, synergistic effect of gold nanoparticles in inhibiting microbial activity, and creation of a physical barrier against secondary contamination. The interaction effect of time and cold plasma treatment (AB) showed that the application of cold plasma can effectively reduce the rate of microbial growth during storage time. The interaction effect of time and active packaging (AC) showed the synergistic effect of these two factors in controlling microbial growth during longer storage periods. The interaction effect of cold plasma treatment and active film (BC) was not statistically significant, which probably indicates different mechanisms of action of the two technologies. The final regression equation was obtained as follows:

TVC = 6.26 + 1.17*A + 0.29*B + 0.24*C + 0.29*A*B + 0.21*A*C + 0.051*B*C

The response surface and contour plots clearly showed that the simultaneous application of cold plasma treatment and active packaging could effectively prevent the increase of microbial counts during the storage period. This study suggests that the combination of cold plasma treatment and active packaging with a film containing antimicrobial compounds could be an effective strategy for controlling microbial growth and enhancing the shelf life of fish paste.

Both the current investigation and the research performed by Chen *et al*. (2020) independently validate the efficacy of active packaging in controlling microbial growth in fishery products. In Chen's study, the use of active film containing chitosan and plant extract significantly reduced the total viable count (TVC) in fried fish fillets during storage. This finding is fully consistent with the results of the present study, by which active packaging with polycaprolactone/beta-glucan nanocomposite film containing plant extracts and gold nanoparticles effectively inhibited microbial growth (TVC) in fish paste. Both studies show that natural active compounds incorporated in packaging films including plant extracts in the present study and Herba lophatheri extract in Chen's study can inhibit bacterial growth and extend the shelf life of various fishery products by gradually releasing antimicrobial compounds. This consistency of results confirms the effectiveness of the strategy of using natural compounds in active packaging to control microbial spoilage in different fish products (Chen *et al*., 2020).

Overall Acceptance

Fig. 6 shows the three-dimensional diagram and interaction curve of the effect of storage time, cold plasma treatment and active film packaging on the overall acceptance of fish paste. The changes in the overall sensory acceptance of fish paste during the storage period were observed based on the results of the analysis of variance of the quadratic response surface model. The presented model was statistically significant at 99.99% confidence level (p-value < 0.0001) and was able to explain 98.7% of the response changes. The R^2 values (0.987) and adjusted R^2 (0.954) indicate the optimal fit of the model. Storage time (A) was identified as the most effective factor in reducing overall acceptance. This reduction was mainly due to undesirable sensory changes caused by lipid oxidation, formation of off-odor compounds resulting from microbial spoilage, leading to textural changes and loss of product freshness characteristics. Both cold plasma treatment (B) and active film packaging (C) significantly maintained sensory quality, which can be attributed to the following: reducing the rate of spoilage by inhibiting microbial activity, reducing the rate of lipid oxidation, and maintaining the desired sensory properties of the product for a longer period of time.

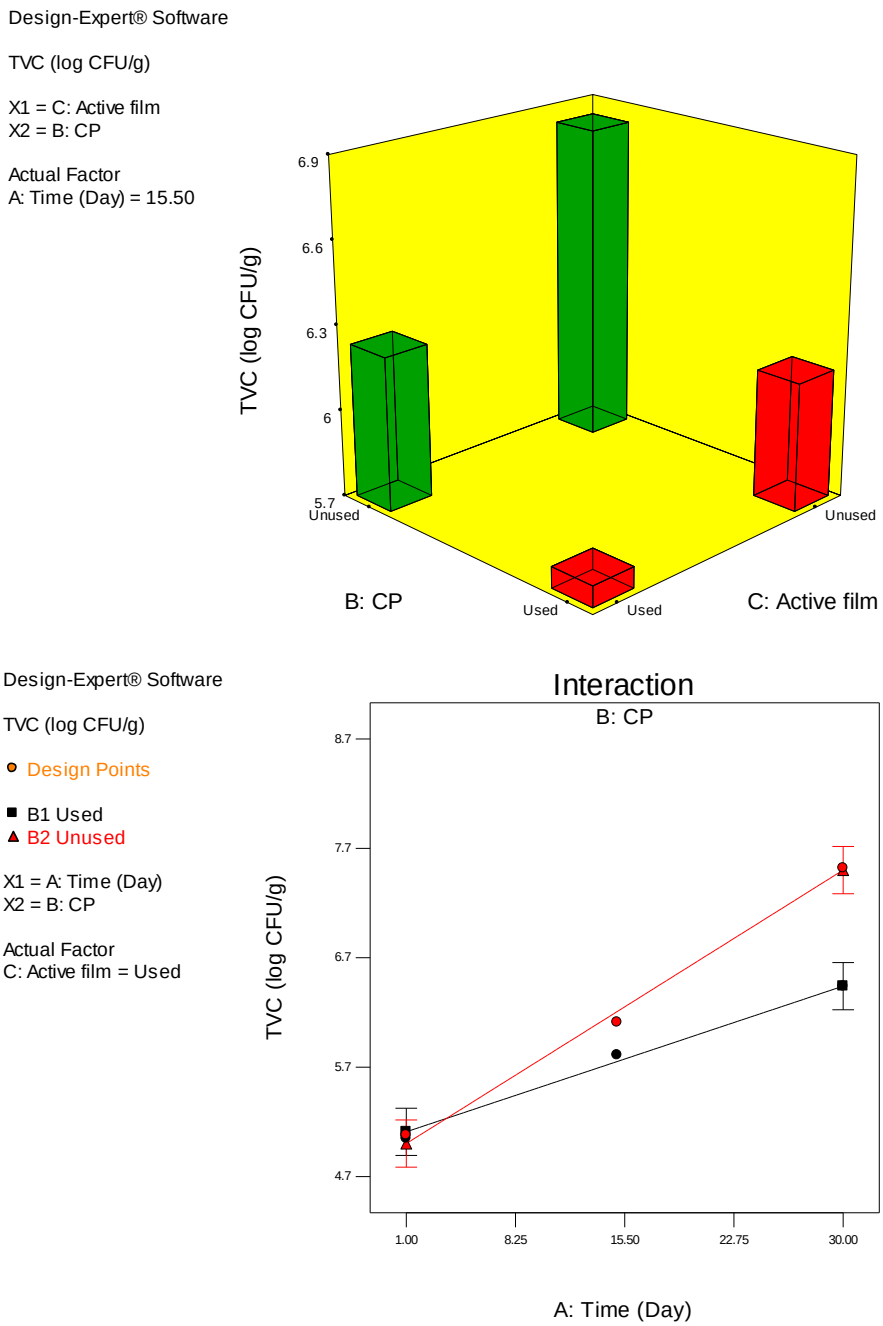

Fig. 5. 3D diagram and interaction curve of the effect of storage time, cold plasma treatment, and active film packaging on the total microbial count of fish paste

The interaction effects of time and cold plasma treatment (AB) and time and active packaging (AC) were all significant, indicating that these treatments can reduce the negative effects of time on sensory quality. The interaction effect of cold plasma treatment and active film (BC) was not statistically significant, possibly indicating that these two technologies affect sensory quality independently. The quadratic effect of time (A^2) was significant, indicating a nonlinear pattern of overall acceptance decline over time. The

final regression equation was obtained as follows:

Total acceptance=+4.47-1.00*A-0.25*B-0.25*C-0.25*A*B-0.25*A*C+0.00*B*C-0.47*A2

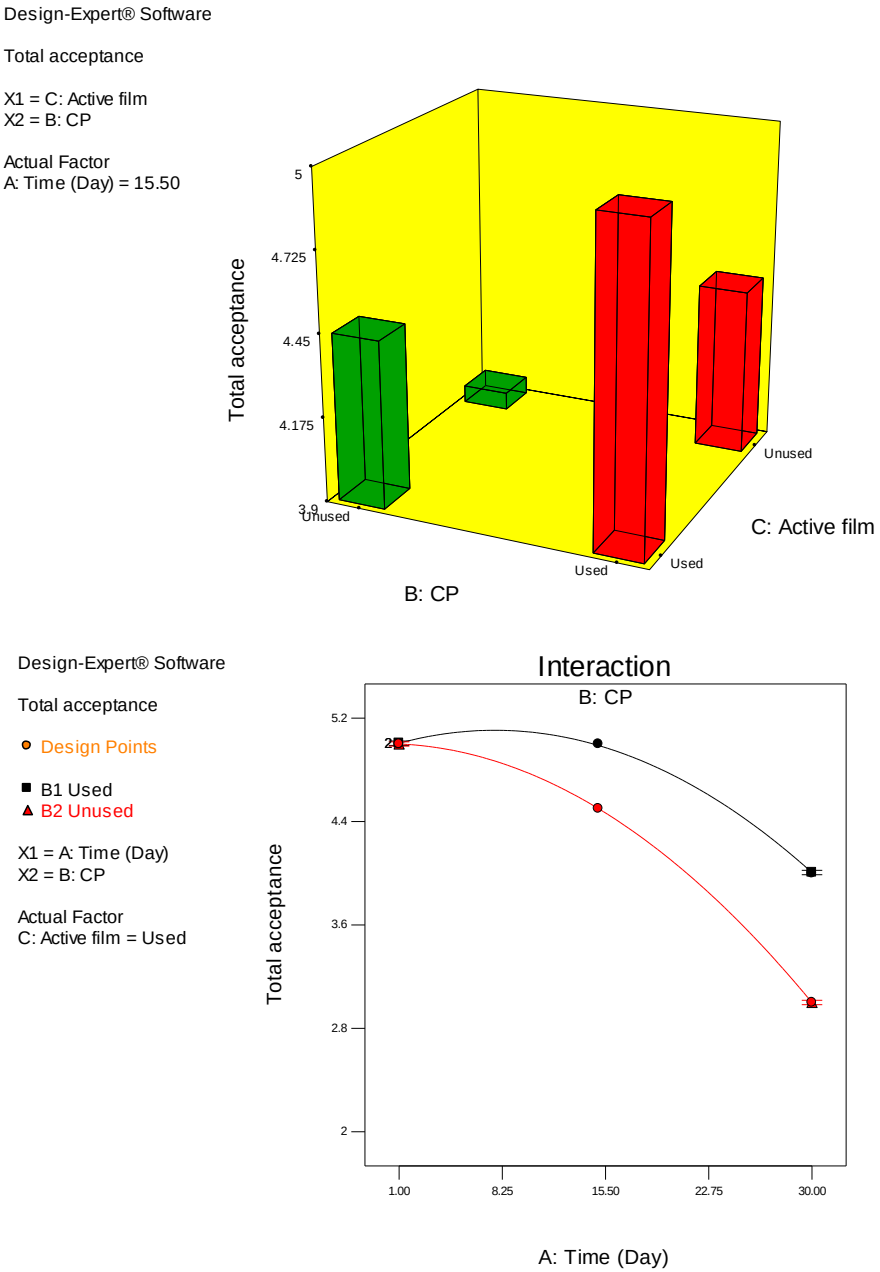

Fig. 6. 3D diagram and interaction curve of the effect of storage time, cold plasma treatment, and active film packaging on the overall acceptance of fish paste

The negative coefficients in the equation indicate the reducing effect of the variables on the overall acceptance. This study shows that the combination of cold plasma treatment and

active packaging can be an effective strategy to maintain the overall sensory acceptance of fish paste during the storage period. Although both treatments individually are able to reduce the sensory quality, their synergistic effect together can significantly increase the overall acceptance during the storage of the product.

Conclusion

The findings of this study clearly show that the prepared nanocomposite film containing plant extracts and gold nanoparticles, along with cold plasma treatment, is a comprehensive and effective solution in maintaining the quality, safety and shelf life of fish paste. This active packaging system, with its strong antibacterial and antioxidant properties, has the ability to simultaneously inhibit the main factors of spoilage, including the growth of microorganisms, lipid oxidation and the production of volatile compounds. The synergistic interaction between the active compounds in the film and the effects of cold plasma has led to the creation of a multiple protective barrier against spoilage. Cold plasma treatment effectively delays the spoilage process through the inactivation of surface enzymes and microorganisms, while the active film concurrently mitigates spoilage progression during storage by gradually releasing antibacterial compounds and establishing a physical barrier against oxygen ingress. The results of thermal analyses also confirmed the stability and appropriate compatibility of the film components. The presence of gold nanoparticles not only improved the thermal properties of the film, but also enhanced the overall performance of the packaging system by enhancing the antibacterial effect. Given its demonstrated efficacy, this integrated approach shows considerable promise for implementation in the food packaging sector, particularly for preserving perishable fishery products, and presents a viable alternative to conventional preservation techniques. It is suggested that further studies focus on optimizing the production scale, economic evaluation of this technology, and also investigating the synergistic effects of other natural active compounds.

Author Contribution

Sajad Pirsa: conceived of the presented idea, verified the analytical methods and wrote the manuscript and revised it. **Amir Afshar Asdagh:** developed the theory and performed the computations. **Mir Khalil Pirouzifard**: discussed the results and contributed to the final manuscript. **Amir Afshar Asdagh**: out the experiment.

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مقاله پژوهشی
جلد ۲۲، شماره ۱، فروردین- اردیبهشت ۱۴۰۵، ص. ۷۲-۵۱

توسعه فیلم‌های فعال-PCL/β گلوکان غنی‌شده با عصاره‌های طبیعی و نانوذرات طلا برای بسته‌بندی خمیر ماهی تیمار شده با پلاسمای سرد

امیر افشار اصدق[۱]- سجاد پیرسا[۱*]- میر خلیل پیروزی فرد[۱]

تاریخ دریافت: ۱۴۰۴/۰۸/۲۰
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چکیده

نگرانی‌های فزاینده در مورد بسته‌بندی‌های پلاستیکی مرسوم به‌دلیل عدم تجزیه‌پذیری، خطر انتقال ترکیبات سمی به مواد غذایی و آلودگی محیط زیست، توسعه جایگزین‌های سازگار با محیط‌زیست مانند فیلم‌های خوراکی و زیست‌تخریب‌پذیر را ضروری کرده است. این مطالعه با هدف توسعه یک سیستم بسته‌بندی فعال بر اساس فیلم نانوکامپوزیتی پلی‌کاپرولاکتون/بتا-گلوکان (PCL-BG) اصلاح‌شده با عصاره جفت میوه بلوط (OFPE)، عصاره هسته زردآلو (AKE) و نانوذرات طلا (AuNPs) و استفاده از آن برای بسته‌بندی خمیر ماهی که قبلاً با پلاسمای سرد تیمار شده بود، انجام شد. ارزیابی خواص ضد باکتریایی فیلم نشان داد که ترکیبات فعال اثر مهاری قوی دارند، به‌طوری‌که بزرگترین هاله‌های مهاری ۲۷٫۷۸ میلی‌متر در برابر باسیلوس سرئوس و ۲۱٫۳۸ میلی‌متر در برابر اشریشیا کلی در فیلمی با بالاترین غلظت عصاره مشاهده شد. تجزیه و تحلیل حرارتی (DSC) بهبود قابل توجهی در پایداری حرارتی نشان داد، به‌طوری‌که دمای ذوب فیلم کامپوزیت نهایی به ۸۱٫۶ درجه سانتی‌گراد افزایش یافت. ارزیابی‌های میکروبیولوژیکی و شیمیایی طی ۳۰ روز نگهداری نشان داد که تیمار ترکیبی به‌طور قابل توجهی ($p < 0.05$) رشد میکروبی (شمارش کل میکروبی زنده) را کاهش داد، تولید ترکیبات نیتروژن فرار (TVB-N) را کاهش داد، اکسیداسیون لیپید (TBA) را کنترل کرد و پایداری pH و پذیرش حسی را حفظ کرد. مدل‌های رگرسیون برای همه پارامترهای کیفی، دقت و قدرت پیش‌بینی بالایی را نشان دادند ($R^2 > 99.27\%$). این مطالعه به وضوح اثربخشی استراتژی ترکیبی پلاسمای سرد و بسته‌بندی فعال را در حفظ کیفیت و افزایش ماندگاری خمیر ماهی نشان می‌دهد.

واژه‌های کلیدی: آنتی‌باکتریال، بسته‌بندی فعال، پلاسمای سرد، فیلم زیست‌تخریب‌پذیر

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