



The impact of nanobubbles-O₂ and chitosan coating on physicochemical and microbial quality of refrigerated shrimp (*Litopenaeus vannamei*) stored at 4 °C

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ABSTRACT

This study assessed the effect of chitosan coating in the presence of O₂ nanobubbles (NBO₂) on shrimp quality (*Litopenaeus vannamei*). The chemical [pH, peroxide value (PV), thiobarbituric acid (TBA) and total volatile base nitrogen (TVBN)] and microbial [total viable count (TVC), total psychrophilic bacteria count (TPBC), *Enterobacteriaceae*, *Listeria* (LIS), and *Salmonella* (SAL)] analysis, sensory evaluation, textural and color profile was carried out for control and coated samples with chitosan, NBO₂ and chitosan+NBO₂ (Chi+NBO₂) throughout the storage period of 12 days. The NBO₂ had a particle size of 303.2 nm, a polydispersity index (PDI) of 0.544, and a zeta potential of -29.6 mV. PV, TBARS, and TVBN values in the control increased to 2.35 ± 0.21 meq O₂/kg, 1.43 ± 0.04 mg MDA/kg, and 42.81 mg N/100 g, respectively, during storage. Chitosan or NBO₂ alone slowed these increases, while the Chi+NBO₂ coating significantly reduced them to 1.41 ± 0.01 , 0.86 ± 0.01 , and 25.18 mg N/100 g, respectively. Moreover, the TVC, TPBC, LIS, and SAL counts of the Chi+NBO₂ coating reduced to 5.95 ± 0.35 log CFU/g, 6.48 ± 0.04 log CFU/g, 5.60 ± 0.14 log CFU/g, and 2.45 ± 0.07 log CFU/g, respectively. Chi+NBO₂ treatment showed negative correlations with spoilage parameters and positive correlations with hardness and acceptability, therefore maintained product quality during storage compared to other treatments.

1. Introduction

Shrimp, an extensively consumed marine food, is valued for its nutritional composition and desirable organoleptic characteristics, owing to the presence of disodium guanosine-5'-monophosphate and disodium inosine-5'-monophosphate, as well as high levels of free glycine and arginine (Lin et al., 2022). However, it is highly perishable, which is attributed to a combination of biochemical and microbial factors, including the endogenous protease activity (Li et al., 2022), melanosis (Mu et al., 2012), microbial spoilage (Qian et al., 2018), and protein and lipid oxidation (Deng et al., 2019). Such degradation processes during storage and transportation not only compromise product quality and market value but may also threaten consumer health (Lin et al., 2022). Maintaining the quality and freshness of shrimp is a critical

challenge in the international shrimp industry. Therefore, numerous techniques have been employed to extend shrimp shelf life, including radiation, various packaging methods, and active edible coatings loaded with bioactive compounds (Shafiei et al., 2024). Among different methods for enhancing marine quality, the use of films or coatings has become increasingly noticeable (Abedi et al., 2024). Chitosan is a natural, non-toxic, biodegradable, and biocompatible compound composed of a linear polysaccharide with β -(1→4)-linked 2-amino-2-deoxy-D-glucose residues, derived from the deacetylation of chitin (Aranaz et al., 2021). Chitosan-based coatings can suppress microbial growth and extend shrimp shelf life, likely by forming an oxygen barrier and electrostatically disrupting microbial membranes (Chen et al., 2022). They may also inhibit proteolytic enzymes during storage (Gan et al., 2021). Therefore, it has been successfully used to coat and preserve various

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products. To effectively utilize chitosan coating, the chitosan can be combined with other extracts, essential oils, bioactive compounds, and other preservation techniques (El-Aidie, 2018). To date, numerous studies have used chitosan coating to extend the shelf life of shrimp incorporated with essential oils or extracts (Alparslan et al., 2019; Chen et al., 2021; Rezaei et al., 2023; Rezaeifar et al., 2020; Wang et al., 2018).

Nano bubbles (NBs) technology represents a significant advancement in green food processing (Abedi, Gahruie, et al., 2025; Abedi, Sayadi, et al., 2025; Abedi & Wietstock, 2026). Bubbles form in aqueous solutions when the pressure is reduced below a critical threshold. This induces a phase change, creating the characteristic gas-filled cavities. Simultaneous rapid pressure fluctuations and velocity variations in a liquid flow initiate hydrodynamic cavitation. This leads to the formation of vapor bubbles due to pressure differences applied over milliseconds (Sollid et al., 2012). This technology offers a range of advantages, including high efficacy, environmental friendliness, and reduced processing time and costs (Pal et al., 2022). Over the last few years, NBs technology has gradually attracted attention and has been successfully applied in water purification and disinfection, the agricultural, and biomedical industries (Chen et al., 2021). The antibacterial and antiviral activities of NBs are associated with the free radicals they generate, which disrupt microbial cell walls, increase membrane permeability, and ultimately lead to cell death (Huang et al., 2025). There are various types of NBs, including ozone, oxygen, and carbon dioxide, which could have been used for the treatment of vegetables, aquaculture products (e.g., fish and shrimp), and surface disinfection (de Oliveira Ramiro et al., 2024; Imaizumi et al., 2018; Mahasri et al., 2018; Mauladani et al., 2020; Rahmawati et al., 2021).

While nanobubbles and chitosan individually exhibit antimicrobial activity, their combined effect has not been previously investigated. In the present study, NBs were generated through hydrodynamic (HC) O₂-HC, and the impact of the independent and simultaneous application of chitosan coating and NBO₂ treatment on shrimp deterioration criteria (microbial and oxidation reaction) was evaluated. In this regard, the treatments are divided into four groups: control, NBs treatment, chitosan coating, and chitosan+NBO₂ (Chi+NBO₂). The chemical (pH, PV, TBARS, and TVBN) and microbial quality (*Salmonella typhi*, *Listeria monocytogenes*, total psychrophilic bacteria, *Enterobacteriaceae*, and total viable count) parameters, texture and color profiles, and sensory evaluation of shrimp were assessed during cold storage (4°C) for 12 days.

2. Materials and methods

2.1. Materials

All reagents used were of analytical grade and supplied by Merck Chemical Co., Limited (Darmstadt, Germany). Deionized water was employed for the experimental procedures.

2.2. NBO₂ characteristics

2.2.1. Particle size, polydispersity index (PDI), and zeta potential of NBO₂

A modified procedure of Abedi, Gahruie, et al. (2025) and Abedi & Wietstock (2026) was applied to produce NBs. High-purity oxygen gas (99.999%, medical/industrial grade) was supplied from a compressed gas cylinder equipped with a two-stage precision pressure regulator. The gas was introduced into 10 L of sterile distilled water using a hydrodynamic nanobubble generator (pressure-drop/cavitation-based system). The regulator was initially set to level 5 (approximately 55–60 psi) for the first 1 min to achieve rapid saturation, then reduced to level 1 (approximately 30–35 psi) for the remainder of the process. The nanobubble generator was operated in pulsed mode for a total of 15 s (5 cycles of 3 s each). The resulting oxygen nanobubble water (NBO₂) was immediately collected and stored in sterile, gas-tight containers at 4°C. The DO immediately after generation typically ranges from ~30 to ~43

mg/L. The final bulk O₂ nanobubbles exhibited a mean hydrodynamic diameter of 303.2 nm. Dynamic light scattering (DLS, SZ-100, Horiba, Japan) was used to determine the mean particle size and PDI of nanobubbles at 25°C.

2.2.2. Chitosan coating solution

Chitosan solution (2 g/100 mL) (Sigma-Aldrich Co., Germany) with a degree of deacetylation (76.5%) and molecular weight (200 kDa) was prepared by dissolving acetic acid (1% w/v) and stirring continuously at room temperature until completely homogeneous. Then, chitosan solution and glycerol (0.75 mL/g) were blended. The solution was further mixed at 12000 rpm for 10 min (Lab 60 high-pressure homogenizer, APV Gaulin, AxFlow, Durham, UK).

2.3. Preparation and coating of shrimps (*Litopenaeus vannamei*)

Fresh shrimps were purchased from a local market, transferred to the laboratory, and washed. The shrimp fillets were then cut into 10 g pieces. The pieces were soaked in 70% ethanol (v/v) for sterilization for about 1 min (Sihamok et al., 2025). Finally, all samples were washed with sterile water. For coating, shrimps were separated into 4 groups. The initial group was uncoated (Control). Dipping shrimp prepared the second and third groups in a chitosan coating. Shrimps were immersed in 100 mL of coating solution for 3 min at room temperature (25°C), then drained for 5 min, and then immersed again in the coating solution for 30 s. Subsequently, the treated fillets were placed under sterile conditions on a metal grid at 10°C for 30 min to allow the coating solution to dry completely. The third group was treated with NBO₂ for 10 min. The fourth shrimp group was sequentially dipped in chitosan under the above conditions and then treated with NBO₂ (Chi + NBO₂). Then, each group was stored at 4 ± 1°C for 12 days. Chemical and microbial tests were performed on days 0, 3, 6, 9, and 12, with three replicates per test.

2.4. Chemical analysis

2.4.1. pH measurement

The pH of 10-g samples from each treatment was measured using a pH meter (OHAUS, Starter3000, USA) after homogenization with 90 mL distilled water (6000 rpm, 5 min) using a Digital Ultra-Turrax (IKA, Germany).

2.4.2. Peroxide value (PV)

PV of shrimps was measured based on the technique described by Behbahani et al. (2020). Approximately 3 g of the sample was homogenized with 25 mL of chloroform-acetic acid solution (3:2 v/v), and 0.5 mL of saturated potassium iodide solution was then added. After 5 min of dark storage, the reaction mixture was titrated with the standard sodium thiosulfate solution (0.1 N). The following equation was used to express the PV as meq O₂/ kg sample:

$$PV = (V \times N) / W \times 100 \quad (1)$$

V₂ is the volume (mL) of sodium thiosulfate solution,

N is the normality of the sodium thiosulfate solution,

W is the weight of the sample

2.4.3. Thiobarbituric acid reactive substances (TBARS)

TBARS values for the groups were determined using the colorimetric method. About 10 g of fish tissue was thoroughly homogenized with 25 mL of distilled water using a chilled homogenizer. Subsequently, 1 mL of the resulting homogenate was transferred into a test tube and mixed with 2 mL of the TBARS reagent, which consisted of 0.37% (w/v) TBARS, 15% (w/v) trichloroacetic acid (TCA), and HCl (0.25 N) hydrochloric acid and then was heated (80°C for 90 min) to create a pink color. After cooling, the solution's absorbance at 530 nm was measured

using a UV-Vis spectrophotometer (PerkinElmer, Lambda 25). The TBARS value was expressed as mg of malonaldehyde (MDA)/kg sample.

2.4.4. Determination of total volatile basic nitrogen (TVBN)

Total volatile basic nitrogen (TVBN) was determined using a modified micro-Kjeldahl method. Minced samples underwent sulfuric acid digestion. The TVBN concentration (mg N/100 g) was calculated using Eq. (2)

$$\text{TVBN} = [(V_1 - V_2) \times N \times 14 \times 10] / W \quad (2)$$

V_1 is the volume (mL) of sulfuric acid used for the sample titration,

V_2 is the volume (mL) of sulfuric acid for the blank titration,

N is the normality of the sulfuric acid,

W is the weight (g) of the sample

The factor 10 accounts for the dilution and conversion to mg N/100g.

2.5. Microbial analysis

Antimicrobial activity was assessed against representative pathogenic strains (*Salmonella typhi* [SAL] and *Listeria monocytogenes* [LIS]) and spoilage-associated bacterial groups, namely *total psychrophilic bacteria* (TPBC), *Enterobacteriaceae* (EB), and *total viable count* (TVC). To prepare the sample for microbial analysis, 10 g of chopped shrimp were aseptically mixed with 90 mL of sterile peptone water (0.1 g/100 mL) and homogenized for 2 min to ensure uniform suspension. Microbiological analysis was conducted on days 0, 3, 6, 9, and 12. TVC and TPBC were measured with plate count agar (PCA) (Merck, Germany), incubated at 37°C for 48 h, and at 7°C for 10 days, respectively. LIS and SAL were counted using PALCAM agar (Sigma Aldrich, UK) for 48 h at 37°C (Zainal Abidin et al., 2023) and XLD agar following a 24 h incubation period at 37°C (Rabiey et al., 2013), respectively. *Enterobacteriaceae* (EB) counts were determined using Violet Red Bile Glucose Agar (Oxoid, Thermo Scientific™, Italy), followed by incubation at 37°C for 48 h (Amiri et al., 2024).

2.6. Texture profile analysis

Texture profile analysis (TPA) was conducted using a Brookfield CT3-4500 texture analyzer (AMETEK Inc., Middleboro, MA, USA). A TA5 cylindrical probe was used at a temperature 25°C; Samples in cylindrical form (1 × 1 cm²) were subjected to a double compression test, reducing them to 30% of their original height at a speed of 0.5 mm/s with a 10 s interval, with a 0.05 N triggering point load, using a 6 cm diameter aluminum cylindrical probe. These parameters were measured: hardness, elasticity, cohesiveness, and adhesiveness (Huang et al., 2025).

2.7. Color measurements

A Hunter-Lab Colorflex Colorimeter (Hunter Associates Lab, Inc., Reston, Virginia, USA) was used to assess color attributes, with color values expressed as L* (lightness), a* (red/green), and b* (yellow/blue).

2.8. Sensory analysis

The sensory assessment was conducted according to Huang et al. (2025), with minor modifications. A total of 30 trained panelists (18-35 years; balanced gender) evaluated shrimp under various treatments. The sensory attributes measured included appearance, aroma, flavor, texture, and overall acceptability, using a nine-point hedonic scale (1 = extremely disliked, 5 = neither liked nor disliked, 9 = extremely liked). Color, odor, texture, and overall acceptability were evaluated. The tests were conducted in a room maintained at 25 ± 2°C, with minimal noise to minimize distractions. Panelists were informed in advance of the study's purpose, the composition of the samples, and the safety

procedures, and they voluntarily agreed to participate in the sensory evaluation.

2.9. Statistical analysis

Experimental data were collected from three independent replicates, and the average values were computed for analysis. Statistical analyses were conducted using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) was performed to evaluate the effect of different treatments on the measured parameters. Before conducting ANOVA, data were tested for normality and homogeneity of variances using the Shapiro-Wilk and Levene's tests, respectively. Upon confirmation of significant treatment effects ($P < 0.05$), Duncan's multiple range test was utilized to separate the means and identify substantial pairwise differences. The significance level was set at $\alpha = 0.05$ for all analyses.

3. Results and discussion

3.1. NBO₂ water characteristics

3.1.1. Particle size, zeta potential, and PDI

The particle size, PDI, and zeta potential of NBO₂ water were 303.2 nm, 0.544, and -29.6, respectively (Fig. 1). The dispersity and size of nanobubbles in different research are varied and are related to the nanobubble machine condition, type of air or gas, rate of water flow, gas pressure, and generation time (Nghia et al., 2021; Phan et al., 2021). Moreover, during NB production, water temperature may increase, affecting NBs' diameter and concentration. Indeed, NBs become scorched at higher temperatures, reducing their concentration (Nghia et al., 2021). The size of NBO₂ in the present study was lower than the results reported by Phan et al. (2021) who reported a Z-average of NB-CO₂ around 520 nm at 300 kPa. The prolonged stability of NBs is attributed to the accumulation of negative charges at the gas-water interface. It has been proposed that NBs are encapsulated by an electrical double layer. The long-term stability of NBs has been linked to their negatively charged surfaces and the preferential adsorption of anions at the gas-liquid boundary. This double layer generates strong electrostatic repulsion, preventing bubble aggregation and coalescence. The stability of micro- and nanobubbles in aqueous media is further enhanced when they exhibit a high absolute ζ -potential value. However, the ζ -potential of NBs is influenced by several factors, including the type of gas used, the electrolyte composition of the aqueous phase, and the presence of chemical surfactants (Malahlela et al., 2024). A reduction in NB diameter increases their surface area, making smaller bubbles particularly advantageous. Owing to their nanoscale dimensions, these bubbles readily adhere to suspended contaminants, thereby enhancing contact duration and improving interaction efficiency (Abedi, Gahruei, et al., 2025; Abedi, Sayadi, et al., 2025; Malahlela et al., 2024).

3.2. Microbial analysis

The changes in TVC, TPBC, LIS, and SAL counts of different treated shrimps during the storage at 4°C are shown in Fig. 2. The initial TVC was around 3.65-3.30 log CFU/g (Fig. 2 a). Generally, an increasing trend of TVC in all groups was seen during the 12 days of storage. The maximum TVC was observed in the control group, reaching 9.53 ± 0.25 log CFU/g on the 12th day ($p < 0.05$). According to the results, shrimps treated with Chi+ NBO₂ significantly reduced the rising trend of TVC (3.61-5.95 log CFU/g). The final TVC of the chitosan-coating alone and NBO₂-treated groups was 6.48 ± 0.18 log CFU/g and 6.85 ± 0.21 log CFU/g, respectively. The combination of chitosan coating and NBO₂ exhibited significantly greater antibacterial activity ($p < 0.05$). The considerable TVC reduction was attributed to the coating's role as an effective gas barrier and to reduced oxygen diffusion, which prevented aerobic bacterial proliferation (Nikmanesh et al., 2023). Our results

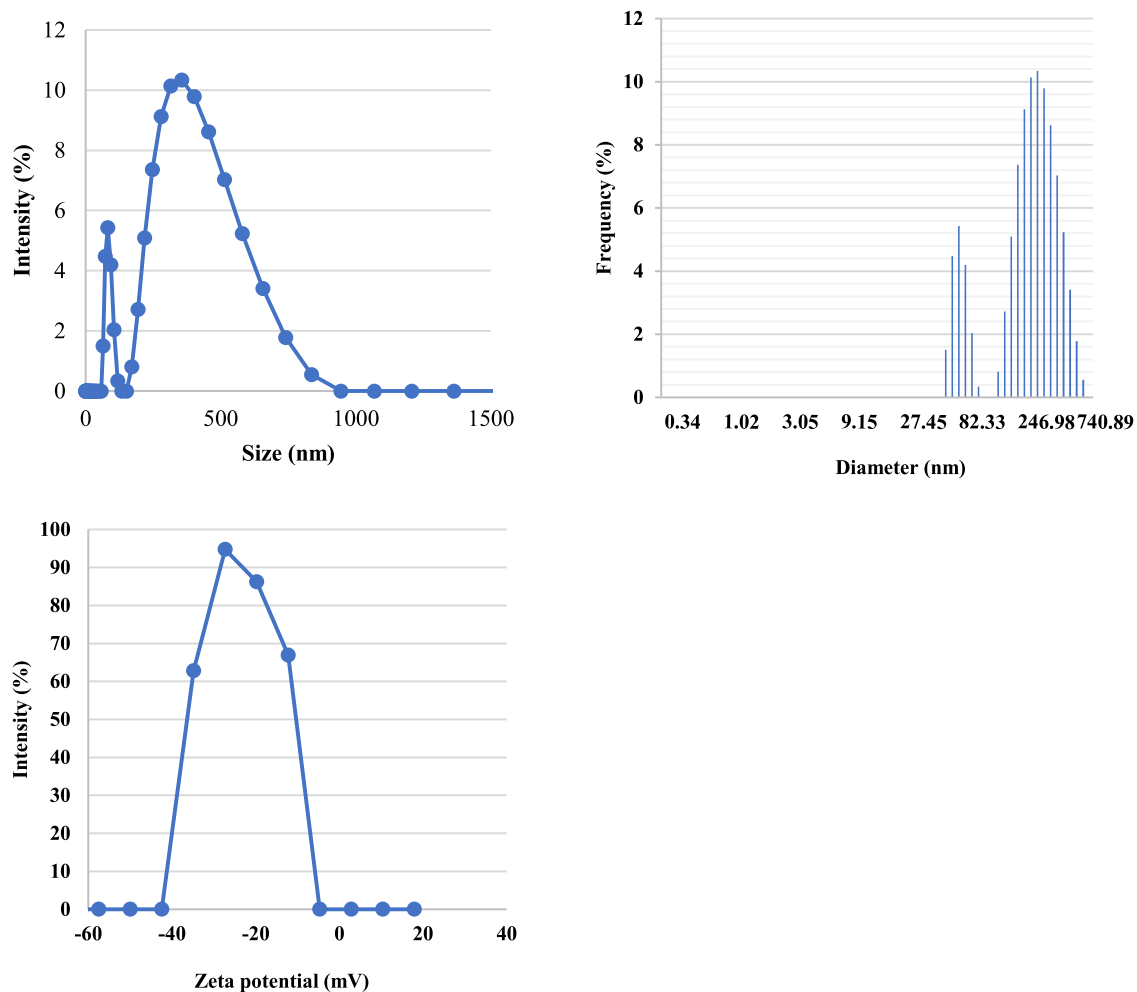


Fig. 1. Average size, zeta potential, and PDI of NBO₂.

agreed with those of [Yuan et al. \(2016\)](#), who studied the shelf-life extension of chitosan coatings containing pomegranate peel extract during cold storage. Their results showed that the TVC of the uncoated group increased during storage. In contrast, the coated groups had lower microbial counts, reflecting the antibacterial activity of the polyphenolic compounds in pomegranate peel extract and chitosan. [Liu et al. \(2020\)](#) also observed a decrease of 2-3 log CFU/g of total aerobic plate counts in shrimp coated with an active chitosan coating loaded with kojic acid.

Psychrophilic bacteria are crucial in causing spoilage of marine products within 12 days ([Sae-Leaw et al., 2017](#)). The initial TPBC of the control was 2.50 ± 0.14 log CFU/g ([Fig. 2 b](#)), which increased throughout the storage period at a remarkably higher rate (7.55 log CFU/g; $P < 0.05$) than in the chitosan (2.55 ± 0.07 log CFU/g to 6.90 ± 0.28 log CFU/g) and Chi+NBO₂-treated shrimp group was 6.48 ± 0.04 log CFU/g on the 12th day. However, the TPBC of the control and NBO₂-treated groups exceeded the allowable limit (7.0 log CFU/g) ([ICMSF & ICMSF, 1986](#)). Our results were consistent with those of [Na et al. \(2018\)](#), who reported that chitosan and ϵ -polylysine coatings markedly prevented mesophilic and psychrotrophic bacteria, including *Pseudomonas* spp., and reduced 3-log CFU/g in Pacific white shrimp after 9 days of cold storage.

[Fig. 2 c](#) shows that the LIS count increased significantly across all groups throughout storage ($P < 0.05$). The chitosan coating and NBO₂ treatment were relatively effective compared with the control, yielding LIS counts of 6.05 ± 0.21 and 6.45 ± 0.07 log CFU/g, respectively. The combination of Chi-NBO₂ treatments significantly intensified the LIS inhibitory effect, and its LIS count reached 5.10 ± 0.14 log CFU/g

($p < 0.05$). This inhibitory effect was due to the antibacterial potential of chitosan and NBO₂. Similar studies also confirmed that (2 g / 100 mL) chitosan coating enriched with organic acid (2 g/ 100 mL) and (1 g/ 100 mL) chitosan solution loaded with organic acid (2 g/ 100 mL) can decline the *L. innocua* count (about 2 log CFU/g) and *L. monocytogenes* count (about 5 log CFU/g) inoculated on ready-to-eat shrimps, respectively ([Guo et al., 2013](#); [Li et al., 2013](#); [Na et al., 2018](#)). [Zainal Abidin et al. \(2023\)](#) also revealed that the N,N'-Diacetylchitobiose chitin oligosaccharide coating can slow the growth of *L. monocytogenes* on coated shrimp, resulting in a 0.5 log CFU/g reduction compared with the control.

The effects of chitosan coating and NBO₂ treatment on SAL in refrigerated shrimps are shown in [Fig. 2 d](#). The SAL count in all groups (except control) decreased significantly with increasing storage time ($p < 0.05$). Initial SAL count was around 3.85-3.95 log CFU/g, and showed an increasing pattern and reached 6.03 ± 0.18 log CFU/g for the control on day 12 ($p < 0.05$). In contrast, the respective counts for the coated groups were lower throughout the entire storage period ($p < 0.05$). When storage was over, the SAL count was lower in the chitosan-coated (2.65 ± 0.21 log CFU/g) and NBO₂-treated (2.75 ± 0.21 log CFU/g) groups compared to the control, indicating that SAL growth was inhibited by the chitosan coating or NBO₂ treatment ($p < 0.05$). Moreover, SAL proliferation was lower in shrimps stored under the Chi+NBO₂ coating, from an initial value of 3.85 ± 0.21 log CFU/g to 2.45 ± 0.07 log CFU/g ($p < 0.05$). Our findings were in close agreement with [Sorour et al. \(2021\)](#), who investigated the effect of chitosan coating on the quality of fresh tilapia. The authors observed a reduction in the

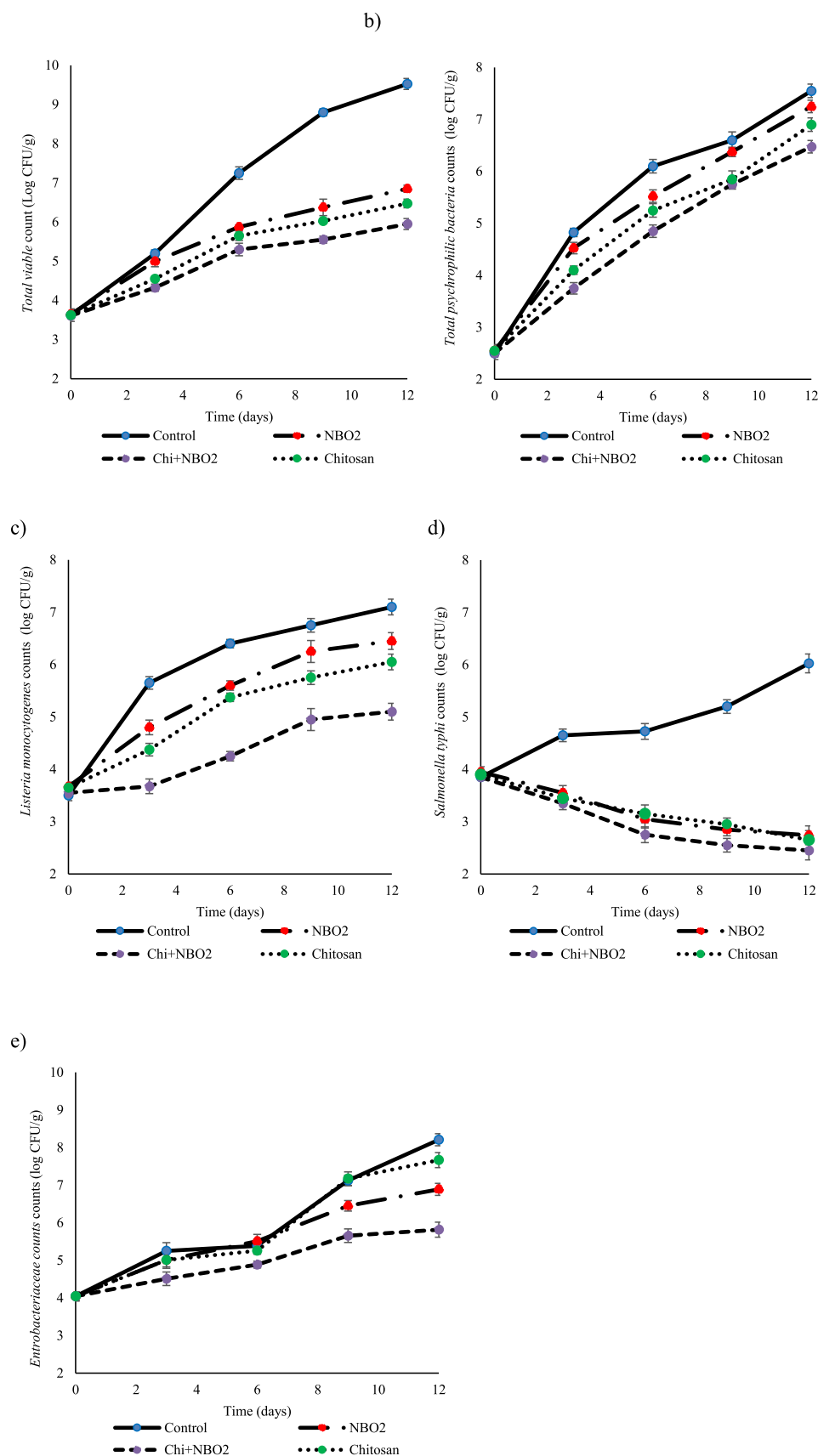


Fig. 2. Microbial analysis of different treated shrimp groups: total viable count (a), total psychrophilic bacteria count (b), *Listeria* (c), *Salmonella* (d), and Enterobacteriaceae (e) of different treatments of shrimp during cold storage. Control: uncoated group; Chitosan: coating with chitosan; NBO₂: treating with NBO₂; Chi+NBO₂: coated with chitosan+NBO₂.

SAL population over storage time and reported that chitosan coating with nanochitosan effectively lowered the *S. typhimurium* count. This inhibitory activity was associated with changes in the outer membrane lipopolysaccharide of Gram-negative bacteria and with membrane dysfunction that impairs oxygen permeation. Similarly, Johnson et al. (2022) reported that a chitosan coating loaded with *M. piperita* and *P. amboinicus* inhibited *Salmonella* spp. in coated chicken. Sotoudeh et al. (2020) also noted that the chitosan–nisin coating had antimicrobial potential against SAL in refrigerated chicken fillets, and that the SAL count was below 109 log CFU/g on the 7th day of storage. El-Khawass et al. (2020) reported that chitosan coating and lactic acid exhibited antibacterial activity against *S. typhimurium* and *Escherichia coli* in chicken fillets during cold storage. The authors reported that a chitosan coating containing lactic acid exhibited potent inhibition against *S. typhimurium* and *E. coli*, reducing CFU/g by 1.4 and 1, respectively.

The results demonstrated that the most potent inhibition of *Enterobacteriaceae* growth (Fig. 2 e) occurred in Chi+NBO₂ (4.04–5.81 Log CFU/g), followed by chitosan (4.04–7.66 Log CFU/g), NBO₂ (4.03–6.88 Log CFU/g), and the control sample (4.04–8.20) after 12 days of storage.

Several studies have confirmed chitosan's antibacterial activity against numerous pathogens. The positively charged amino group on the chitosan structure is thought to be responsible for its antibacterial feature. It may interact with negatively charged proteins on the bacterial cell membrane, altering membrane permeability, causing autolysis, and inhibiting microbial metabolism (Farajzadeh et al., 2016). The study by Wei et al. (2022) evaluated conductivity and ATPase activity as indicators of chitosan-induced cell membrane damage. The conductivity values of the shrimp treated with chitosan were significantly higher than those of the control group ($P < 0.05$), indicating that chitosan altered cell membrane permeability and the leakage of electrolytes and nucleic acids into the surrounding suspension, thereby inhibiting bacterial growth. Moreover, Na⁺/K⁺-ATPase, located in the bacterial cell membrane, plays a crucial role in bacterial growth and reproduction. When membrane integrity is compromised, this enzyme may leak into the extracellular environment. Therefore, alterations in membrane permeability increased ATPase activity in the chitosan group compared with the control groups. Furthermore, chitosan can chelate nutrients and essential metals and block bacterial protein synthesis through binding to DNA. Therefore, chitosan can inhibit DNA replication in microorganisms, reducing bacterial proliferation and inducing cell death (Yilmaz Atay, 2020). Wang et al. (2018) reported that a chitosan coating containing carvacrol synergized with microbial growth inhibition and reduced the aerobic bacterial count in Pacific white shrimps during cold storage.

The oxidation–reduction potential (ORP) of an aqueous solution is the potential at which the solution can oxidize or reduce other substances and varies logarithmically with oxygen concentration. As such, ORP is regarded as an essential factor governing microbial inactivation. The ORP decreased to approximately 408.3 mV (CO₂), 326.7 mV (O₂), 308.4 mV (air), and 290.8 mV (N₂). It should be concluded that the insertion of NBO₂ causes a significant preservative impact on shrimp's microbial quality due to ROS and hydroxyl radical production, which can destroy microbial cells (Javed et al., 2023). These are distinct mechanisms arising from the interaction between chitosan and OH[•]: (A) Chi+NBO₂ exhibited a markedly greater capacity to disrupt bacterial membrane integrity than either chitosan or NBO₂ alone. Such effects

likely result from interactions between hydroxyl and amino groups in chitosan and bacterial cell wall components, or from hydrogen bonding with membrane phospholipids (Fig. 3). (B) OH[•] attack at hydroxyl groups or the anomeric carbon (C₁) induces cleavage of the glycosidic bond, promoting polymer chain scission and the formation of low-molecular-weight fragments, which are likely to enhance antioxidant and antimicrobial activity (Kim & Thomas, 2007). The antioxidant properties of chitosan with molecular weights of 30, 90, and 120 kDa were examined in salmon (*Salmo salar*). Among them, the 30 kDa chitosan exhibited superior water solubility and significant antioxidant capacity, suggesting its effectiveness in retarding lipid oxidation and enhancing the stability of lipid-rich foods (Kim & Thomas, 2007; Z. Wang et al., 2024). (C) The protonation state (NH₃⁺ or NH₂) is determined by the solution pH rather than radical attack since the pK_a of chitosan's –NH₂ groups is around 6.3–6.7. At pH < 6.3, most –NH₂ groups are protonated and –NH₃⁺ form dominates. It should be noted that the reaction medium was 5.2 due to chitosan solubilization in acetic acid. This facilitates the formation of –NH₃⁺ (Tamer et al., 2016). Electrostatic attraction between protonated amine groups and negatively charged microbial membranes may further enhance permeability. The superior antimicrobial activity of NBO₂ can be attributed to its hydroxyl (OH[•]) groups, whose deprotonation lowers pH and increases amino group protonation, thereby strengthening interactions with bacterial surfaces (Batista et al., 2021).

Numerous investigations have reported that NBs exhibit antimicrobial properties for sterilization. The bactericide pathways generate acoustic waves, cavitation bubbles, and subsequent shock waves, as well as free radicals that destroy bacteria. Furthermore, the generated OH[•] and ROS exhibit antibacterial activity (Mokudai et al., 2024; H. Zhang & Tikekar, 2021). Thanh Dien et al. (2021) reported that multiple ozone NBs (NBO₃) significantly reduced *Aeromonas hydrophila* levels (15.9–35.6%) in Nile tilapia farming water and decreased infection and mortality rates over 14 days. The antibacterial activity of NBO₃ was due to the disruption of bacterial cell membrane glycoproteins and glycolipids. Therefore, binding of the sulfhydryl groups could inactivate enzymes involved in DNA replication, leading to cell death (Thanh Dien et al., 2021). Banche et al. (2022) also reported that chitobiocomposites loaded with NBO₂ exhibited antimicrobial activity against human pathogens, including *Staphylococcus aureus* and *Candida albicans*. This interaction disrupts the integrity of the cell membrane, leading to cell death via leakage of cellular constituents and impairment of cellular function. Similarly, Yamaguchi et al. (2021) investigated the antibacterial potential of NBs of O₂, CO₂, and N₂ against *E. coli*. The authors reported that NBO₂ generated much higher levels of hydroxyl radicals and ROS than NBS and NBS, and that it markedly reduced *E. coli* proliferation. Munir et al. (2023) also used NBO₃ to improve curcumin's antimicrobial feature. The authors reported that incorporating NBO₃+curcumin as the core within a chitosan shell, with photo-activation treatment, exhibited strong antimicrobial activity against *E. coli*, *Enterococcus faecalis*, and *S. aureus*.

3.3. Chemical characteristics of shrimps

3.3.1. pH

According to Table 1, the initial pH of the fresh shrimp was approximately 6.23–6.42. This value corresponds to the pH values

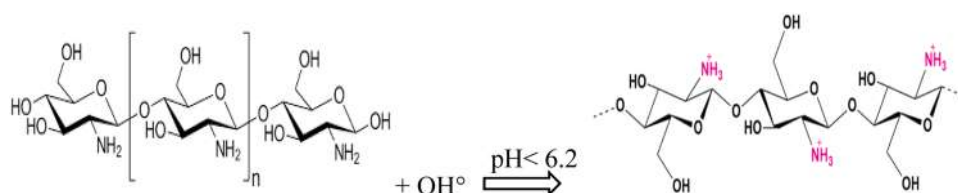


Fig. 3. The effect of hydroxyl radical on the amine group of chitosan.

Table 1

Changes in pH, PV, TBARS, and TVBN values of different treated shrimp groups.

	Samples	0	3	6	9	12
pH	Control	6.42 ± 0.12 ^{Ea}	6.83 ± 0.09 ^{Da}	7.36 ± 0.07 ^{Ca}	7.95 ± 0.09 ^{Ba}	8.55 ± 0.14 ^{Aa}
	Chitosan	6.41 ± 0.04 ^{Da}	6.52 ± 0.10 ^{Cc}	6.85 ± 0.11 ^{Dc}	7.12 ± 0.06 ^{Bc}	7.49 ± 0.09 ^{Ac}
	NBO ₂	6.48 ± 0.06 ^{Ea}	6.75 ± 0.08 ^{Db}	7.06 ± 0.07 ^{Cb}	7.46 ± 0.12 ^{Bb}	7.78 ± 0.12 ^{Ab}
	Chi-NBO ₂	6.43 ± 0.05 ^{Ea}	6.42 ± 0.09 ^{Dd}	6.47 ± 0.08 ^{Cd}	6.75 ± 0.08 ^{Bd}	6.83 ± 0.12 ^{Ad}
	Control	0.48 ± 0.04 ^{Db}	0.82 ± 0.05 ^{Ca}	1.95 ± 0.06 ^{Ba}	2.05 ± 0.07 ^{Ba}	2.35 ± 0.21 ^{Aa}
PV (meq/kg)	Chitosan	0.50 ± 0.01 ^{Eb}	0.68 ± 0.04 ^{Dc}	1.13 ± 0.04 ^{Cc}	1.50 ± 0.28 ^{Bbc}	1.91 ± 0.13 ^{Ac}
	NBO ₂	0.51 ± 0.01 ^{Ea}	0.75 ± 0.01 ^{Db}	1.66 ± 0.08 ^{Cb}	1.88 ± 0.11 ^{Bab}	2.12 ± 0.03 ^{Ab}
	Chi-NBO ₂	0.51 ± 0.02 ^{Ea}	0.61 ± 0.01 ^{Dd}	0.85 ± 0.07 ^{Cd}	1.20 ± 0.06 ^{Bc}	1.41 ± 0.01 ^{Ad}
	Control	0.12 ± 0.01 ^{Ea}	0.37 ± 0.02 ^{Da}	0.72 ± 0.02 ^{Ca}	1.15 ± 0.01 ^{Ba}	1.43 ± 0.04 ^{Aa}
	Chitosan	0.12 ± 0.00 ^{Ea}	0.31 ± 0.01 ^{Db}	0.60 ± 0.03 ^{Cc}	0.71 ± 0.01 ^{Bc}	1.00 ± 0.02 ^{Ac}
TBARS (mg MDA/kg)	NBO ₂	0.12 ± 0.01 ^{Ea}	0.34 ± 0.00 ^{Dab}	0.66 ± 0.01 ^{Cb}	0.86 ± 0.01 ^{Bb}	1.17 ± 0.05 ^{Ab}
	Chi-NBO ₂	0.11 ± 0.01 ^{Ea}	0.24 ± 0.02 ^{Dc}	0.51 ± 0.01 ^{Cd}	0.61 ± 0.03 ^{Bd}	0.86 ± 0.01 ^{Ad}
	Control	7.48 ± 0.05 ^{Ea}	15.06 ± 0.08 ^{Da}	24.03 ± 0.09 ^{Ca}	35.11 ± 0.11 ^{Ba}	41.81 ± 0.08 ^{Aa}
	Chitosan	7.52 ± 0.06 ^{Ea}	11.18 ± 0.11 ^{Dc}	16.94 ± 0.06 ^{Cc}	28.10 ± 0.12 ^{Bc}	31.13 ± 0.09 ^{Ac}
	NBO ₂	7.46 ± 0.09 ^{Ea}	12.83 ± 0.08 ^{Db}	18.25 ± 0.11 ^{Cb}	29.03 ± 0.07 ^{Bb}	31.62 ± 0.10 ^{Ab}
TVBN (mg N/100 g shrimp)	Chi-NBO ₂	7.46 ± 0.07 ^{Ea}	8.84 ± 0.08 ^{Dd}	12.79 ± 0.12 ^{Cd}	18.51 ± 0.11 ^{Bd}	21.18 ± 0.13 ^{Ad}

Data are expressed as mean±SD (n=3). Small letters in each column and capital letters in each row indicate significant differences between samples (p<0.05). Control: uncoated group; Chitosan: coating with chitosan; NBO₂: treating with NBO₂; Chi+NBO₂: coated with chitosan+NBO₂.

reported by Liu et al. (2020) and Sayadi et al. (2025), who reported an initial pH of approximately 6.3 for shrimp. Throughout the entire storage period, the pH of all the samples increased significantly, and the control group had a higher pH than the other samples (p<0.05). At the beginning of storage, the control pH was 6.42 ± 0.12, which increased to 8.55 ± 0.14 after 12 days. However, the best performance was achieved by shrimp coated with Chi-NBO₂, whose pH was relatively low and remained constant for the first six days of storage (p > 0.05), before increasing slightly to 6.83 on the 12th day of storage (p < 0.05). This increase was significantly smaller than that observed in shrimp treated with NBO₂ (6.48-7.78) and chitosan (6.41-7.49). The preservation effect observed with the chitosan coating is likely due to its barrier properties, which resulted in significantly less pH variation than in other treatments (p < 0.05). This value was much lower than that of pH chitosan coatings containing *Salvia officinalis* L. nano-essential oil reported by Sayadi et al. (2025). Importantly, the pH of shrimp coated with chitosan and Chi-NBO₂ remained below the acceptable limit of 7.6 (Shafiei et al., 2024) until day 9. These results corroborate findings by Liu et al. (2020), who linked similar pH increases in chitosan-coated shrimp to microbial growth, amino acid decomposition, and subsequent ammonia production.

3.3.2. PV

Lipid oxidation is one of the most critical interactions affecting meat and seafood products and can be monitored by PV and TBARS. Generally, PV is a standard quality indicator of lipid oxidation status and reflects hydroperoxide production as the primary lipid oxidation product.

Hydroperoxides are unstable molecules that decompose into aldehyde and ketone radicals, which contribute to unpleasant taste and odor in seafood products (Shafiei et al., 2024). Table 1 presents the PV of shrimp treated with different coatings during refrigerated storage. According to the results, shrimps initially had a PV value of about 0.47-0.51 meq/kg. As shown, PV increased significantly with storage time in both coated and uncoated shrimp (p < 0.05). A rapid and sustained rise in PV levels was observed from the 3rd day to the peak after storage (P<0.05). Furthermore, compared with the treated groups, the PV level in the control group was substantially higher (p < 0.05) and reached 2.35 ± 0.21 (meq O₂/kg) on the 12th day of storage. While the chitosan coating alone was more effective than NBO₂ treatment, the final PV values were 1.91 ± 0.13 meq O₂/kg and 2.12 ± 0.03 meq O₂/kg, respectively. Moreover, the PV level in the NBO₂-treated group was slightly lower than that in the control group. This result was due to the antibacterial potential of NBO₂, which can reduce PV by reducing the bacterial population, thereby lowering the production of microbial enzymes such as lipase and phospholipase (Farajzadeh et al., 2016). Among all groups, shrimp treated with Chi+NBO₂ exhibited the lowest PV value (1.41 ± 0.01 meq O₂/kg) throughout storage (P<0.05). The lower PV value in the chitosan-coated group (Chitosan and Chi+NBO₂) was attributed to several reasons. (1) In Chi+NBO₂, the interaction of OH[•] with the amine group of chitosan results in amine protonation and increases the antioxidant capacity. (2) Additionally, increased hydroxylation enhances antioxidant activity by providing additional sites for free radical scavenging (Soria & Villamiel, 2010). Chitosan-based coatings have been reported to function as oxygen barriers. It has been confirmed that the edible chitosan coating is a robust gas barrier with lower oxygen permeability, effectively controlling oxygen transport across the coating (Morachis-Valdez et al., 2021). HC is recognized as a physical method that significantly degrades chitosan. The degradation mechanism is primarily associated with the collapse of cavitation bubbles, during which intense mechanical shear and localized chemical reactions occur. These effects cleave chitosan's glycosidic and other chemical bonds, yielding a complex distribution of chitosan degradation products with enhanced antioxidant capacity (Chokradjareon et al., 2018; Kumar & Moholkar, 2011; Rajoriya et al., 2017; Zhang et al., 2021). However, the similarity between PV and the control group might be due to reactive oxygen species generated by NBO₂. Lipid peroxidation results from ROS attacking fatty acids, producing additional free radicals that initiate further lipid reactions and auto-oxidation (Abedi et al., 2024; Alparslan et al., 2019; Alparslan & Baygar, 2017; Aranaz et al., 2021; Banche et al., 2022; Bao & Huang, 2024; Behbahani et al., 2020; Zainal Abidin et al., 2023). Okpala et al. (2016) reported similar observations regarding the ozonated water treatment of shrimp during iced storage. However, it should be noted that the effects of ozone and NBO₂ on lipid oxidation might differ slightly, as ozone decomposes into oxygen and free radicals, which are favored for lipid peroxidation. Its activity depends on the exposure time (Goncalves & Santos, 2019).

Our results were in agreement with those of Liu et al. (2022), who reported that coated shrimp (*Litopenaeus vannamei*) with a squid Maillard peptide-chitosan coating had a lower PV value than the control group and maintained relatively high shrimp quality (Rezaeifar et al., 2020). They also claimed that rainbow trout (*Oncorhynchus mykiss*) coated with chitosan incorporated with lemon verbena extract greatly retarded oxygen penetration and had a lower PV level than the control. Sabu et al. (2020) applied an edible chitosan coating loaded with various lemon peel extracts to extend the shelf life of yellowfin tuna. They reported that coating at higher extract concentrations reduced lipid oxidation and PV and TBARS values, likely due to its higher antioxidant activity.

3.3.3. TBARS

The production of lipid peroxides and hydroperoxides as secondary metabolites contributes to TBARS values and lipid oxidation. TBARS value indicates the flavor quality and spoilage index of marine-based

products (Morachis-Valdez et al., 2021). The TBARS results are shown in Table 1. There is a positive relation between PV and TBARS. TBARS values increased significantly across all groups during storage ($P < 0.05$). However, the increase was moderate in shrimps coated with Chi+NBO₂. Indeed, bacterial spoilage and specific enzymes are responsible for quality degradation and increased TBARS levels during storage (Shafiei et al., 2024). The initial TBARS levels across all groups ranged from 0.11 to 0.12 mg MDA/kg. TBARS levels in the uncoated shrimp (control) increased gradually from the 3rd day (0.37 ± 0.02 mg MDA/kg) and reached 1.43 ± 0.04 mg MDA/kg on the 12th day ($p < 0.05$). Other treated groups showed a similar upward trend from the 3rd day, and the TBARS levels of the chitosan- and NBO₂-treated groups increased to 1.00 ± 0.02 mg MDA/kg and 1.17 ± 0.05 mg MDA/kg, respectively, at 12th storage time. While chitosan coating alone was more effective than NBO₂ alone throughout the storage period, the lower TBARS level in the chitosan-coated group was associated with the coating's O₂ barrier activity. Moreover, chitosan inhibits bacterial growth and effectively retards those related chemical interactions. Additionally, the lower TBARS levels may be due to chitosan's antioxidant activity via ion chelation (Kumar et al., 2020). The antioxidant activity of chitosan also reduces TBARS values and MDA content, as its primary amino groups can bind volatile aldehydes, such as MDA generated during lipid oxidation, forming a stable fluorophore (Muñoz-Tebar et al., 2023). Additionally, treatment with NBO₂ delayed lipid oxidation and reduced the rate of MDA production, possibly due to its antimicrobial activity, which reduced microbial enzyme activity and lowered oxidation (Farajzadeh et al., 2016). Furthermore, Chi+NBO₂ significantly affected TBARS levels and effectively reduced the rising TBARS ($P < 0.05$). Among the various treatments, the Chi+NBO₂-treated group had the lowest TBARS value compared with the other groups throughout storage. The final TBARS level in this group was 0.86 ± 0.01 mg MDA/kg ($P < 0.05$), which remained below the maximum TBARS limit of 1–2 mg MDA/kg (Nagarajan et al., 2021). Moreover, TBARS and PV values altered similarly throughout the storage. These results revealed that chitosan coating combined with NBO₂ causes higher oxidative stability than other groups. Our results were consistent with those of Zhang et al. (2019), who used a chitosan-based edible coating for the preservation of Chinese shrimp; chitosan coating alone and its combination with ϵ -polylysine reduced lipid oxidation and significantly reduced oxygen contact with the shrimp surface. Similarly, Chen et al. (2022) and Nagarajan et al. (2021) confirmed the protective effect of a chitosan-based edible coating against lipid oxidation in shrimps and the reduction in TBARS values.

3.3.4. TVBN values

TVBN accumulation, as an indicator of enzymatic activity and spoilage, was monitored to assess meat quality. Initial TVBN levels (7.46–7.52 mg N/100 g) indicated acceptable initial hygiene. However, a significant increase in TVBN ($p < 0.05$) was observed across all samples during storage (Table 1). The control group exhibited the most rapid spoilage, as indicated by the highest TVBN values (41.81 mg N/100 g on day 12). In contrast, treatments with Chi+NBO₂ (21.18 mg N/100 g), followed by chitosan (31.13 mg N/100 g) and NBO₂ (31.62 mg N/100 g) significantly ($p < 0.05$) inhibited TVBN generation after 12-day storage. The Chi+NBO₂ treatment proved most effective at maintaining low TVBN values (approximately 31.62 mg N/100 g) throughout the storage period, significantly extending the meat's shelf life. This improvement is attributed to the treatments' ability to retard microbial growth and/or enzymatic activity (Alizadeh-Sani, Mohammadian, et al., 2020). The infusion of gases, such as O₂, into liquids generates reactive species. As mentioned, the generation of NBO₂ from O₂ yields ROS, such as hydrogen peroxide (H₂O₂) and OH[•], which facilitate microbial and enzymatic inactivation and, in turn, TVBN reduction. Malahlela et al. (2025) demonstrated that microbubbles (MBs) can stimulate the production of OH[•] radicals during ozonation. Similarly, Singh et al. (2025) found that ROS generated by NBs are highly reactive with organic

compounds, making them suitable for a wide range of disinfection applications. As TVBN in shrimp results from bacterial-driven protein degradation into non-protein nitrogen compounds via proteolytic enzymes (Alizadeh-Sani, Rhim, et al., 2020), the antibacterial effects of these treatments likely reduced enzyme activity, thereby lowering TVBN levels. This consistent finding suggests that inhibiting bacterial growth by incorporating NBO₂ into coatings or packaging is an effective strategy for lowering TVBN accumulation, a key indicator of meat spoilage.

3.4. TPA analysis

TPA results (Table 2) revealed significant differences ($P < 0.05$) in various treatments. The Ch+NBO₂ group exhibited significantly higher hardness (12.85–4.59), elasticity (16.65–4.83), adhesion (5.57–9.58),

Table 2

Textural attributes of different coated shrimp during storage.

	Treatments	Storage time (day)				
		First day	3	6	9	12
Hardness (N)	Control	12.76 ± 0.39 ^{Aa}	8.37 ± 0.25 ^{Bd}	5.17 ± 0.21 ^{Cd}	3.06 ± 0.23 ^D	ND
		12.73 ± 0.41 ^{Aa}	11.15 ± 0.27 ^{Bb}	9.52 ± 0.26 ^{Cc}	5.23 ± 0.34 ^{Dc}	1.63 ± 0.54 ^{Ec}
		12.82 ± 0.54 ^{Aa}	10.89 ± 0.52 ^{Bc}	9.87 ± 0.59 ^{Cb}	5.48 ± 0.46 ^{Cb}	1.91 ± 0.39 ^{Eb}
	Chitosan	12.85 ± 0.50 ^{Aa}	12.31 ± 0.58 ^{Aa}	11.69 ± 0.62 ^{Ba}	8.84 ± 0.71 ^{Ca}	4.59 ± 0.42 ^{Da}
		16.55 ± 0.12 ^{Aa}	10.29 ± 0.42 ^{Bc}	5.23 ± 0.41 ^{Cc}	1.37 ± 0.27 ^{Dc}	ND
		16.59 ± 0.26 ^{Aa}	13.43 ± 0.42 ^{Bb}	10.59 ± 0.49 ^{Cb}	6.59 ± 0.51 ^{Db}	1.86 ± 0.28 ^{Ec}
	NBO ₂	16.62 ± 0.37 ^{Aa}	13.67 ± 0.58 ^{Bb}	10.73 ± 0.53 ^{Cb}	6.89 ± 0.40 ^{Db}	2.11 ± 0.17 ^{Eb}
		16.65 ± 0.42 ^{Aa}	15.92 ± 0.37 ^{Ba}	13.74 ± 0.35 ^{Ca}	10.58 ± 0.44 ^{Da}	4.83 ± 0.55 ^{Ea}
		5.67 ± 0.31 ^{Da}	8.59 ± 0.37 ^{Ca}	11.19 ± 0.17 ^{Ba}	14.56 ± 0.73 ^{Aa}	ND
	Ch+NBO ₂	5.61 ± 0.34 ^{Da}	6.36 ± 0.49 ^{Cb}	9.25 ± 0.41 ^{Bb}	12.24 ± 0.51 ^{Ab}	ND
		5.69 ± 0.47 ^{Da}	6.84 ± 0.45 ^{Cb}	9.58 ± 0.36 ^{Bb}	12.47 ± 0.39 ^{Ab}	ND
		5.57 ± 0.53 ^{Da}	5.78 ± 0.28 ^{Cc}	7.13 ± 0.38 ^{Bc}	9.58 ± 0.26 ^{Ac}	ND
Elasticity	Control	0.87 ± 0.03 ^{Aa}	0.57 ± 0.04 ^{Bc}	0.35 ± 0.04 ^{Cc}	ND	ND
		0.84 ± 0.04 ^{Aa}	0.71 ± 0.005 ^{Bb}	0.48 ± 0.03 ^{Cb}	ND	ND
		0.83 ± 0.05 ^{Aa}	0.73 ± 0.04 ^{Bb}	0.43 ± 0.03 ^{Cb}	ND	ND
	Chitosan	0.85 ± 0.03 ^{Aa}	0.81 ± 0.03 ^{Aa}	0.59 ± 0.05 ^{Ba}	0.28 ± 0.05 ^{Ca}	ND
		0.87 ± 0.03 ^{Aa}	0.57 ± 0.04 ^{Bc}	0.35 ± 0.04 ^{Cc}	ND	ND
		0.84 ± 0.04 ^{Aa}	0.71 ± 0.005 ^{Bb}	0.48 ± 0.03 ^{Cb}	ND	ND
	NBO ₂	0.83 ± 0.05 ^{Aa}	0.73 ± 0.04 ^{Bb}	0.43 ± 0.03 ^{Cb}	ND	ND
		0.85 ± 0.03 ^{Aa}	0.81 ± 0.03 ^{Aa}	0.59 ± 0.05 ^{Ba}	0.28 ± 0.05 ^{Ca}	ND
		0.87 ± 0.03 ^{Aa}	0.57 ± 0.04 ^{Bc}	0.35 ± 0.04 ^{Cc}	ND	ND
	Ch+NBO ₂	0.84 ± 0.04 ^{Aa}	0.71 ± 0.005 ^{Bb}	0.48 ± 0.03 ^{Cb}	ND	ND
		0.83 ± 0.05 ^{Aa}	0.73 ± 0.04 ^{Bb}	0.43 ± 0.03 ^{Cb}	ND	ND
		0.85 ± 0.03 ^{Aa}	0.81 ± 0.03 ^{Aa}	0.59 ± 0.05 ^{Ba}	0.28 ± 0.05 ^{Ca}	ND

Data are expressed as mean ± SD (n=3). Small letters in each column and capital letters in each row indicate significant differences between samples ($p < 0.05$). Control: uncoated group; Chitosan: coating with chitosan; NBO₂: treating with NBO₂; Chi+NBO₂: coated with chitosan+NBO₂. ND: not detected.

and cohesiveness (0.89–0.28) than other groups. This positive correlation between NBO₂ treatment and increased hardness, adhesion, and chewiness suggests that NBO₂ induces myofibrillar fiber contraction. The results were consistent with those of Huang et al. (2025), demonstrating improved textural properties following MB treatment. They suggested that the observed increase in TPA parameters following MB treatment is consistent with the moisture content, water loss, and shrinkage rates. While MB treatment can cause protein denaturation, it maintains shrimp freshness, possibly by transiently inactivating endogenous enzymes (trypsin, cathepsins, and calpains), thereby inhibiting muscle protein degradation. Previous studies highlight the context-dependent effects of MB treatment. Hou et al. (2023) demonstrated that a 30-second wash with MB and ozone (100 ppm) maintained the sensory attributes (aroma, texture, color) of tomatoes. In contrast, Bao and Huang (2024) showed that MB treatment improved the freezing process in tomatoes, lowering drip loss by 13.7–17.0% and increasing firmness and lycopene content. This variability suggests that the optimal treatment duration varies by product. In the context of shrimp, the impact of MB treatment duration on endogenous enzyme activity and subsequent protein degradation warrants further exploration, as the microbial counts and TVBN in Chi-NBO₂ were lower than those in other treatments.

3.5. Color evaluation

Shrimp color is a key factor significantly influencing customer satisfaction and marketability. As shown in Table 3, the concentration of coating compounds affected the lightness of uncoated shrimp; shrimp coated with chitosan, shrimp treated with NBO₂, and shrimp with Chi+NBO₂ were exhibited. The L* value of shrimp was significantly reduced over time. There is no significant difference between Chi-NBO₂ and NBO₂, which had higher L* than the control and chitosan groups. The implosion of NBs generates shockwaves and ROS, including OH[•], thereby increasing oxidative stress and causing a greater reduction in brightness of Chi-NBO₂ and NBO₂ than in chitosan and control samples

Table 3
Color attributes of different coated shrimp during storage.

	Treatments	Storage time (day)				
		First day	3	6	9	12
L*	Control	68.36 ± 0.42 ^{Ab}	58.19 ± 0.31 ^{Bc}	46.27 ± 0.36 ^{Cd}	35.61 ± 0.43 ^{Dc}	28.52 ± 0.26 ^{Ec}
	Chitosan	62.56 ± 0.39 ^{Ad}	56.74 ± 0.40 ^{Bd}	49.53 ± 0.68 ^{Cc}	37.83 ± 0.19 ^{Db}	34.81 ± 0.16 ^{Eb}
	NBO ₂	71.73 ± 0.41 ^{Aa}	67.38 ± 0.19 ^{Ba}	59.71 ± 0.32 ^{Ca}	48.92 ± 0.13 ^{Da}	41.27 ± 0.57 ^{Ea}
	Chi-NBO ₂	67.11 ± 0.12 ^{Ac}	60.46 ± 0.39 ^{Bb}	53.75 ± 0.41 ^{Cb}	48.48 ± 0.38 ^{Da}	41.59 ± 0.26 ^{Ea}
	Control	-6.55 ± 0.18 ^{Eb}	-4.78 ± 0.12 ^{Dd}	-1.49 ± 0.11 ^{Cb}	3.75 ± 0.16 ^{Ba}	6.75 ± 0.16 ^{Aa}
a*	Chitosan	-6.32 ± 0.19 ^{Ea}	-3.67 ± 0.14 ^{Da}	0.73 ± 0.12 ^{Ca}	1.89 ± 0.11 ^{Bb}	3.34 ± 0.15 ^{Ab}
	NBO ₂	-7.59 ± 0.06 ^{Ec}	-7.29 ± 0.12 ^{Dd}	-5.45 ± 0.08 ^{Cd}	-6.59 ± 0.10 ^{Bd}	-3.15 ± 0.07 ^{Ad}
	Chi-NBO ₂	-6.63 ± 0.11 ^{Eb}	-5.57 ± 0.14 ^{Dc}	-3.28 ± 0.13 ^{Cc}	-1.17 ± 0.12 ^{Bc}	0.83 ± 0.09 ^{Ac}
	Control	5.45 ± 0.11 ^{Eb}	7.31 ± 0.07 ^{Db}	10.63 ± 0.06 ^{Ca}	15.19 ± 0.03 ^{Ba}	19.51 ± 0.08 ^{Aa}
	Chitosan	5.89 ± 0.04 ^{Ea}	8.47 ± 0.08 ^{Da}	9.82 ± 0.11 ^{Cb}	11.49 ± 0.09 ^{Bb}	14.11 ± 0.07 ^{Ab}
b*	NBO ₂	5.23 ± 0.07 ^{Ec}	5.84 ± 0.05 ^{Dd}	7.39 ± 0.08 ^{Cc}	10.28 ± 0.09 ^{Bc}	12.37 ± 0.06 ^{Ac}
	Chi-NBO ₂	5.61 ± 0.09 ^{Eb}	6.19 ± 0.07 ^{Dc}	7.45 ± 0.08 ^{Cc}	9.64 ± 0.06 ^{Bd}	11.64 ± 0.08 ^{Ad}

Data are expressed as mean±SD (n=3). Small letters in each column and capital letters in each row indicate significant differences between samples (p<0.05). Control: uncoated group; Chitosan: coating with chitosan; NBO₂: treating with NBO₂; Chi+NBO₂: coated with chitosan+NBO₂.

(Huang et al., 2025). The entire treatment results in increased redness (a*) and yellowness (b*). The a* and b* values in the chitosan samples were higher than those in the other samples. It is suggested that changes in the pH of the chitosan coating may affect the pigments in the shrimp, potentially leading to a shift toward redness. Chitosan's interaction with shrimp's natural pigments (e.g., astaxanthin and carotenoids) may alter their light-absorption properties. This interaction could involve binding or changes in the pigment's molecular structure, resulting in a perceived increase in redness. The lower content of a* and b* in Chi-NBO₂ and NBO₂ was ascribed to several reasons: (a) Radicals oxidize the molecules responsible for color, disrupting their conjugated double bonds, which are crucial for color absorption. (b) Radicals can break chemical bonds within pigment molecules, leading to a loss of color. (c) The chemical structure of pigments becomes unstable, resulting in color fading (Abedi et al., 2025).

3.6. Sensory evaluation

According to Table 4, differences in organoleptic parameters over 12 days were significant, and all organoleptic attributes decreased during storage (P<0.05). Conversely, shrimp coated with Chi-NBO₂ had a higher texture score at the end of storage (P<0.05). Moreover, no significant differences in the sensor properties (P > 0.05) were detected between Chi+ NBO₂.

While all groups experienced off-odor deterioration associated with lipid oxidation and volatile compound formation, the Chi-NBO₂ achieved significantly higher odor scores (5.38 ± 0.29; P < 0.05) than the other treatments, consistent with PV and TBARS results. This improved odor perception likely results from the protective effect of lipid oxidation prevention. The sensory evaluation at the end of storage showed a preference for the Chi-NBO₂ in terms of texture, color, and overall acceptability. There were no significant differences in overall acceptability between the chitosan-coated samples and the NBO₂ treatments. These findings are linked to the protective effects of the coating and NBs, which minimized microbial growth and protein degradation, thereby reducing volatile compound formation and preserving sensory qualities. This finding supports the work of Huang et al. (2025), who demonstrated the efficacy of MB treatment in extending shelf life by inhibiting lipid oxidation in shrimp products. The sensory evaluation of Chi+NBO₂ was higher than that of chitosan, NBO₂, and the control, and was correlated with lower oxidation indices (PV and TBARS) and microbial populations.

3.7. Pearson correlation heatmap between different treatments, storage time, and quality parameters

The Pearson correlation coefficients (Fig. 4) highlight distinct patterns among the (control, chitosan, NBO₂, Chi+NBO₂), storage time (days), and various physicochemical, microbiological, and sensory attributes. Storage time exhibited strong positive correlations with microbial growth and spoilage indicators, including total viable count (TVC, r = 0.807), psychrotrophic bacteria count (TPBC, r = 0.958), lipid oxidation (TBARS, r = 0.870), and total volatile basic nitrogen (TVBN, r = 0.931). These relationships indicate that prolonged storage promotes microbial proliferation and oxidative spoilage. In contrast, day showed strong negative correlations with instrumental quality attributes, such as hardness (r = -0.899) and lightness (L*, r = -0.917), confirming that texture softens and color darkens as storage progresses. Moreover, overall acceptability decreased significantly over time (r = -0.786), indicating deterioration in sensory quality. Among treatments, the Chi+NBO₂ group demonstrated notably weak or negative correlations with spoilage-associated variables (e.g., SAL, pH, PV), and positive associations with hardness (r = 0.278) and overall acceptability (r = 0.299), indicating its potential to retard microbial growth, oxidative degradation, and textural decline. The NBO₂ treatment alone also showed moderate inhibition of spoilage (negative correlation with

Table 4
Sensorial attributes of different coated shrimp during storage.

		Storage time (day)					
		First day	3	6	9	12	
Texture	Control	9.00±0.00 ^{Aa}	7.26±0.61 ^{Db}	3.29±0.18 ^{Cc}	1.00±0.00 ^{Dd}	1.00±0.00 ^{Dd}	
	Chitosan	9.00±0.00 ^{Aa}	8.07±0.62 ^{Cb}	6.05±0.32 ^{Bc}	5.33±0.54 ^{Bd}	3.31±0.28 ^{Be}	
	NBO ₂	9.00±0.00 ^{Aa}	8.56±0.36 ^{Bb}	6.52±0.41 ^{Bc}	4.59±0.38 ^{Cd}	2.84±0.34 ^{Ce}	
	Ch-NBO ₂	9.00±0.00 ^{Aa}	9.00±0.00 ^{Aa}	7.58±0.58 ^{Ab}	6.12±0.54 ^{Ac}	4.38±0.28 ^{Ad}	
	Control	8.23±0.38 ^{Ab}	6.67±0.34 ^{Bc}	1.56±0.45 ^{Cd}	1.00±0.00 ^{Cc}	1.00±0.00 ^{Cc}	
Odor	Chitosan	8.46±0.59 ^{Aab}	8.74±0.50 ^{Ab}	3.96±0.27 ^{Bc}	3.41±0.37 ^{Bb}	2.12±0.38 ^{Cb}	
	NBO ₂	9.00±0.00 ^{Aa}	8.42±0.58 ^{Bb}	5.84±0.34 ^{Cb}	3.73±0.41 ^{Db}	2.56±0.31 ^{Eb}	
	Ch-NBO ₂	9.00±0.00 ^{Aa}	9.00±0.00 ^{Aa}	8.28±0.38 ^{Ba}	7.83±0.26 ^{Ca}	5.38±0.29 ^{Da}	
	Control	9.00±0.00 ^{Aa}	5.89±0.74 ^{Bc}	2.38±0.48 ^{Cc}	1.00±0.00 ^{Db}	1.00±0.00 ^{Dc}	
	Chitosan	9.00±0.00 ^{Aa}	6.38±0.48 ^{Bb}	6.05±0.32 ^{Bb}	4.58±0.58 ^{Cb}	2.67±0.25 ^{Db}	
Color	NBO ₂	9.00±0.00 ^{Aa}	9.00±0.00 ^{Aa}	8.27±0.15 ^{Ba}	6.19±0.25 ^{Ca}	4.48±0.28 ^{Da}	
	Ch-NBO ₂	9.00±0.00 ^{Aa}	8.54±0.50 ^{ABa}	8.19±0.58 ^{Ba}	6.37±0.37 ^{Ca}	4.51±0.32 ^{Da}	
	Control	9.00±0.00 ^{Aa}	6.48±0.24 ^{Bc}	1.45±0.34 ^{Cd}	1.00±0.00 ^{Cc}	1.00±0.00 ^{Cc}	
	Chitosan	9.00±0.00 ^{Aa}	7.87±0.61 ^{Bb}	6.52±0.50 ^{Cc}	5.59±0.46 ^{Db}	2.61±0.34 ^{Eb}	
	NBO ₂	9.00±0.00 ^{Aa}	8.85±0.35 ^{Aa}	7.39±0.43 ^{Bb}	5.83±0.39 ^{Cb}	2.78±0.42 ^{Db}	
Overall acceptability	Ch-NBO ₂	9.00±0.00 ^{Aa}	9.00±0.00 ^{Aa}	8.74±0.62 ^{Ba}	6.31±0.51 ^{Ca}	4.86±0.48 ^{Da}	

Data are expressed as mean±SD (n=3). Small letters in each column and capital letters in each row indicate significant differences between samples (p<0.05). Control: uncoated group; Chitosan: coating with chitosan; NBO₂: treating with NBO₂; Chi+NBO₂: coated with chitosan+NBO₂.

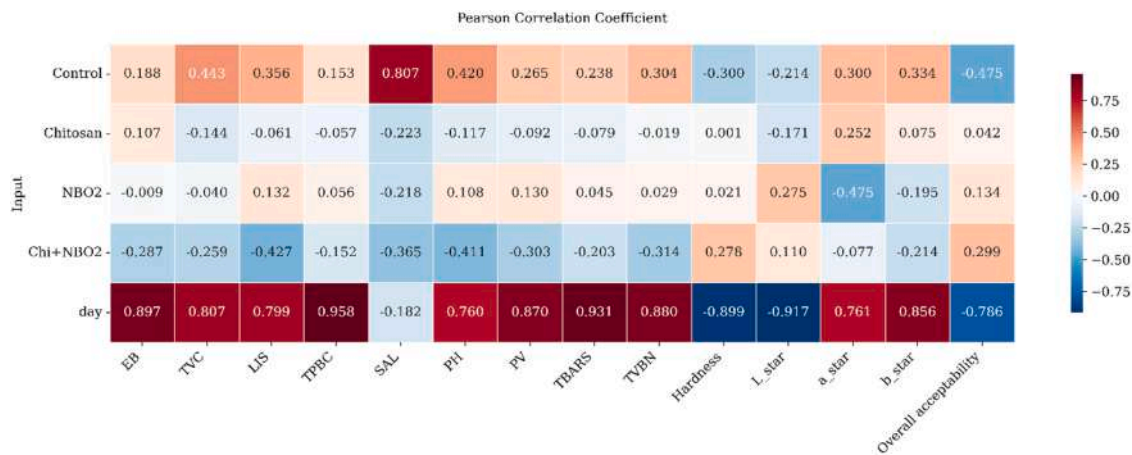


Fig. 4. The correlation between response and different treatments. EB: Enterobacteriaceae; TVC: total viable count; LIS: Listeria monocytogenes; pH, PV: peroxide value; TBARS: thiobarbituric acid value; TVBN: total volatile nitrogen.

TBARS and overall TVBN), though to a lesser extent than the combined treatment. The Chitosan-only group showed minor improvements compared to the control, with slightly negative or near-zero correlations with spoilage indices, suggesting limited preservation capacity when used independently. In contrast, the control samples correlated positively with microbial and spoilage parameters (e.g., SAL, $r = 0.807$; TVC, $r = 0.443$) and negatively with overall acceptability ($r = -0.475$), confirming that untreated samples deteriorated most rapidly.

4. Conclusion

This investigation assessed the influence of chitosan coating and NBO₂ treatment, individually and in combination, on the physico-chemical and microbiological quality of shrimp during refrigerated storage. In untreated samples, PV and TBARS values increased sharply within 12 days, confirming rapid oxidative degradation and loss of quality. Conversely, chitosan and NBO₂ treatments significantly retarded lipid oxidation and inhibited microbial proliferation, as indicated by reductions in TVC, TPBC, Listeria monocytogenes, Salmonella typhi, and Enterobacteriaceae. The synergistic Chi+NBO₂ treatment produced the most pronounced preservative effect, yielding the lowest PV, TBARS, and microbial counts throughout the storage period. These findings demonstrate that both chitosan and NBO₂ contribute to quality preservation, but their combination offers superior antioxidant and

antimicrobial efficacy. Consequently, this integrated approach may serve as a novel, energy-efficient strategy for decontaminating and preserving seafood and other perishable food products. For further development, the molecular mechanisms underlying the synergistic antioxidant and antimicrobial effects of Chi+NBO₂ require further investigation. For example, the degradation of chitosan under NBO₂ treatment could be assessed by determining its molecular weight using chromatographic techniques.

Ethical statement

In this study, human and animal testing were not necessary.

Ethical animal science

The authors declare that the manuscript does not contain experiments using animals and it does not involve any human studies.

CRedit authorship contribution statement

Mohammad Reza Kanani: Resources, Methodology. **Mehran Sayadi:** Resources, Project administration, Methodology, Investigation, Funding acquisition. **Elahe Abedi:** Writing – original draft, Project administration, Methodology. **Sayed Mohammad Bagher Hashemi:**

Project administration, Methodology. **Mohammad Hashemi:** Methodology. **Roghayeh Nejati:** Methodology. **Amene Nematollahi:** Resources, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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