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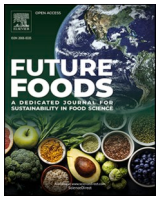

Dual valorization of yeast cells and pomegranate peel to enhance microbial safety and quality of salmon fillets

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ABSTRACT

The valorization of food by-products offers a sustainable strategy to reduce waste and enhance food safety. This study developed a novel antimicrobial delivery system by modifying modified yeast cell particles (YCP) with betaine hydrochloride (BHC-YCP) for encapsulating pomegranate peel extract (PGPE), aimed at improving the microbial quality and safety of refrigerated salmon fillets. Confirmed through Fourier transform infrared spectroscopy (FTIR) and green fluorescent protein (GFP)-tagged *Salmonella*, PGPE was successfully encapsulated into YCP and BHC-YCP using vacuum infusion. The YCP and BHC-YCP treatments significantly reduced *Listeria monocytogenes* on salmon fillets by 1.52 ± 0.32 log and 1.93 ± 0.30 log ($P < 0.05$) compared to the control group at the end of 8 days refrigeration storage (4 ± 1 °C). *Salmonella* was reduced to undetectable levels within 4 and 2 days, respectively by YCP and BHC-YCP treatments. From a spoilage perspective, YCP and BHC-YCP extended the microbial shelf life of salmon by 4 and 6 days, respectively. Moreover, the production of biogenic amines, including cadaverine, putrescine, histamine, and tyramine, was significantly retarded ($P < 0.05$) by the encapsulated PGPE treatments. This study highlighted the potential of integrating by-product valorization, encapsulation technology and antimicrobial innovations to advance sustainable food preservation.

1. Introduction

The global food industry generates significant amounts of food by-products and waste, posing both environmental and economic challenges. Direct disposal of food waste places a heavy burden on natural land and water resources, compromising environmental sustainability. In response, many governments increasingly apply fines and taxes to discourage unsustainable practices. However, these measures are often economically burdensome for companies, thus prompting them to seek alternative strategies that not only mitigate costs but also create value from waste (De Iseppi et al., 2020). This shift aligns with the principles of the Circular Economy, which emphasizes keeping materials in a continuous loop to maximize efficiency and resource utilization. One sector with considerable waste generation is the alcoholic drinks industry. Spent brewer's yeast, predominantly composed of *Saccharomyces cerevisiae* (Perez-Bibbins et al., 2015), is a major by-product of beer production (Liu et al., 2016; Olivares-Galván et al., 2022). In 2023, global beer production reached about 1.88 billion hectoliters (Statista, 2025), with spent beer yeast accounting for 1.5–2.5 % of total production – equivalent to 282,000 to 470,000 kg (Puligundla et al., 2020). Despite its abundance, spent yeast remain underutilized, highlighting the need for innovative applications to integrate it into bioeconomy value chains. One promising application of spent yeast lies in its natural encapsulation properties. The unique capsule-like morphology of yeast cell particles (YCP) makes them an excellent bio-vehicle for encapsulation, capable of carrying both hydrophobic and hydrophilic bioactive compounds. Major structural constituents of the cell wall are polysaccharides, which are responsible for hydrophilic compounds diffusion, and mannoproteins can be implicated in the intake of hydrophobic compounds (Pham-Hoang et al., 2013). Studies have demonstrated that yeast encapsulation effectively protects compounds such as essential oil (Ru et al., 2024), curcumin (Young et al., 2020) and fisetin (Young et al., 2017), shielding them from external environmental stresses, facilitating controlled release, and offering biocompatibility and biodegradability (Tan et al., 2021).

Similarly, fruit peels – another significant stream of food waste – are rich in bioactive compounds that can be encapsulated into yeast cells for enhanced functionality. Generated in large quantities across agricultural and food processing sectors, fruit peels contain valuable compounds such as phenolic acids, flavonoids, anthocyanins, carotenoids and

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vitamin C. These compounds exhibit strong antimicrobial properties (Dhakal et al., 2024; Rifna et al., 2023), making them ideal candidates for encapsulation to improve their stability and efficacy in food systems. Among these, pomegranate (*Punica granatum* L.) peel is particularly noteworthy, as it constitutes nearly 50 % of fruit weight and is abundant in phenolics, ellagitannins, proanthocyanidins, complex polysaccharides, flavonoids and appreciable quantities of microelements (Akhtar et al., 2015; Siddiqui et al., 2024). Scientific studies have further demonstrated that pomegranate peel extract (PGPE) exhibits potent antimicrobial activity against various foodborne pathogens and enhances the shelf life of food products (Chen et al., 2020; Nabeel Ahmad et al., 2024; Yu et al., 2022). Such findings present an opportunity to convert pomegranate peel waste into value-added products, supporting eco-friendly and economical solutions for the food industry. However, direct application of PGPE in food systems faces several challenges, including its inherently unpleasant taste (Andishmand et al., 2023), diminished functional effects caused by interactions with food ingredients during processing or storage (Andrade et al., 2019), and the sensitivity of phenolic compounds to light, high temperatures, and pH variations (Kaderides and Goula, 2019). To address these limitations, encapsulation methods based on yeast cells can enhance the stability, bioavailability, and functionality of PGPE, enabling its broader application in food systems. This approach not only facilitates the practical application of PGPE in food systems but also supports sustainable waste valorization, aligning with the principles of Circular Economy.

The proposed encapsulation strategy holds significant potential for food applications, particularly for its antimicrobial properties. In recent years, seafood consumption has increased due to its exceptional nutritional value, being an excellent source of high-quality protein, unsaturated fatty acids, and essential vitamins and minerals (Jami et al., 2014). Among seafood, salmon is particularly popular and is often consumed raw without heat processing. However, this practice poses significant risks to human health from foodborne pathogens. Two key pathogens of concern in salmon are *Listeria monocytogenes* and *Salmonella. L. monocytogenes* is a major pathogen responsible for frequent seafood recalls worldwide and poses a significant public health risk due to its high case-fatality rate, which can reach up to 16 % (Belias et al., 2024; Jami et al., 2014). A recent review reported a pooled prevalence of *L. monocytogenes* in raw fish at 5.8 %, with higher rates in ready-to-eat products at 14.5 % (Zakrzewski et al., 2024). From 2012 to 2024, a prolonged outbreak in Europe linked to fish products resulted in 73 cases and 14 deaths (EFSA, 2024). In the past five years, multiple recalls of salmon due to *L. monocytogenes* contamination were issued by both the U.S. Food and Drug Administration (FDA) and the Singapore Food Agency (SFA) (FDA, 2024a; 2024b; SFA, 2019). *Salmonella* is also one of the leading causes of foodborne illness worldwide, and infections linked to the consumption of contaminated ready-to-eat seafood can result in symptoms such as diarrhea, fever, and abdominal cramps, posing a significant public health concern (Finstad et al., 2012; Roy et al., 2024). In 2022, the FDA reported a multistate outbreak of *Salmonella* infections associated with raw salmon, causing 39 illnesses across four states (FDA, 2022). Earlier in 2021, FDA inspections identified *Salmonella* contamination in multiple seafood products, including salmon, leading to 115 reported illnesses and 20 hospitalizations (FDA, 2021a; 2021b). In the meantime, salmon are highly perishable with a limited shelf life due to the rapid growth of spoilage microorganisms, which generate metabolites responsible for off-putting odors and flavors (Bita and Sharifian, 2023). Common spoilage organisms in salmon include *Pseudomonas, Shewanella*, and *Enterobacteriaceae* during storage (Jaaskelainen et al., 2019). Biogenic amines (BAs) are basic nitrogenous compounds with low molecular weight produced during seafood processing and storage due to microbial contamination. This occurs through microbial decarboxylase enzyme activity, which convert amino acids into their respective Bas (Biji et al., 2016). Excessive oral intake of BAs can lead to adverse health effects, including nausea, migraines, hypertension, rashes and blood pressure fluctuations (Ahmad et al., 2020). These incidents underscore the urgent need to explore sustainable and effective strategies for controlling and mitigating these threats. One promising solution is the encapsulation of PGPE in yeast cell-based carriers to enhance antimicrobial efficacy and stability.

To further enhance antimicrobial efficacy, YCP can be chemically modified to improve their biological properties. The primary structural components of yeast cell wall - β-glucan and mannan - can be modified to alter their chain structure, thereby improving their functional properties (Liu et al., 2021). Among the chemical modification methods, esterification reactions are widely employed due to their controllability. These reactions target hydroxyl groups on the main or side chains, as well as functional groups in intracellular proteins, resulting in modifications such as carboxymethylation, sulphation, phosphation and chlorination (Huang et al., 2022; Shi et al., 2014). Cationic charge modification is particularly relevant for enhancing antimicrobial activity, as most bacteria, despite structural differences between Gram-positive and Gram-negative types (e.g., Gram-negative bacteria's outer lipopolysaccharide layer and thin peptidoglycan versus Gram-positive bacteria's thick peptidoglycan cell wall), exhibit a net negative surface charge. Cationic chemicals are known to bind to microbial cell membranes via electrostatic interactions, integrating with lipid domains through hydrophobic components, ultimately leading to membrane disruption and microbial inactivation (Engler et al., 2012; Jurko et al., 2023; Ribeiro de Carvalho et al., 2024). However, conventional cationic agents used are usually synthetic compounds with potential toxicity, high cost or origins from non-renewable materials (Karic et al., 2021). Betaine hydrochloride (BHC), a natural derivative of betaine found in microorganisms, plants and animals (Arumugam et al., 2021), offers an environmentally friendly and sustainable alternative for cationic modification of YCP, aiming to enhance their antimicrobial properties.

The objectives of this study were to develop a novel antimicrobial delivery system by modifying YCP with betaine hydrochloride (BHC-YCP) and encapsulating pomegranate peel extract (PGPE) for application on refrigerated salmon fillets. The structural modification of BHC-YCP was characterized using Fourier transform infrared spectroscopy (FTIR), and the electrostatic interaction between BHC-YCP and bacterial cells was visualized using fluorescence microscopy with green fluorescent protein (GFP)-tagged *Salmonella* as a model microorganism. PGPE was encapsulated into YCP and BHC-YCP through an optimized vacuum infusion method to enhance the stability and functionality of the bioactive compounds. The antimicrobial efficacy of encapsulated PGPE was assessed for its ability to inhibit *L. monocytogenes, Salmonella*, and spoilage-causing microorganisms on salmon fillets, thereby ensuring food safety and extending shelf life. Additionally, the study evaluated the effectiveness of encapsulated PGPE in retarding the accumulation of biogenic amines during storage. By integrating structural modification, encapsulation techniques, and antimicrobial assessments, this study aimed to demonstrate the potential of YCP and BHC-YCP as effective carriers for PGPE in food preservation applications. This approach not only offers a sustainable method for utilizing food by-products but also addresses pressing food safety challenges in seafood.

2. Material and methods

2.1. Bacteria cultures

All bacterial cultures were pre-activated by two consecutive passages before the experiments. Strains were cultured in tryptone soya broth (TSB; OXOID, UK) first and streaked onto tryptone soya agar (TSA; OXOID, UK) plates and incubated at 37 °C for 24 h as a single passage. The *L. monocytogenes* strains included *L. monocytogenes* serovar 1/2a SSA 81, *L. monocytogenes* serovar 1/2b LM 13, *L. monocytogenes* serovar 3a LM 8, and *L. monocytogenes* serovar 4b LM 5, which were isolated from pre-packaged smoked salmon (Li et al., 2018). *Salmonella enterica* serovar Typhimurium ATCC 14028, and *S.* enterica serovar Newport

ATCC 6962 were obtained from American Type Culture Collection (ATCC). *S*. Thompson 889, isolated from fresh basil produced in Israel, was provided by Ghent University (Delbeke et al., 2015), and GFP-tagged *S*. Thompson 889 was constructed by our research group (Zwe et al., 2021). The frozen stock of GFP-tagged *S*. Thompson 889 was initially grown in TSB with 100 μg/mL ampicillin overnight and subsequently streaked onto TSA supplemented with ampicillin.

2.2. Preparation of YCP and modification with BHC

Active dry baker's yeast cells were washed extensively with deionized (DI) water and pelleted by centrifugation at 3000 × *g* for 5 min, repeated three times. The washed yeast cells were resuspended in DI water at a ratio of 1:20 (w/v) and autoclaved at 121 °C for 20 min. The autoclaved cells were then pelleted at 3000 × *g* for 5 min, washed with DI water five times, and freeze-dried to obtain YCP.

Modified YCP was prepared using an aqueous solution of BHC supplemented with lactic acid, following a method reported by Karic et al. (2021) with slight modifications. Initially, 3.0 g of BHC was dissolved in 5 mL of DI water. After complete dissolution, 0.5 mL of lactic acid (90 %; Sigma-Aldrich) was added to the mixture. Freeze-dried YCP (0.5 g) was then mixed with the prepared solution, and the resulting slurry was stirred at room temperature for 5 min in a glass petri dish. The slurry was spread as a thin layer on the dish and heated at 120 °C for 6 h, with remixing every 0.5 h. The crude product was thoroughly washed with DI water under stirring until a neutral pH was achieved, removing residual reagents (BHC and lactic acid). Finally, the purified product was frozen overnight and freeze-dried (−80 °C; 0.5 mbar; Lyovapor ™ l-300, BÜCHI Labortechnik AG, Flawil, Switzerland) for 48 h to obtain powdered cationic BHC-YCP.

2.3. FTIR analysis

FTIR of all samples were collected using a PerkinElmer Spectrum ONE FTIR spectrometer (L1600400, PerkinElmer, UK). Freeze-dried YCP and BHC-YCP were mixed well with potassium bromide (KBr) powder, at a ratio of 1:150 (w/w), respectively, then pressed into pellets for FTIR analysis. The spectra were acquired with a wavelength range between 4000 and 500 cm^{-1} at a resolution of 4 cm^{-1}.

2.4. Fluorescence microscopy imaging for the interaction between YCP and BHC-YCP with GFP-expressing Salmonella

A single pure colony of GFP-tagged *S*. Thompson 889, cultured on a TSA plate, was inoculated into TSB broth supplemented with 100 μg/mL ampicillin, and incubated overnight to obtain bacterial pellets. The harvested bacterial pellets were washed thoroughly and diluted in phosphate buffer saline (PBS) solution to achieve a concentration of approximately 6.00 log CFU/mL.

YCP and BHC-YCP were each suspended in diluted GFP-tagged bacteria suspension at a concentration of 5 % (w/v), ensuring uniform interaction. The interactions between GFP-tagged bacteria and YCP or BHC-YCP were visualized using a fluorescence microscope (BX51; Olympus Corporation, Tokyo, Japan).

2.4. PGPE preparation and encapsulation into YCP and BHC-YCP by vacuum infusion

Dried pomegranate peel was acquired from local supermarket in Singapore. The husks were pulverized in an electric mill and stored in a dark glass bottle to prevent degradation. Pulverized husks (40 g) were added to 400 mL of 70 % ethanol in a sealed container and shaken in the dark at room temperature for 24 h. Subsequently, the mixture was centrifuged at 10,000 × *g* for 10 min, and the supernatant was concentrated to 5 ml using rotary evaporator (IKA VACSTAR D S099, IKA-Werke, Germany) at 40 °C. The obtained concentrate was further adjusted to a final volume of 30 mL with DI water.

Lyophilized YCP or BHC-YCP were initially washed with excess DI water, and centrifuged at 3000 × *g* for 5 min. The collected YCP or BHC-YCP was resuspended in the concentrated PGPE, maintaining a final YCP or BHC-YCP concentration of 5 % (w/v) in the encapsulation solution. PGPE was encapsulated into YCP or BHC-YCP through vacuum infusion in a glass desiccator, with hold times of 2, 5, 10, and 20 min, respectively. The obtained PGPE@YCP or PGPE@BHC-YCP microcarriers were decanted into 50 mL falcon tubes, centrifuged at 3000 × *g* for 5 min, and washed five times with excess DI water to remove unencapsulated extract.

The amount of PGPE encapsulated in YCP or BHC-YCP was quantified with 1 mL methanol from 0.01 g of wet yeast microcarriers. The samples were vortexed, sonicated in a bath for 10 min, and centrifuged at 10,000 × *g* for 10 min. Total phenolic contents (TPC) of the released PGPE were determined using Folin-Ciocalteu method, with gallic acid as a standard (Singleton and Rossi, 1965). A diluted 0.5 mL aliquot of the sample was mixed with 2.5 mL of 10-fold diluted Folin Ciocalteu Reagent. After a 3 min incubation, 2 mL of 7.5 % Na_2CO_3 was added, and the reaction mixture was kept in the dark for 30 min. The absorbance was measured at 760 nm using a spectrophotometer (Touch DUO; BioDrop, Cambridge, UK). Gallic acid standard (10–100 mg/L) were prepared and processed under identical conditions to generate a standard curve. The TPC of encapsulated PGPE was expressed as mg gallic acid equivalent (GAE) per gram of yeast microcarriers. The encapsulation yield was calculated based on the equation:

$$\text{Encapsulation yield} = \frac{C_E(mg)}{C_Y(g)}$$

Where C_E is the content of phenol from YCP or BHC-YCP, and C_Y is the dry mass of YCP or BHC-YCP.

2.5. Coating of salmon fillets

Fresh salmon fillets were purchased from a local market in Singapore and promptly delivered to our laboratory within 30 min with ice-packed containers to maintain their freshness. All subsequent procedures were conducted in a biosafety cabinet to minimize environmental contamination. For coating solutions, YCP and BHC-YCP were mixed with concentrated PGPE at the concentration (5 %, w/v) and vacuum treated for 10 min to establish the treatment groups "YP-PE" and "b-YP-PE". A coating solution containing only concentrated PGPE was designated as "PE". Similarly, the YCP and BHC-YCP were resuspended in sterile DI water at the same concentration of 5 % (w/v) and vacuum treated for 10 min to prepare the treatment groups "YP" and "b-YP", respectively. The salmon fillets were sliced and immersed into the respective coating solution (YP, b-YP, PE, YP-PE, and b-YP-PE) with a fillets-to-coating solution ratio of 1: 4 (w/v). After 5 min of immersion, the fillets were drained on sterilized draining basket for 30 min. The control group underwent identical procedures except for immersion in the coating solutions labeled as "Control". Each sample was individually packaged in sterile containers and stored at 4 ± 1 °C for subsequent analysis on day 0, 2, 4, 6, and 8. Samples from day 0 were coated, drained, and analyzed immediately after preparation.

For the pathogenic bacteria challenge test, *L. monocytogenes* (SSA 81, LM 13, LM 8, and LM 5) or *Salmonella* (ATCC 14,028 and ATCC 6962) were cultivated individually in TSB at 37 °C overnight to achieve a concentration of 8.5 log CFU/mL. Bacterial pellets were collected by centrifugation at 3000 × *g* for 15 min at 4 °C, washed twice with 0.1 % (w/v) peptone water and combined into a cocktail of 1:1:1:1 for *L. monocytogenes* and 1:1 for *Salmonella*. A 200 μL aliquot of the respective bacterial cocktail of *L. monocytogenes* or *Salmonella* was spotted onto the surface of each salmon fillet (final concentration: ~ 3.00 log CFU/g), and these fillets were then held in a biosafety cabinet for 30 min to facilitate bacteria attachment before proceeding to coating procedures described above.

2.6. Microbiological analysis

Each salmon fillet was aseptically transferred from the individual package to a sterile stomacher bag and homogenized with 0.1 % peptone water (1:9, w/v) for 2 min using a slapping stomacher (Interscience Bag Mixer 400, France). The decimal dilutions were prepared, and the diluent was aseptically spread onto the corresponding agar plates for microbial enumeration. *L. monocytogenes* on salmon fillets was enumerated on PALCAM agar (Difco™, France) with Bacto™ Palcam Antimicrobic Supplement and incubated at 37 °C for 48 h. *Salmonella* counts were enumerated on xylose lysine deoxycholate (XLD; OXOID, UK) agar and incubated at 37 °C for 24 h. Psychrotrophic total counts were determined on plate count agar (PCA; OXOID, UK) at 15 °C for 7 days. Counts of *Pseudomonas* spp. were obtained by incubating on *Pseudomonas* CFC selective agent (OXOID, UK) at 30 °C for 48 h. Enumeration of *Enterobacteriaceae* was conducted on violet red bile glucose (VRBG; OXOID, UK) agar at 37 °C for 24 h. All microbial counts were expressed as log CFU/g sample.

2.7. Biogenic amine (BA) analysis

A 2 *g* fish sample without pathogen inoculation was homogenized with 10 mL of 0.6 M perchloric acid using an IKA T25-digital ultra turrax homogenizer (IKA, Germany). The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C. The pellet underwent a second extraction, and the supernatants from both centrifugations were combined and adjusted to a final volume of 50 mL with 0.6 M perchloric acid. Pre-column derivatization of the extract with dansyl chloride, and high-performance liquid chromatography (HPLC) analysis were performed according to the method described by Hua et al. (2022). The HPLC system (Alliance 2695, Waters, MA, USA) was equipped with a C18-Diamondsil column (25 cm × 4.6 mm, 5 μm) and a photodiode array (PDA) detector. Both samples and standard BA compounds (putrescine [PUT], cadaverine [CAD], histamine [HIS], and tyramine [TYR]) were analyzed at 254 nm under identical chromatographic conditions. Individual BA was identified and quantified by comparing the retention time and peak area with those of the BA standards.

2.8. Statistical analysis

All experiments were performed in triplicate. The statistical analyses were accomplished using the SPSS statistic 19.0 (SPSS, USA). Analysis of variance (ANOVA) and Duncan's multiple range test ($P < 0.05$) were executed to assess the significance of the parameters.

3. Results and discussion

3.1. FTIR analysis of YCP and BHC-YCP

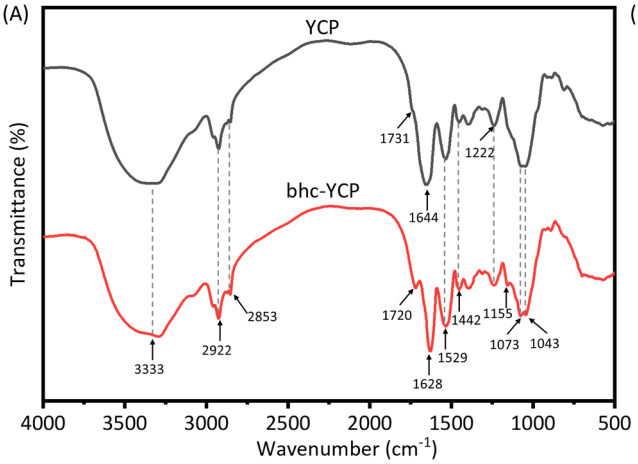
FTIR is an effective way for structural elucidation and characterization of functional groups. The FTIR spectra and characteristic wavenumbers of YCP and BHC-YCP are shown in Fig. 1. The FTIR spectra of BHC-YCP displayed a profile similar to that of unmodified YCP in the fundamental region. A broad band observed around 3333 cm^{-1} corresponds to -OH stretching vibrations due to polysaccharides in yeast cells (Liu et al., 2021). The bands at 2922 cm^{-1} and 2853 cm^{-1} are attributed to C–H stretching vibrations, likely contributed by CH_2 stretching in CH_2OH groups of sugars (Novák et al., 2012). The carbonyl vibration region revealed characteristic bands at 1644 cm^{-1} (amide I and in-plane water bending) and 1529 cm^{-1} (amide II), along with a minor peak at 1731 cm^{-1} in YCP samples. The amide I and II bands are associated with the R—NH—*C*═*O*—CH_3 group in yeast cell wall chitin and possibly linked to sugars (Mohammad Nejad Khiavi et al., 2020). Additionally, intense, overlapping bands between 990 and 1200 cm^{-1}, attributed to C—O—C and C—C stretching vibrations, are dominant characteristic of different polysaccharides (Kacurakova et al., 2000).

Notably, a reduction in the intensity of hydroxyl group peaks in BHC-YCP suggested substitution of hydroxyl groups in the YCP molecule with cationic groups. In BHC-YCP, distinct spectral changes were observed. An intensified absorption peak at 1720 cm^{-1} was assigned to *C*═*O* stretching vibrations, indicating the formation of ester bonds between YCP and betaine hydrochloride. A new absorption band at 1155 cm^{-1}, attributed to C—O—C stretching, further supports ester bond formation. Additionally, enhanced intensities of bands at 1442 cm^{-1}, 1073 cm^{-1} and 1043 cm^{-1}, corresponding to C—N, C—O and C—C vibrations, highlight structural modifications in the modified samples. These changes confirmed structural modifications in BHC-YCP by the reaction of betaine hydrochloride with YCP.

3.2. Interaction between unmodified YCP and BHC-YCP with GFP-expressing Salmonella

A key feature of BHC is its quaternary ammonium groups, which carry a positive charge and can bind microbial cell membranes via electrostatic interactions. When yeast cells are modified with BHC, the attached cationic charge is expected to attract bacteria from the surrounding environment, concentrating them around the yeast cells. This property could enhance the antimicrobial effect of BHC-modified yeast cell particles, particularly when combined with bioactive and antimicrobial substances.

To directly visualize these electrostatic interactions, GFP-expressing



(B)

Main assignment	YCP	bhc-YCP
	Absorption band (cm^{-1})	
-OH stretching	3333	3333
-CH stretching	2922	2922
-CH stretching	2853	2853
-C=O stretching	1731	1720
Amide I: -C=O vibrations of different protein structures	1644	1628
Amide II: -N–H and -C–N vibrations of the peptide bond in different protein conformations	1520	1529
-CN stretching	1442	1442
-COC stretching	-	1155
-CO stretching	1073	1073
-CC stretching	1043	1043

Fig. 1. FTIR spectra (A) and characteristic wavenumber (cm^{-1}) (B) of YCP and BHC-YCP.

S. Thompson 889 was selected as a representative microorganism. The interactions between unmodified YCP and BHC-YCP with bacterial cells were assessed using fluorescence microscopy as shown in Fig. 2. In the YCP dispersion system, *Salmonella* cells were observed to be evenly distributed around the YCP, with some cells showing a tendency to adhere to the particle surfaces. This pattern reflects the inherent properties of yeast cell walls, which are composed predominantly of 1,3-β-glucan and chitin. These polysaccharides exhibit moderate affinity for microbial binding, likely through specific molecular interactions or weak electrostatic forces (Liu et al., 2021; Ru et al., 2024). In contrast, a distinct interaction pattern was observed in the BHC-YCP dispersion system, where *Salmonella* cells formed tightly lustered aggregated around the modified particles, indicating a significantly stronger adhesion relationship. This enhanced aggregation can be attributed to the introduction of positive charges on the YCP surface via BHC modification. The cationic surface facilitates robust electrostatic interactions with the negatively charged bacterial cell walls, resulting in closer attachment and pronounced clustering. The different behavior of BHC-YCP compared to unmodified YCP confirmed the successful modification of BHC molecules. Furthermore, these findings highlight the critical role of surface charge modification in significantly improving the binding efficiency of YCP, enhancing its potential for targeted antimicrobial applications.

In this study, GFP-labeled *S. Thompson* was used as a model pathogen to visualize interactions with BHC-YCP via microscopy. *Salmonella* was chosen for its well-defined surface charge, rod-shaped morphology, and significance as a major foodborne pathogen. Its negative surface charge - a trait shared with other pathogens like *L. monocytogenes* - makes it a suitable representative for initial binding assessments. However, structural differences, such as Gram status (*Listeria* Gram-positive vs. *Salmonella* Gram-negative), may limit the broader translation of these findings. Future work should include diverse bacterial species with varying surface properties to validate the generalizability of the observed interactions.

3.3. Effect of vacuum time on encapsulation yield of PGPE in YCP and BHC-YCP

Giving the superior binding ability of BHC-YCP, PGPE was designed to be encapsulated within it to achieve synergistic antibacterial effect in food matrix. For bioactive substances encapsulation, conventional processing methods, such as passive diffusion, typically require extended treatment times and are associated with higher energy costs. In contrast, vacuum infusion is a rapid, efficient technique that requires only a few minutes and does not involve heating. Previous research by Young et al. (2017) demonstrated that a 99 % vacuum achieved the highest encapsulation efficiency and yield for curcumin and fisetin compared to 75 % and 50 % vacuum levels when applied to YCP. Notably, the 99 % vacuum process required 288 times less processing time than passive diffusion. Based on these findings, we also employed a 99 % vacuum in our study to investigate the effect of vacuum infusion time on the encapsulation efficiency of concentrated PGPE.

PGPE contains phenols as its primary bioactive compounds; therefore, the encapsulation yield was assessed using total phenol content as a descriptor. As illustrated in Fig. 3, for both YCP and BHC-YCP samples, the encapsulation yields significantly increased with longer vacuum infusion times ($P < 0.05$). At a vacuum treatment time of 10 min, the encapsulation yield peaked at 103.50 ± 2.69 mg/g (dry basis) for YCP and 107.20 ± 2.33 mg/g (dry basis) for the BHC-YCP group. Interestingly, when the vacuum infusion time was extended beyond 10 min to 20 min, a decline in encapsulation yield was observed. This reduction may be attributed to the structural degradation of the cell wall particles under prolonged exposure to a 99 % vacuum environment in the presence of concentrated PGPE solution. A similar optimal vacuum infusion time of 10 min was also reported for curcumin encapsulation in *Geotrichum candidum* arthrospore cell wall particles, though no subsequent decline in encapsulation efficiency was observed within the extended time range of 10–30 min (Wu et al., 2023).

3.4. Effect of YCP and BHC-YCP encapsulated PGPE on microbial quality of salmon fillets

PGPE was encapsulated into YCP or BHC-YCP using vacuum infusion method for 10 min according to the above results. Meanwhile, different ratios of YCP or BHC-YCP to PGPE solution were prepared, including 2 % (w/v), 5 % (w/v), and 10 % (w/v). After application to salmon fillets under refrigeration (4 ± 1 °C), 5 % (w/v) ratio exhibited superior antimicrobial effectiveness compared to 2 % (w/v), while no significant additional improvement was observed at 10 % (w/v) (Table S1). Based on these findings, YCP and BHC-YCP were mixed with PGPE solution at a 5 % (w/v) ratio before application to salmon fillets.

In this study, for *L. monocytogenes* contamination, treatments involving PGPE (PE, YP-PE, b-YP-PE) significantly reduced the bacterial count to below the detection limit (2.00 log CFU/g) on day 0 and maintained it below this threshold during the first two days of storage (Fig. 4A; $P < 0.05$). In contrast, samples without PGPE supplementation (control, YP, and b-YP) exhibited gradual bacterial growth throughout the entire storage period ($P < 0.05$). By day 6, *L. monocytogenes* levels on YP-PE samples (2.63 ± 0.06 log CFU/g) and b-YP-PE samples (2.30 ± 0.30 log CFU/g) were significantly lower ($P < 0.05$, Fig. 4A) than those on PE samples (3.03 ± 0.35 log CFU/g). Additionally, on day 8, *L. monocytogenes* counts on YP-PE and b-YP-PE samples were 1.52 ±

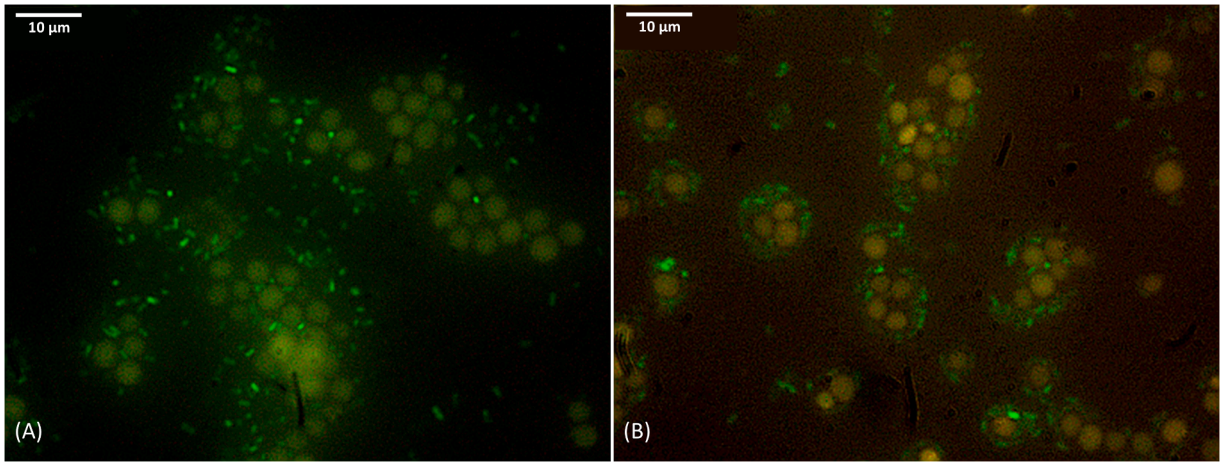


Fig. 2. Fluorescence imaging between GFP-tagged *Salmonella* Thompson 889 and YCP (A) and BHC-YCP (B).

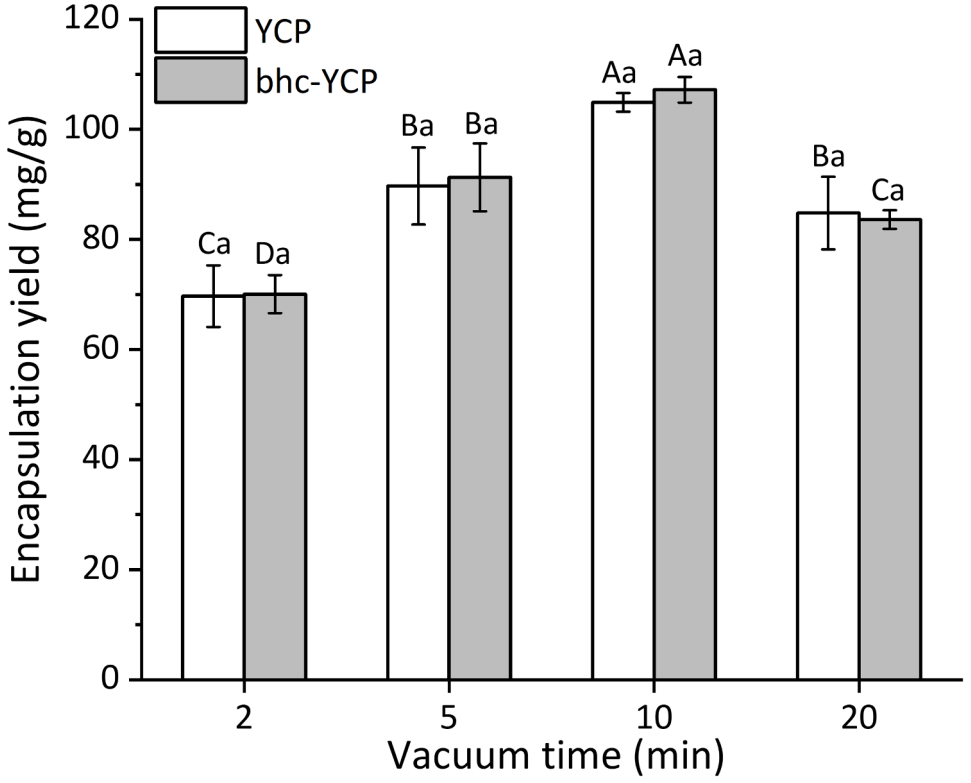


Fig. 3. Encapsulation yield (mg/g) of PGPE in YCP and BHC-YCP under different vacuum time (min). A-C: The mean values with different letters of the same treatment are significantly different between different vacuum time ($P < 0.05$). a: The mean values with different letters of the same vacuum time are significantly different between different treatments ($P < 0.05$).

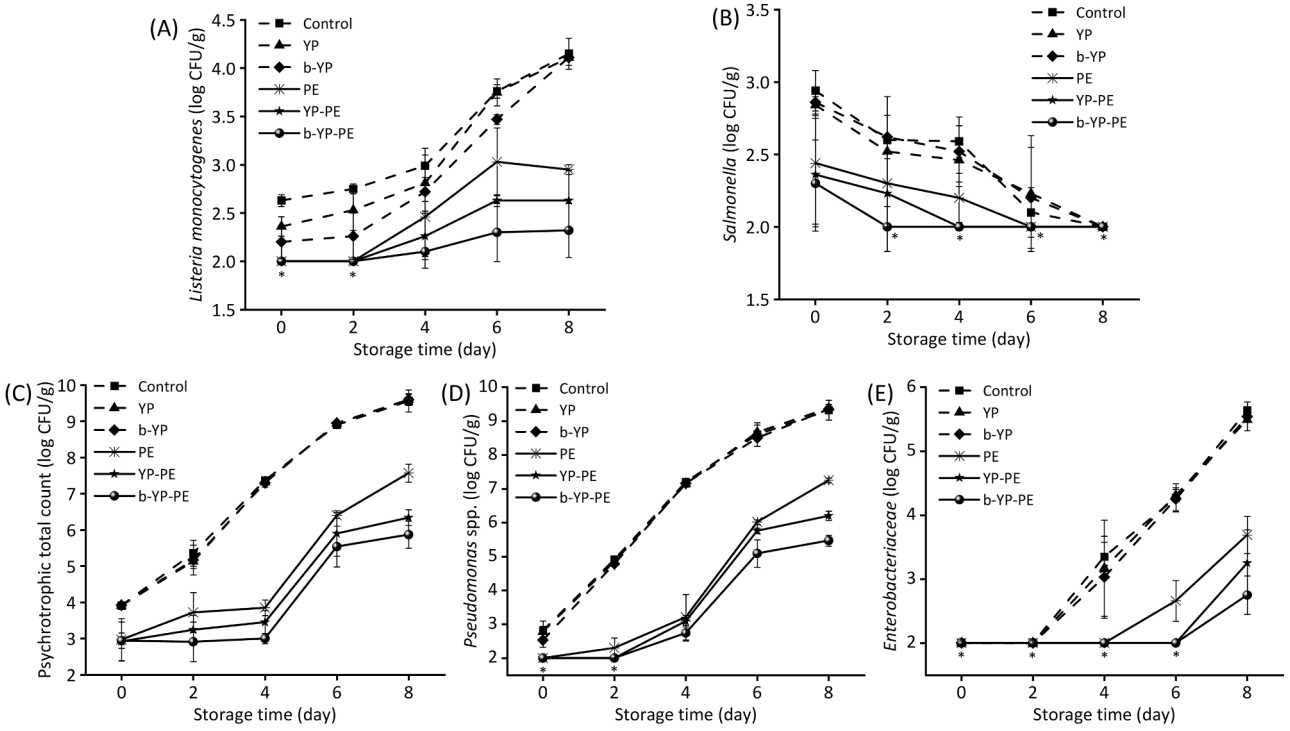


Fig. 4. Changes in *L. monocytogenes* (A), *Salmonella* (B), psychrotrophic total count(C), *Pseudomonas* spp. (D), and *Enterobacteriaceae* (E) on salmon fillets during a refrigeration storage period of 8 days. Error bars represent the standard deviations ($n = 3$). *: below detection limit (2.0 log CFU/g).

0.32 log and 1.93 ± 0.30 log lower ($P < 0.05$, Fig. 4A), respectively, compared to the control samples. These findings demonstrate that PGPE effectively inhibits the growth of *L. monocytogenes*, and the encapsulation of YCP and BHC-YCP further enhances its anti-*L. monocytogenes* efficacy. According to U.S. Food Safety and Inspection Service (FSIS) guidelines (FSIS, 2014), antimicrobial agents (AMAs) or antimicrobial processes (AMPs) are considered effective if they limit the growth of L. *monocytogenes* to no more than a 2-log increase over the expected shelf life of the product. Our results clearly demonstrate that the application of BHC-YCP encapsulating PGPE meets this criterion and should be considered an effective control measure for *L. monocytogenes* in ready-to-eat foods from a regulatory standpoint.

In current study, *Salmonella* counts exhibited a decreasing trend across all samples with different treatments throughout the 8-day storage period at 4 °C (Fig. 4B). This trend aligns with the fact that *Salmonella* are mesophilic microorganisms, with a minimum growth temperature of 5.2 °C under near-optimal conditions (e.g., pH and water activity), as reported by the International Commission on Microbiological Specifications for ICMSF (1996). Notably, samples treated with PGPE-containing formulations demonstrated a reduction of approximately 0.50 log compared to control samples on day 0 (Fig. 4B). Furthermore, *Salmonella* counts on b-YP-PE, YP-PE, and PE-treated samples decreased to below the detection limit (2.00 log CFU/g) on days 2, 4, and 6, respectively ($P < 0.05$, Fig. 4B). In contrast, *Salmonella* on control samples only decreased to below the detection limit by day 8. These results highlight the enhanced antimicrobial effectiveness of PGPE-based treatments, particularly when encapsulated with YCP and BHC-YCP, in accelerating the reduction of *Salmonella* under refrigeration conditions.

Seafood spoilage, driven by the metabolic activity of associated microbiota, leads to the degradation of sensory characteristics during storage, rendering the product unacceptable and unfit for consumption (Anagnostopoulos et al., 2022). The key spoilage microorganisms in salmon include psychrotrophic total counts, *Pseudomonas* spp., and *Enterobacteriaceae*. In this research, significant initial reductions (~1.00 log) in psychrotrophic total counts and *Pseudomonas* spp. (~1.00 log) were observed immediately after treatment (day 0) in PE, YP-PE, and b-YP-PE treated salmon samples (Fig. 4C and 4D). Furthermore, PE encapsulated with YCP and BHC-YCP effectively inhibited *Pseudomonas* spp. to below the detection limit during the first two days of storage (Fig. 4D). Although increasing trends were observed in all tested groups along with the storage ($P < 0.05$), PE-treated samples maintained a 1.99 ± 0.49 log reduction of psychrotrophic total counts compared to control samples at day 8, with YP-PE and b-YP-PE achieving further reductions of 1.22 ± 0.33 log and 1.70 ± 0.45 log, respectively, compared to the PE group. A similar trend was observed in *Pseudomonas* spp., with b-YP-PE showing the highest inhibition (3.85 ± 0.33 log reduction), followed by YP-PE (3.12 ± 0.32 log reduction) and PE (2.07 ± 0.30 log reduction) by day 8. *Enterobacteriaceae* counts were below the detection limit across all samples on day 0 and remained undetectable during the first two days of refrigeration (Fig. 4E), indicating the high hygienic and microbial quality of the salmon. In PE-treated samples, *Enterobacteriaceae* levels remained undetectable for 4 days, while YP-PE and b-YP-PE treatments extended this period to 6 days (Fig. 4E), highlighting their superior antimicrobial efficacy. According to the international commission for microbiological standards of food (ICMSF, 1986), 6.0 log CFU/g of aerobic counts and 4.0 log CFU/g *Enterobacteriaceae* are regarded as acceptable limit for fish products. Based on the criterion, PGPE only, YCP encapsulated PGPE and BHC-YCP encapsulated PGPE extended the shelf life for 2, 4 and 6 days compared to control salmon samples, respectively.

Overall, enhanced antimicrobial effectiveness was observed in treatments involving YCP and BHC-YCP encapsulated PGPE. The excellent antimicrobial properties and enhancement in this study could be attributed to several factors. First, PGPE has been demonstrated with excellent antimicrobial properties with both agar based (Al-Zoreky, 2009; Ul-Islam et al., 2023) and food based experimental set-ups (Chen et al., 2020; Kaderides et al., 2021) against various pathogenic and spoilage-causing microorganisms. Its antibacterial activity might be attributed to a synergistic effect of multiple bioactive compounds, including polyphenols, sterols, and pentacyclic triterpenoids compounds (Sun et al., 2021). Second, unencapsulated PGPE has been reported with high susceptibility to volatilization and quick deterioration of the phytochemical compounds during storage when incorporated into food matrix (Kaderides et al., 2019; Rahnemoon et al., 2021), thereby resulting in reduced antimicrobial performance on salmon fillets accordingly over time. In contrast, YCP encapsulation of PGPE is a promising technique and can offer several advantages. YCP encapsulation can provide protection for bioactive compounds of PGPE from detrimental factors such as light and oxygen from storage environment. This protective mechanism preserves the stability and functionality of PGPE's active ingredients during refrigeration storage. YCP encapsulation also facilitates controlled release of PGPE in the organic-rich environment of the salmon surface, preventing undesirable interactions with surface moisture and organic matter that could dilute or inactivate its bioactive components. Moreover, the localized high concentration of encapsulated PGPE enhances its antimicrobial potency. Last, the modification of YCP with BHC further enhances its functionality. BHC-modified YCP increases bacterial affinity, facilitating stronger binding between the encapsulated PGPE and microbial cells. This modification ensures a more targeted and localized release of PGPE in proximity to the bacteria, thereby enhancing its antimicrobial effectiveness. The sustained release of PGPE in this manner enables prolonged inactivation of microbes, effectively reducing their proliferation in the surrounding environment. These combined mechanisms highlight the superior antimicrobial performance of YCP and BHC-YCP encapsulated PGPE, making them effective solutions for controlling microbial growth and extending the shelf life of refrigerated salmon. This approach underscores the potential of combining natural antimicrobial agents with innovative encapsulation techniques to address food microbial safety challenges.

3.5. Effect of YCP and BHC-YCP encapsulated PGPE on BA accumulation on salmon fillets

As shown in Table 1, treatments involving PGPE (PE, YP-PE, and b-YP-PE) exhibited a remarkable inhibitory effect on BA accumulation compared to salmon samples treated with pure YP and b-YP. By the end of the storage period (day 8), very low levels of CAD were generated in PE (7.55 ± 2.34 mg/kg), YP-PE (2.10 ± 0.56 mg/kg), b-YP-PE (1.47 ± 0.52 mg/kg) treated samples, significantly lower than the control groups (66.37 ± 5.68 – 80.31 ± 9.99 mg/kg). Notably, PUT, HIS and TYR remained undetectable in YP-PE and b-YP-PE treated salmon throughout the entire storage period, while PE-treated salmon showed minimal accumulation of PUT (3.00 ± 0.19 mg/kg), HIS (0.43 ± 0.03 mg/kg) and TYR (0.54 ± 0.04 mg/kg). In contrast, control groups displayed continuous increases in all four Bas reaching significantly higher levels of CAD (~70 mg/kg), PUT (~15 mg/kg), HIS (~5 mg/kg), and TYR (~40 mg/kg) by day 8. This result showed high accordance to the analysis of the microbial spoilage analysis as demonstrated above, where psychrotrophic total counts, *Pseudomonas* spp. remained below 4 log CFU/g during the first four days, and *Enterobacteriaceae* stayed below 3 log CFU/g during the first six days in PGPE-treated samples (Fig. 4C–E). In control samples, these spoilage microorganisms exhibited significant and continuous growth, correlating with increased biogenic amine accumulation. This suggests that spoilage-associated microflora contribute substantially to BA production. Similar conclusions were reported previously that maximal BA production occurred during the stationary phase of specific bacteria or at the later stage of culture growth (Barbieri et al., 2019; Kim et al., 2000; Masson et al., 1997).

Among these detected BAs, HIS is the most frequently implicated in foodborne illnesses. Although salmon contains relatively low levels of

Table 1
Changes of biogenic amine contents of salmon fillets during a refrigeration storage period of 8 days.

Time	Group	BA contents (mg/kg)			
		CAD	PUT	HIS	TYR
Day 0		ND	ND	ND	ND
Day 2	Control	0.94 ± 0.62 [a]	0.46 ± 0.04 [a]	0.57 ± 0.12 [a]	0.38 ± 0.23 [a]
	YP	0.90 ± 0.50 [a]	0.41 ± 0.07 [a,b]	0.51 ± 0.15 [a]	0.62 ± 0.14 [a]
	b-YP	0.35 ± 0.03 [a]	0.34 ± 0.06 [b]	0.35 ± 0.08 [a]	0.46 ± 0.08 [a]
	PE	ND	ND	ND	ND
	YP-PE	ND	ND	ND	ND
	b-YP-PE	ND	ND	ND	ND
Day 4	Control	9.64 ± 1.60 [a]	5.24 ± 1.94 [a]	3.67 ± 0.81 [a]	14.83 ± 5.03 [a]
	YP	9.04 ± 4.46 [a]	3.89 ± 1.26 [a]	2.00 ± 1.00 [a]	15.81 ± 7.70 [a]
	b-YP	7.86 ± 3.86 [a]	4.99 ± 1.85 [a]	2.15 ± 1.42 [a]	15.79 ± 6.58 [a]
	PE	ND	ND	ND	ND
	YP-PE	ND	ND	ND	ND
	b-YP-PE	ND	ND	ND	ND
Day 6	Control	69.56 ± 10.43 [a]	16.27 ± 1.57 [a]	4.67 ± 0.42 [a]	34.75 ± 3.10 [a]
	YP	52.06 ± 10.54 [a]	15.79 ± 3.47 [a]	2.94 ± 0.75 [a]	23.48 ± 8.85 [a]
	b-YP	55.75 ± 10.99 [a]	11.04 ± 5.64 [a]	3.25 ± 1.43 [a]	26.80 ± 8.37 [a]
	PE	3.85 ± 0.80 [b]	2.64 ± 0.51 [b]	ND	ND
	YP-PE	ND	ND	ND	ND
	b-YP-PE	ND	ND	ND	ND
Day 8	Control	80.31 ± 9.99 [a]	18.51 ± 2.00 [a]	6.45 ± 1.23 [a]	42.31 ± 6.50 [a]
	YP	66.37 ± 5.68 [b]	10.99 ± 1.65 [b]	5.23 ± 1.18 [a]	36.44 ± 8.62 [a]
	b-YP	70.99 ± 7.34 [ab]	14.45 ± 2.61 [b]	5.91 ± 1.82 [a]	41.83 ± 1.93 [a]
	PE	7.55 ± 2.34 [c]	3.00 ± 0.19 [c]	0.43 ± 0.03 [b]	0.54 ± 0.04 [b]
	YP-PE	2.10 ± 0.56 [c]	ND	ND	ND
	b-YP-PE	1.47 ± 0.52 [c]	ND	ND	ND

Error bars represent the standard deviations ($n = 3$).
[a-c] : Different lowercase letters mean significant differences among different groups at the same storage time ($P < 0.05$).
ND: Not detected.

histidine, the precursor to histamine (Prester, 2011), our results demonstrate that microbial activity during storage can still lead to measurable histamine formation. Moreover, the presence of CAD and PUT can potentiate histamine toxicity, increasing the risk of adverse symptoms such as flushing, headache, diarrhea, and hypotension (Shakila and Kumudavally, 2001; Visciano et al., 2020). Many regulatory bodies have set maximum histamine limits in fish and fishery products. The Europe Union (EU), and New Zealand permit up to 200 mg/kg in raw fish, while Singapore has set a maximum limit of 100 mg/kg and the FDA sets a stricter limit of 50 mg/kg for the U.S. and Canada (Visciano et al., 2020; FDA, 2024c; EU, 2013; SFA, 2020). The Russian Federation have set a maximum allowable concentration of 100 mg/kg for histamine in salmon and similar fish species (Visciano et al., 2020). No official limits exist for TYR, CAD, or PUT in salmon, but concentrations above 100 - 200 mg/kg for CAD and PUT are commonly associated with spoilage, and TYR levels over 100 - 800 mg/kg may pose health risks (Prester, 2011). Although levels in this study remained below these thresholds, the accumulation in untreated samples underscores the importance of preventive strategies used in this study.

Overall, these findings demonstrate that the PGPE-fortified solution effectively suppressed the accumulation of BAs, and its encapsulation in YP and b-YP further enhances this effect. These results support the application of PGPE-loaded yeast carriers as a promising antimicrobial and spoilage-controlling system for extending the shelf life and safety of refrigerated salmon.

There are a few considerations regarding the industrial application potentials of this solution. First, the application of BHC-YCP for seafood preservation introduces a promising clean-label strategy. Pomegranate peel extract, derived from a food-grade source, has also been studied widely for food applications (Xiang, 2022). Both betaine hydrochloride and *S. cerevisiae*-derived yeast cells are generally recognized as safe (GRAS) substances by the U.S. FDA, and are used in food and feed industries (Abid, et al., 2022; Dobrijevic, et al., 2023). However, regulatory approval for this specific composite system would depend on local legislation. Although all components are food-derived and pose minimal risk, toxicological validation and residue analysis should be explored before industrial application. Second, the raw materials for this solution, yeast cells and fruit peels, are by-products and generally incur no additional cost. Therefore, the economic viability of this solution will largely depend on the scalability of the encapsulation and infusion processes. Third, consumer acceptance may depend on education regarding the benefits of natural preservatives and sustainability. Conducting sensory studies with consumer panels would be valuable for future validation.

4. Conclusion

This study successfully modified YCP with BHC, and encapsulated PGPE via a vacuum infusion method to enhance the shelf life and edible quality of refrigerated salmon fillets. FTIR analysis confirmed structural modifications in the BHC-modified YCP (BHC-YCP), including decreased O—H intensity and enhanced vibrations of *C═O*, C—N, C—O, and C—C bonds, compared to unmodified YCP. Fluorescence microscopy, using GFP-tagged *Salmonella* Thompson 889 as a model microorganism, revealed electrostatic interactions between BHC-YCP and bacteria. The vacuum infusion method successfully encapsulated PGPE into both YCP and BHC-YCP. These encapsulated mixtures significantly inhibited *L. monocytogenes* and *Salmonella* on refrigerated salmon and extended the microbial shelf life by 4 and 6 days, respectively, compared to the control samples. Furthermore, the encapsulated PGPE effectively suppressed BAs, including PUT, HIS, and TYR, to undetectable levels throughout storage. This innovative approach demonstrated the potential of BHC-YCP encapsulated PGPE as a natural and sustainable preservative, contributing to waste valorization and improved food safety in seafood applications.

Ethical statement - studies in humans and animals

This study does not involve human or animal objects.

CRediT authorship contribution statement

Qian Hua: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Lingdai Liu:** Validation, Supervision, Conceptualization. **Siyu Chen:** Writing – review & editing, Validation. **Dan Li:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2025.100678.

Data availability

Data will be made available on request.

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