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Chitosan edible coating: a potential control of toxic biogenic amines and enhancing the quality and shelf life of chilled tuna filets

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Edible films and coatings offer great potential to support sustainable food production by lowering packaging waste, extending product shelf life, and actively preserving food quality. Using edible coatings containing plant extracts with antioxidant and antibacterial characteristics could help to enhance the quality and shelf life of fish products. In this study, the combination effect of chitosan with beetroot, curcumin, and garlic extracts on biogenic amines (BAs) reduction, biochemical quality [pH, thiobarbituric acid index (TBA), trimethylamine (TMA), and total volatile base (TVB)], shelf life and sensory characteristics of tuna filets was investigated over 14 days of refrigerated storage compared to control (uncoated) samples. The results showed that the coated samples experienced a lower increase in BAs levels than the control samples. Among the treated samples, chitosan incorporated with curcumin (CH-C) showed the highest reduction in BAs formation (1.45 – 19.33, 0.81 – 4.45, and 1.04 – 8.14 mg/kg), followed by chitosan with garlic (CH-G) (1.54 – 21.74, 0.83 – 5.77, and 1.08 – 8.84 mg/kg), chitosan with beetroot extract (CH-B) (1.56 – 31.70, 0.84 – 6.79, and 1.07 – 10.82 mg/kg), and chitosan without extract addition (CH) (1.62 – 33.83, 0.71 – 7.82 and 1.12 – 12.66 mg/kg) compared to control samples (1.62 – 59.45, 0.80 – 11.96, and 1.14 – 20.34 mg/kg) for histamine, cadaverine, and putrescine, respectively. In addition, the rate of increase in pH, TBA, TMA, and TVB of all coated treatments was lower than in the control samples. Sensory evaluation results revealed that chitosan-treated samples incorporated with beetroot, garlic, and curcumin extracts showed good quality and acceptability characteristics. Overall, chitosan edible coatings incorporated with beetroot, garlic, and curcumin extracts reduced the formation of biogenic amine, delayed biochemical deterioration, and extended the shelf life of tuna filets. Among the treated samples, CH-C demonstrated a remarkable

superiority in all the studied parameters. Therefore, this study provides a promising strategy for the incorporation of active compounds in edible coatings to improve the quality and safety of foods during storage.

KEYWORDS

food contaminants, biogenic amines, chitosan edible coatings, beet root, garlic, curcumin, chilled tuna filets

1. Introduction

To increase the food system's sustainability, it is important to use an integrated strategy that considers every aspect of the supply chain, from primary production to packaging and distribution. Fish is considered one of the main sources of essential nutrients that help billions of people avoid malnutrition and diseases (Troell et al., 2019; Kwasek et al., 2020). The proteins, amino acids, vitamins, and other nutrients of fish offer a potent combination of health benefits to consumers (Hosomi et al., 2012; Islam et al., 2018). Additionally, it provides polyunsaturated fatty acids, particularly docosahexaenoic and eicosapentaenoic acids (Hamilton et al., 2020). Fish spoils quickly after it dies and is considered a highly perishable food. Different mechanisms, particularly lipid oxidation and microbiological spoilage, are responsible for fish deterioration under refrigeration or low-temperature storage (Pereira et al., 2010).

Biogenic amines (BAs) are toxic metabolites that formed in fish and fish products due to microbial growth and fish spoilage (Houicher et al., 2021). Its concentrations are considered a marker for food quality and safety as it increases during microbial contamination (González-Ceballos et al., 2020; Hao and Sun, 2020). Free amino acids from protein degradation may be decarboxylated to form BAs (simple nitrogenous compounds) (Liang et al., 2019). Putrescine, cadaverine, histamine, and tyramine are the main toxigenic BAs in foods, and their consumption leads to different adverse effects in humans, such as food poisoning, food allergy, and altering stomach acidity, blood pressure, and brain activity (Maintz and Novak, 2007). Moreover, higher doses of BAs are involved in food-borne illness and intoxication (Biji et al., 2016). Different authorities set limits for BAs in different foods. The European Food Safety Authority reports that the most toxic BAs are histamine and tyramine; as they cause hypertension, palpitations, headaches, and vomiting in certain consumers (Wakinaka et al., 2019). However, controlling of BAs formation is one of the most difficult applications in the food system. The obstacle in controlling BAs is that they are considered heat-stable compounds, so food preservation techniques such as cooking, freezing, canning, and smoking failed to eliminate them from foods (Arvanitoyannis and Kotsanopoulos, 2012; Sagratini et al., 2012).

Fish could threaten human health if they contained BAs (Soares et al., 2021). Controlling biogenic amine formation in fish by natural food additives is of great interest owing to the consumer's awareness to limit synthetic additives in foods that cause potential toxicity, therefore, many bioactive phytochemicals that enhance the sensory profile, shelf life, and safety of food are applied (Houicher et al., 2021). Moreover, one of the novel approaches to improving fish and fish products' safety and quality is the combination of natural plant extract and food preservation technologies, and also protecting humans from toxic hazards of food (Houicher et al., 2021). Beetroot extract is one of the non-toxic antimicrobials that exhibit anti-bacterial and anti-fungal effects on food systems (Maqbool et al., 2020). It contains many active compounds such as carotenoids, betaines, flavonoids, saponins, and many nutrients such as iron, potassium, calcium, sodium, zinc, and vitamins (A, C, B12, and folic acid) (John et al., 2017). Another non-toxic natural extract is curcumin which has strong anti-inflammatory, antioxidant, antimicrobial, and anticancer properties (Sun et al., 2021, 2023a; Fernandez-Marín et al., 2022). Moreover, garlic extract is considered a powerful antimicrobial and antioxidant compound for both humans and animals (Kothari et al., 2019). The incorporation of active compounds in edible coatings and films is said to be a promising strategy for enhancing the quality and safety of food during storage (Panahirad et al., 2021). Rachtanapun et al. (2021) found that the inclusion of curcumin extract into chitosan-based films enhanced the polymer matrix of chitosan through the interactions that occurred between the phenolic compounds from curcumin extract and chitosan matrix as well as enhanced the antioxidant activity of the film. Wang et al. (2022) and Sun et al. (2023b) successively implemented the Pickering emulsion strategy to improve the storage stability of the bioactive ingredients. Asik and Candogan (2014) investigated the impact of garlic oil inclusion in chitosan coatings as an alternative natural strategy to extend the shelf life of shrimp meat, and the obtained results revealed that garlic oil could be used as a natural antioxidant and antimicrobial in packaging systems. In addition, Ahmadi et al. (2022) provided a novel antibacterial chitosan-based membrane using herbal extracts from thyme and garlic. Yadav et al. (2022) investigated the effect of the incorporation of beetroot extract into starch and chitosan edible films on enhancing the quality and shelf life of chicken patties.

Chitosan is of great interest in edible coating applications in the food industry owing to its non-toxic, antimicrobial, antifungal, biocompatibility, and biodegradability properties (Yu et al., 2017; Morsy and Rayan, 2019; Song et al., 2022). It is considered an excellent coating and a carrier for natural additives to enhance food freshness and safety (Zhang et al., 2018). However, to the best of our knowledge, the combinations of chitosan with beetroots, curcumin, and garlic extracts have never been tested in fresh tuna filets. In this study, the effect of chitosan edible coatings incorporated with beetroots, curcumin, and garlic extracts on the BA formation and the shelf life of tuna filets were studied during chilled storage.

2. Materials and methods

2.1. Tuna filet preparation

Fresh bluefin tuna (*Thunnus orientalis*) fish was purchased from local markets in Toukh city, Egypt. Tuna fish samples were sliced into similar-sized filets (each slice weighed 80 g). The filets were transported in an ice box to the laboratory within 10 min.

2.2. Plant extracts preparation

Beetroots, curcumin, and garlic powders were obtained from the National Research Center, Giza, Egypt. Freshly prepared alcoholic extracts of beetroots *(Beta vulgaris*), curcumin (*Curcuma longa*), and garlic (*Allium sativum*) were prepared according to Riaz et al. (2020). In a nutshell, 300 mg each of beetroot, curcumin, and garlic powder were put into three separate Eppendorf tubes, along with 1 mL of 80% ethanol. The resultant suspensions were shaken vigorously at 1500 rpm for 1 min and maintained at ambient temperature for 30 min. The supernatant was collected after 10 min of centrifugation at 8,000 rpm, and the ethanolic extract (500 μL) was then evaporated under a vacuum. Beetroot, curcumin, and garlic extracts were obtained by re-suspending the residue in the same volume of sterilized ddH_2O.

2.3. Chitosan coating preparation

Chitosan (de-acetylation 93%, moisture ≤10.0%, viscosity >75.0%, and molarity 161.16 MW) used for edible coating preparation was obtained from Oxford lab chem., India. Chitosan edible coatings were prepared according to Caner and Cansiz (2007) with some minor modifications. The chitosan coating solution was prepared by gently dissolving 1.5% chitosan (w/v) in distilled water (at 100°C) while being stirred. The resultant solution was cooled to 45°C, then 1% (v/v) acetic acid and 0.25 mL glycerol/g chitosan were added as a plasticizer to prevent brittleness. After 15 min of stirring (during this time, the coating solution's temperature dropped to approximately 37°C), the plasticizer was fully dispersed. At a concentration of 1.5%, beetroot, curcumin, and garlic extracts were added to the coating solution.

2.4. Application of chitosan edible coating on fresh tuna filet

Five treatments were prepared, namely, uncoated (Control, C) and coated [chitosan only (CH), chitosan + beet root extract (CH-B), chitosan + curcumin extract (CH-C), and chitosan + garlic extract (CH-G)]. Fresh tuna filet samples were dipped in the coating solution for 2 min and then dried for 15 min at room temperature (25°C). The samples were then stored refrigerated in polyethylene plastic bags at 4°C. The coated samples were examined for BA formation, and chemical quality tests and sensory attributes were evaluated during the 14 days with 2 days intervals (Figure 1). The experiments were carried out in triplicate.

2.5. Biogenic amines detection

A measure of 20 mL of perchloric acid ($HClO_4$, 0.4 M) and a representative homogenized sample (5 g) were combined, vortexed, and centrifuged at 3000 $\times g$ for 10 min at 4°C. The resultant material was then extracted once more using the same amount of perchloric acid. After being combined and diluted to 50 mL with perchloric acid (0.4 M), the collected supernatants were filtered and maintained at 4°C ± 1°C for analysis. Standard amines including histamine dihydrochloride, tyramine hydrochloride, putrescine dihydrochloride, and cadaverine dihydrochloride were obtained from Sigma (St. Louis, MO, USA) and dissolved in 0.1 M HCl for 1.0 mg/ml concentration of each amine. Samples of standard biogenic amine solutions and 1 ml of tuna filet extracts were derivatized with dansyl chloride according to the method of Chen et al. (2010). The dansyl derivatives were filtrated through a 0.45-μm filter and 20 μl aliquots were used for HPLC injection. BAs including histamine (HI), tyramine (TY), putrescine (PU), and cadaverine (CA) were determined according to the method of Hwang et al. (2011) using HPLC (Agilent 1100 series; Agilent, Santa Clara, CA, USA). The gradient elution protocol started with a flow rate of 1.0 ml/min of 50:50 (v/v) of acetonitrile:water mixture for 19 min, then increased linearly to 90:10 of acetonitrile:water mixture (1.0 ml/min) for the following 1.0 min. For 10 min, the acetonitrile:water mixture was reduced to 50:50 (1.0 ml/min).

2.6. Biochemical analyses

The pH was measured by using a Jenway pH meter (Jenway 3510, Bibby Scientific Ltd., Stone, Staffs, UK). The 2-thiobarbituric acid extraction method was used to determine lipid oxidation according to Choe et al. (2017). In brief, 10 g of tuna sample was added to 25 mL of a solution containing 2:8 (v/v) trichloroacetic acid and 1 mol L^{-1} phosphoric acid. The slurry was filtered and 5 mL of filtrate was added to a vial containing 0.005 mol L^{-1} of thiobarbituric acid (TBA) in distilled water. After capping, the vials were heated for 10 min in a boiling water bath to develop the chromogen and then cooled to ambient temperature. The TBA values were determined by measuring the absorbance at 532 nm with a spectrophotometer (model 6505 UV/Vis, Jenway Ltd., Felsted, Dunmow, UK). Using freshly made, acidified 1,1,3,3-tetraethoxypropane, a standard curve (8–50 nmol) of malondialdehyde (MDA) was prepared. The obtained results were expressed as MDA mg/kg sample weight.

TMA content was determined using the method of Ward et al. (2009). A 0.50-g tuna sample was homogenized with 5 mL of 10% trichloroacetic acid (TCA). After centrifuging the mixture at 3000 rpm, the supernatant was transferred to a 25-mL test tube. After that, the mixture was rapidly agitated for 10 min while 1.0 mL of 10% formaldehyde solution, 10 mL of anhydrous toluene, and 3.0 mL of 1:1 potassium carbonate solution were added. Anhydrous sodium sulfate (1.0 g) was added to the toluene phase in a plastic tube and thoroughly stirred. After mixing, 5 mL of 0.02% picric acid was added to 1 mL of liquid to develop the yellow TMA picrate complex. With 10% TCA used as a blank, the absorbance was measured using a spectrophotometer (model 6505 UV/Vis,

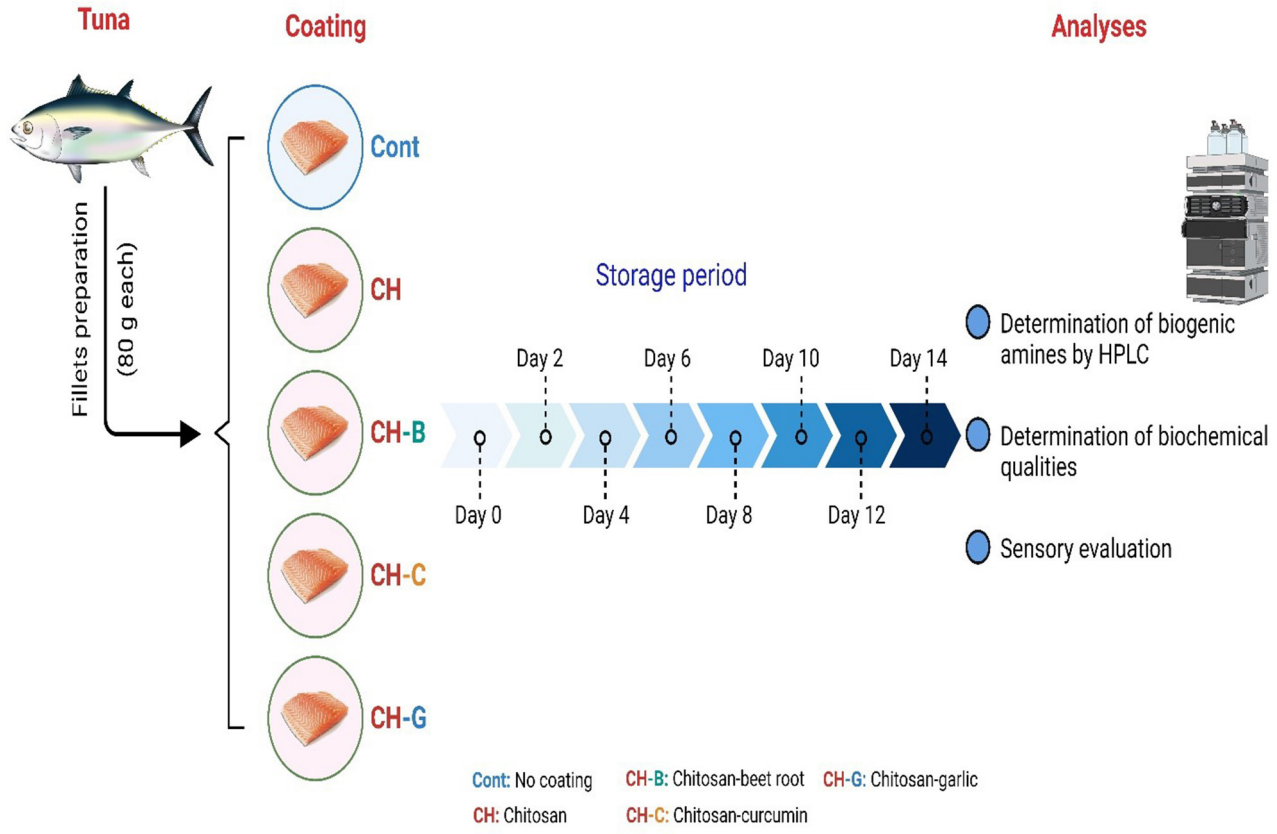

FIGURE 1
Experimental protocol.

Jenway Ltd., Felsted, Dunmow, UK) at its maximum absorption wavelength of 410 nm.

TVB measurement was conducted using the Kjeldahl method according to Cai et al. (2014). A total of 5 g of the minced tuna flesh sample and 50 mL of distilled water were mixed and agitated for 30 min at ambient temperature. The mixture was filtered followed by steam distillation. After being treated with 4% boric acid, the distillate was titrated with 0.01 M HCl. An automated Kjeldahl system was used for the distillation process (VELP Corporation, Italy). The TVB levels were given in mg N/100g of the sample.

2.7. Sensory evaluation

Coated and uncoated tuna filet samples were evaluated for their sensory characteristics (color, odor, appearance, and overall acceptability) during cold storage by 10 trained adult volunteers (Rong et al., 2009). A plate with three-digit numbers was used to assess the samples. Using a 9-point hedonic scale, the samples were judged on their sensory attributes, including color, odor, appearance, and overall acceptability (1 being extremely disliked and 9 being extremely liked).

2.8. Statistical analysis

All of the experiments were performed in triplicate, and the values were expressed as the means ± standard deviations (SD). Data were analyzed by a two-way ANOVA. Using the CoStat statistical program, Duncan's multiple range tests ($p < 0.05$) were used to determine the significance between the various treatments. Moreover, the MetaboAnalyst software was used to create a 3D plot for the multivariate principal component analysis (PCA), variable importance in projection (VIP) score, and clustering heatmap.

3. Results and discussion

3.1. Effect of chitosan-bioactive coatings on biogenic amine formation

Because BAs are an important tool for evaluating the sanitary condition of foods, they can be utilized as indicators of food spoilage and, at high concentrations, can have toxicological consequences on consumers (Saad et al., 2022). Biogenic amine formation depends mainly on the decarboxylase enzyme that is secreted by spoilage or pathogenic bacteria (Xia et al., 2016; Kuley et al., 2019; Burgut, 2020). Controlling pathogenic bacteria

using natural antimicrobials and antioxidants reflects levels of BAs in fish which also represents a quality indicator of fish. Edible coating fortified with plant extracts is one of the novel approaches to food safety and preservation that consumers seek in the food industry (Sapper and Chiralt, 2018). In this study, chitosan edible coatings fortified with natural plants such as beetroot, curcumin, and garlic extracts were applied to chilled tuna filets. It was found that there were significant ($p < 0.05$) delays in BA formation in chilled fish filets coated with chitosan incorporated with bioactive compounds extracted from natural plants. The results in Figures 2A–C show the impacts of chitosan-bioactive coatings on histamine, putrescine, and cadaverine formation in tuna fish filets stored refrigerated at 4°C during storage for 14 days. There were increases in the content of BAs (histamine, putrescine, and cadaverine) during the storage period in both uncoated (control) and coated tuna filets samples. However, the control sample showed a higher increase in BA values than coated samples. Among the treated samples, chitosan incorporated with curcumin (CH-C) showed the lowest increase in BA formation, followed by chitosan with garlic (CH-G), chitosan with beetroot extract (CH-B), and chitosan without extract addition (CH).

Histamine is an indicator of fish quality (Mendes, 1999). Higher levels of histamine cause edema, urticaria, and anaphylactic shock which are life-threatening. In the EU, the histamine legal limit in fish is 100 mg/kg (EC, 2005), and its acute reference dose per meal is approximately 50 mg histamine/meal (EFSA, 2011). The Food and Drug Administration FDA (2011) has established a histamine upper limit of 50 mg/kg as acceptable. Additionally, according to Egyptian standards [Egyptian Organization for Standardization and Quality Control (E.O.S.Q.C), 2005], the maximum allowed amount of histamine for frozen fish cannot exceed 100 mg/kg. In this study, histamine did not exceed the acute reference dose in all studied samples during the 14 days of storage. It is worth mentioning that in chitosan coatings combined with beetroot, curcumin, and garlic extracts, histamine remained lower than 50 mg/kg in the samples. Cadaverine levels in fish are considered an index for spoilage (Al Bulushi et al., 2009). In this study, tyramine was not detected in the investigated tuna samples. Chitosan bioactive coatings greatly reduced putrescine and cadaverine contents in treated tuna samples. According to research by Hao et al. (2017), abalone (*Haliotis discus hannai*), that has been coated with sodium alginate (SAC) and rosemary extract (ROE) or bamboo leaf extract (BLE), contained approximately 75 mg/kg of total BA, and the addition of plant extracts (BLE and ROE) to SAC led to a decrease in putrescine, cadaverine, and tyramine accumulation while histamine was not detected.

The antibacterial property of chitosan could be attributed to the polycationic nature of chitosan, which results from the presence of amine groups (NH^{3+}) of glucosamine and may be a key factor in its ability to interact with negatively charged surface elements of many microorganisms, altering the cell surface extensively and causing the leakage of intracellular substances that causes cell death (Ma et al., 2017). Additionally, chitosan monomers preserve two hydroxyls and an amino group, which can react with free radicals. As a result, they have the scavenging ability (Abd El-Hack et al., 2020).

3.2. Effect of chitosan-bioactive coatings on the biochemical quality of tuna filet samples

3.2.1. pH values

The pH values of the treatments ranged from 6.13 to 6.25 on the first day of storage. During the storage period, the pH values in all of the treated samples considerably increased ($p < 0.05$) (Figure 3A). The pH of the control sample was increased significantly compared to the treated samples. The control samples showed an initial value of 6.25 which increased up to the 12th day of storage. Among the treated samples, the CH-C treatment showed the lowest increase in pH, followed by the CH-G, CH-B, and CH treatments, which were the highest ones. The same pattern in pH rise is consistent with the findings of Hassanzadeh et al. (2018) about the impact of chitosan coating incorporated with grape seed extract on the quality and shelf stability of rainbow trout filet, Kostaki et al. (2009) on the shelf stability of *Dicentrarchus labrax* filet, and Mehrabi et al. (2021) for chicken filet treated with chitosan coating containing *Nepeta pogonosperma* extract. These elevations in pH levels may be caused by the bacteria which produce alkaline substances such as trimethylamine and ammonia (Hassanzadeh et al., 2018, Kostaki et al. (2009). The low pH in chitosan-bioactive coated tuna filet samples during storage might also be attributed to their antioxidant and antibacterial properties and the acidic pH of chitosan (Hassanzadeh et al., 2017; Pabast et al., 2018; Mehrabi et al., 2021).

3.2.2. TBA values

Up until the end of the storage, changes in the TBARS index generally revealed significantly higher levels in all of this study's treatments (Figure 3B). After 14 days of refrigeration, the control sample's TBA level of 0.38 0.01 mg MDA/kg increased to 12.70 0.14 mg MDA/kg when compared with the other treatments. The lowest alteration in TBA was noticed in the CH-C, CH-G, and CH-B treatments compared to chitosan only (CH). The TBA values were 8.05± 0.06, 6.84 ± 0.09, 4.24 ± 0.12, and 4.93 ± 0.05 mg MDA/kg for CH, CH-B, CH-C, and CH-G, respectively, at the end of the storage period. The chitosan-treated samples incorporated with the curcumin, garlic, and beetroot extracts performed better in lipid oxidation reduction than the CH treatment. According to the Egyptian standard (EOSQC, 2005), 4.5 mg MDA/kg in fish flesh is generally regarded as the upper level, after which frozen fish is unsafe for human consumption. On the 6th day of storage, the control sample with a TBA content of 4.77 ± 0.22 mg MDA/kg could not be consumed, but all of the chitosan-coated treatments, except CH treated sample, were within the permissible range for TVB levels of 4.5 mg MDA/kg (EOSQC, 2005) until the end of the 10th day. On the 14th day (end of storage period), however, TBA levels in all treatments were higher than the permitted range, and the chitosan-coated treatment incorporated with curcumin (CH-C) with a TBA content of 4.24 ± 0.12 mg MDA/kg was only consumable on this day. Generally, the secondary constituents of these plant/herb extracts, such as bioactive compounds, could

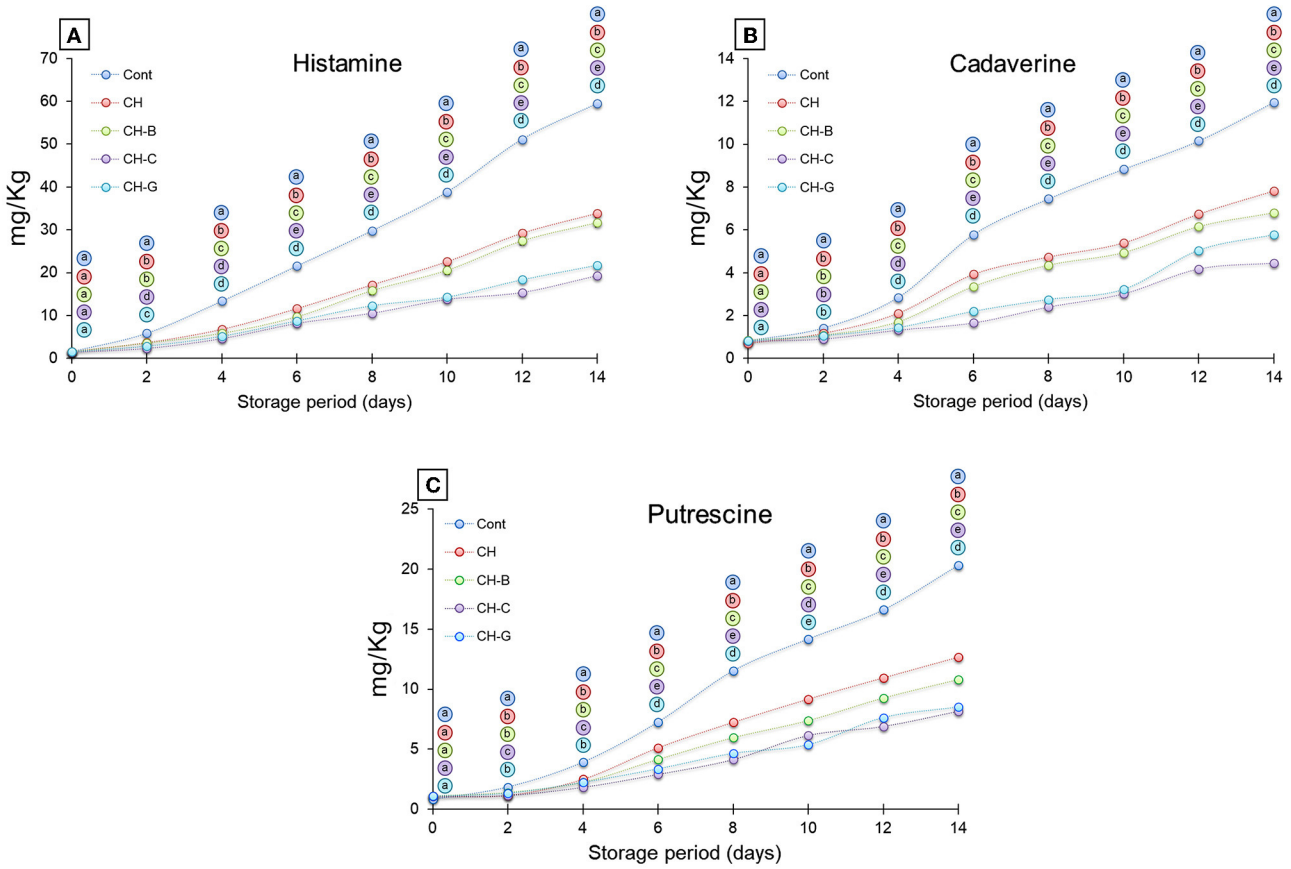

FIGURE 2
Impacts of chitosan bioactive coating fortified with natural extracts on histamine **(A)**, cadaverine **(B)**, and putrescine **(C)** levels (mg/kg) in chilled tuna filets. Values are shown as the mean of triplicates ± SD. Each colored circle with a lowercase letter matches the same colored group at each time point treatment. Different lowercase letters indicate significance at $P < 0.05$ between groups at the same time point.

be linked to their antioxidant activity (Jridi et al., 2018; Mehrabi et al., 2021). Phenolic substances stabilize hydroperoxides to stop their oxidation, further deterioration, and the production of molecules such as malondialdehyde (Hernández-Hernández et al., 2009). According to a study by Mahdavi et al. (2018), chitosan incorporated with anise essential oil (2 and 1.5%) film reduced the rate of TBA alteration in chicken burgers. Additionally, Qin et al. (2013) discovered that combining chitosan coating with tea polyphenols could significantly slow down the oxidation and spoilage of pork. Mehrabi et al. (2021) found that chicken filet samples treated with chitosan coating containing *N. pogonosperma* extract resulted in a reduction in the rate of TBA alterations.

3.2.3. TVB values

Several volatile compounds, including ammonia, methylamine, dimethylamine, trimethylamine, and other compounds formed during storage of meat in refrigerated conditions as a result of microbial activity, are included in TVB, one of the most significant indications of fresh meat detection (Anderson, 2008; Rodríguez et al., 2008). According to Figure 3C, TVB levels increased significantly ($p < 0.05$) with storage time. The lowest alteration in TVB values was noticed in chitosan-coated samples containing curcumin, garlic, and beetroot extracts compared to chitosan only. TVB level of 3.48 ± 0.31 mg/100 g in the control sample on the first day of storage reached 80.71 ± 3.78 mg/100 g after 14 days of refrigerated storage, with the highest increase compared to the coated treatments. On the 6th day of storage, the control sample with a TVB level of 37.69 ± 0.25 mg/100 g could not be consumed, while all treatments coated with chitosan were within the permitted range with TVB levels of 30 mg N/100 g [upper limit according to EOSQC (2005), ES: 3494] until the end of the 8th day. The chitosan-coated treatment containing curcumin (CH-C), with a TVB concentration of 27.96 ± 0.44 mg/100 g, was only consumable on the 14th day (the end of the storage period), as TVB concentration in all treatments was higher than normal permitted range.

3.2.4. TMA values

Uncoated and coated tuna samples showed a similar way in TMA value alterations, where clear increases were noticed during refrigerated storage (Figure 3D). The lowest alteration in TMA values was observed in chitosan-coated samples containing curcumin, garlic, and beetroot extracts compared to chitosan only. TVB level of 1.77 ± 0.28 mg/100 g in the control sample on the

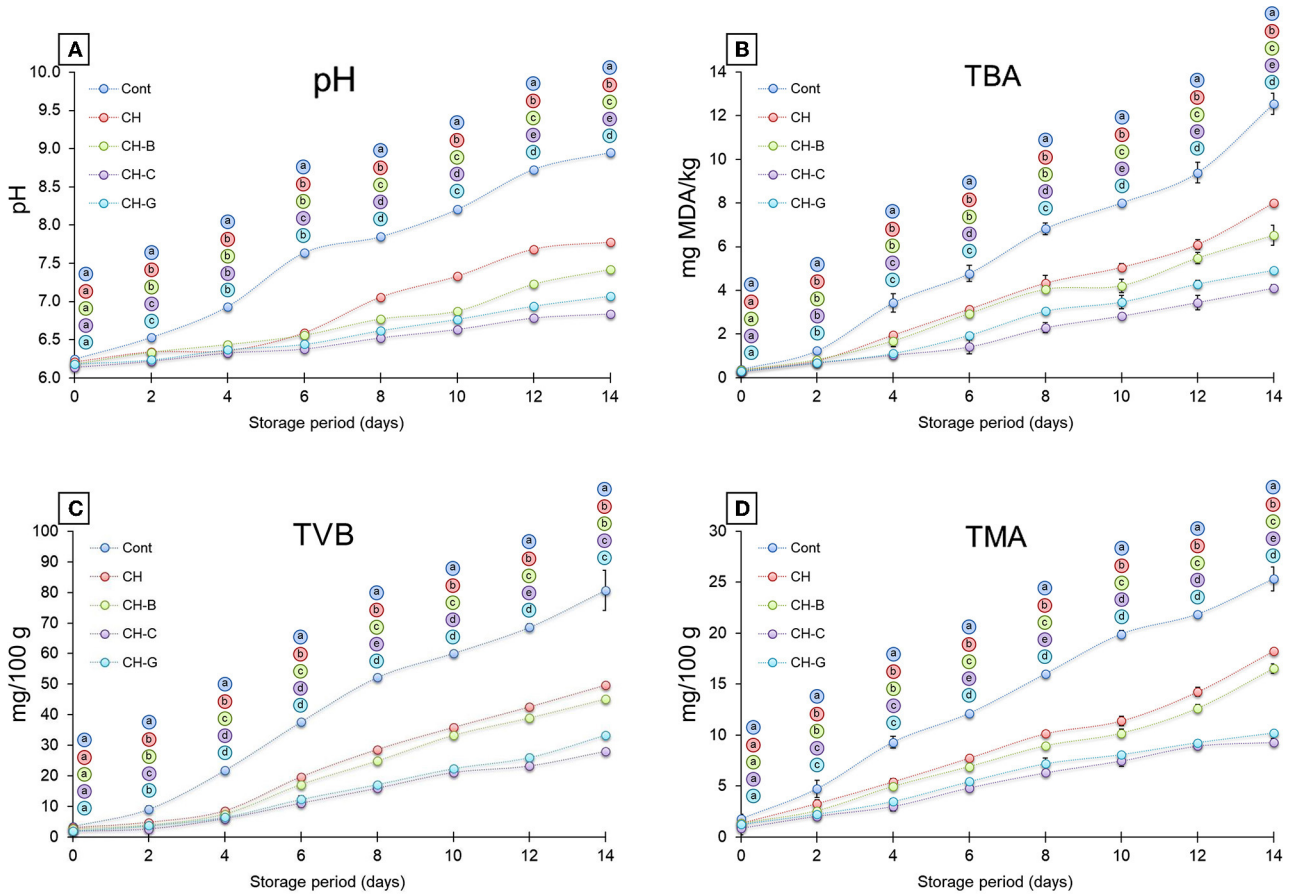

FIGURE 3
Impacts of chitosan bioactive coating fortified with natural extracts on pH **(A)**, TBA **(B)**, TVB **(C)**, and TMA **(D)** values in chilled tuna filets. Values are shown as the mean of triplicates ± SD. Each colored circle with lower case letter matches the same colored group at each time point treatment. Different lowercase letters indicate significance at $P < 0.05$ between groups at the same time point.

first day of storage, reached 25.79 ± 0.21 mg/100 g after 14 days of refrigeration, with the highest increase when compared with the coated treatments. The refrigerated storage of treated Indian white prawn (*Fenneropenaeus indicus*) revealed an increase in TMA, according to the finding of Bindu et al. (2013). TMA level of 1.77 ± 0.28 mg/100 g in the control sample on the first day of storage, reached 25.79 ± 0.21 mg/100 g after 14 days of refrigerated storage, with the highest increase when compared with the coated treatments. On the 6th day of storage, the control sample's TVB level of 12.13 ± 0.11 mg/100 g rendered it inedible, whereas all treatments coated with chitosan remained below the permitted limit of 10 mg/100 g (EOSQC, 2005) until the end of the 8th day. However, at the end of storage (day 14), TMA concentrations in all treatments were higher than the standard permitted range, and curcumin-containing treatment (CH-C) with a TVB content of 9.59 ± 0.19 mg/100 g was only edible on this day. According to the finding of Alboghbeish and Khodanazary (2018), by the end of the 12-day storage period at 4°C, the 2% chitosan-coated fish filets (*Carangoides coeruleopinnatus*) showed considerably lower TMA levels than the control sample filets (2.83 vs. 6.53 mg/100 g). However, Li et al. (2018) found no significant differences in TMA values between the control (untreated) and 1% chitosan-coated fish filets (*Cynoglossus semilaevis*) after 18 days of storage at 4°C (3.7 vs. 3 mg/100 g, respectively).

It can be concluded that among the treated samples, chitosan incorporated with curcumin (CH-C) demonstrated a remarkable superiority in all the studied parameters followed by chitosan with garlic (CH-G) extract and then chitosan with beetroot extract (CH-B).

3.3. Effect of chitosan-bioactive coatings on the sensory characteristics of tuna filet samples

The results of the sensory evaluation of tuna filet samples are presented in Figure 4. Sensory scores exhibited a significant reduction in the color, odor, appearance, and overall acceptability of C and CH treatments during storage. It has been proven that when fish spoils, it develops flavors that are strongly fishy, rancid, and putrid. As a result, the sensory score of 4 was utilized as the upper limit of acceptability in the current investigation (Fan et al., 2009; Wenjiao et al., 2013).

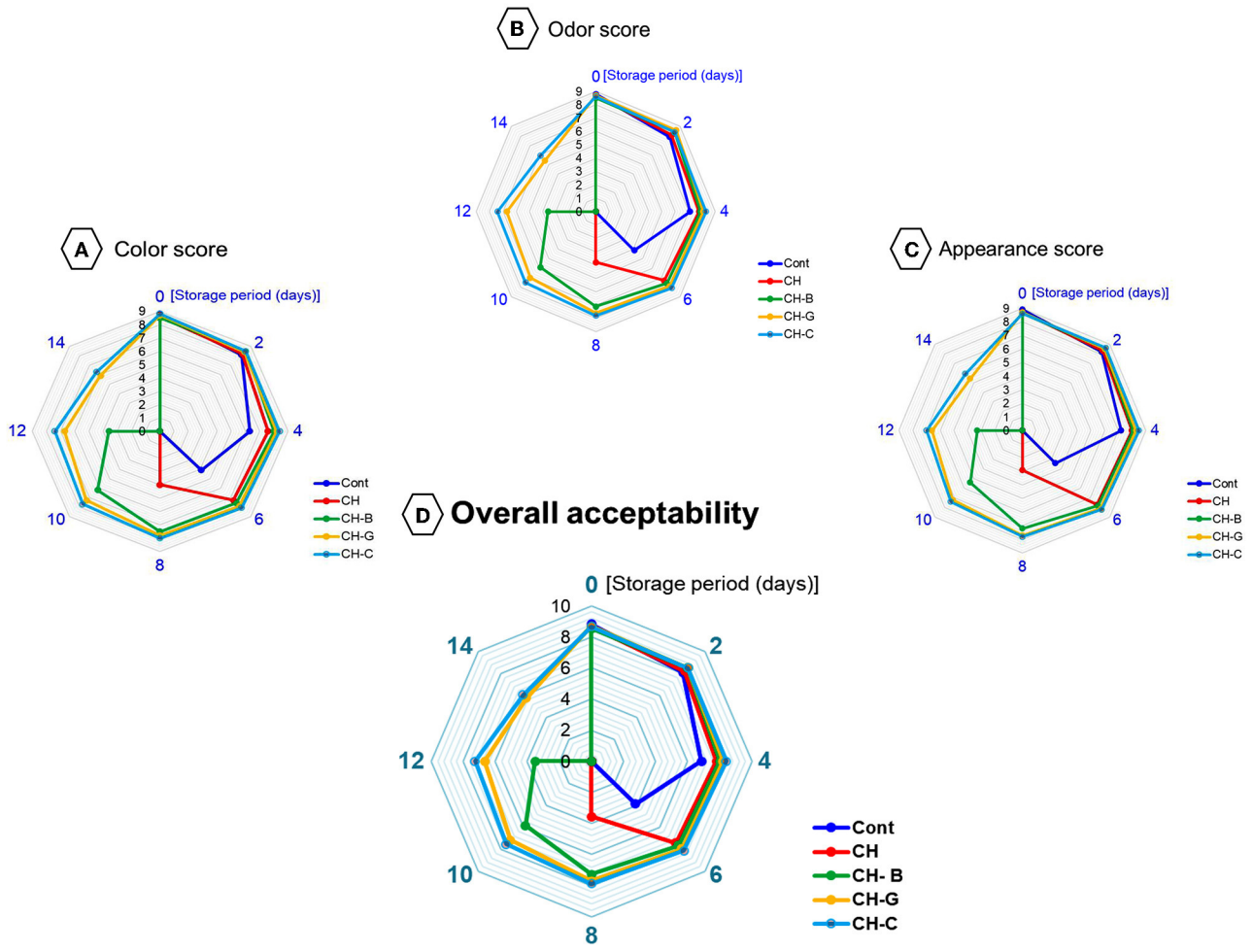

FIGURE 4
Impact of chitosan bioactive coating fortified with natural extracts sensory attributes of chilled tuna filets including color **(A)**, odor **(B)**, appearance **(C)**, and overall acceptability **(D)**. Values are shown as the mean of triplicates.

After 6 days, the C and CH samples were no longer acceptable; however, the CH-B sample remained in good and acceptable form after 10 days, and the CH-G and CH-C samples were still in good and acceptable condition for up to 14 days (end of the storage period).

3.4. PCA, VIPscore, and hierarchical clustering heatmap

A multivariate analysis (principal component analysis, PCA) was used to reveal the relationship between the various treatments and variables. All variables were included in the three principal dimensional components (PC1, PC2, and PC3), which together accounted for 99.5% of the total variance. Most of the examined variables were distinguished by PC1, and hence revealed the greater portion of variance (93.7%), whereas the lesser proportion of variance was reflected by PC2 (5.6%) and PC3 (0.2%) as depicted in Figure 5A. Furthermore, the PCA showed that the CH and CH-B treated samples were clustered together on the upper part of the gel. Similarly, CH-C and CH-G treated samples were clustered together on the bottom of the gel, and both were segregated from the control samples.

The variable importance in projection (VIP) revealed the BAs (putrescine, histamine, and cadaverine), and biochemical quality indicators (pH, TVB, TBA, and TMA) and sensory overall acceptability were the most important variables that affected the quality and shelf life of tuna filet samples (Figure 5B). The clustering heatmap shown in Figure 5C presents a clear overview of all the datasets and highlights the distinct changes in concentration values of all tested parameters in response to effective treatments when compared to other groups. These findings revealed that the CH-C and CH-G showed more improvements in the overall characteristics of tuna filet samples than other groups.

4. Conclusion

The results showed that coating tuna filets with chitosan incorporated with beetroot, garlic, and curcumin extracts

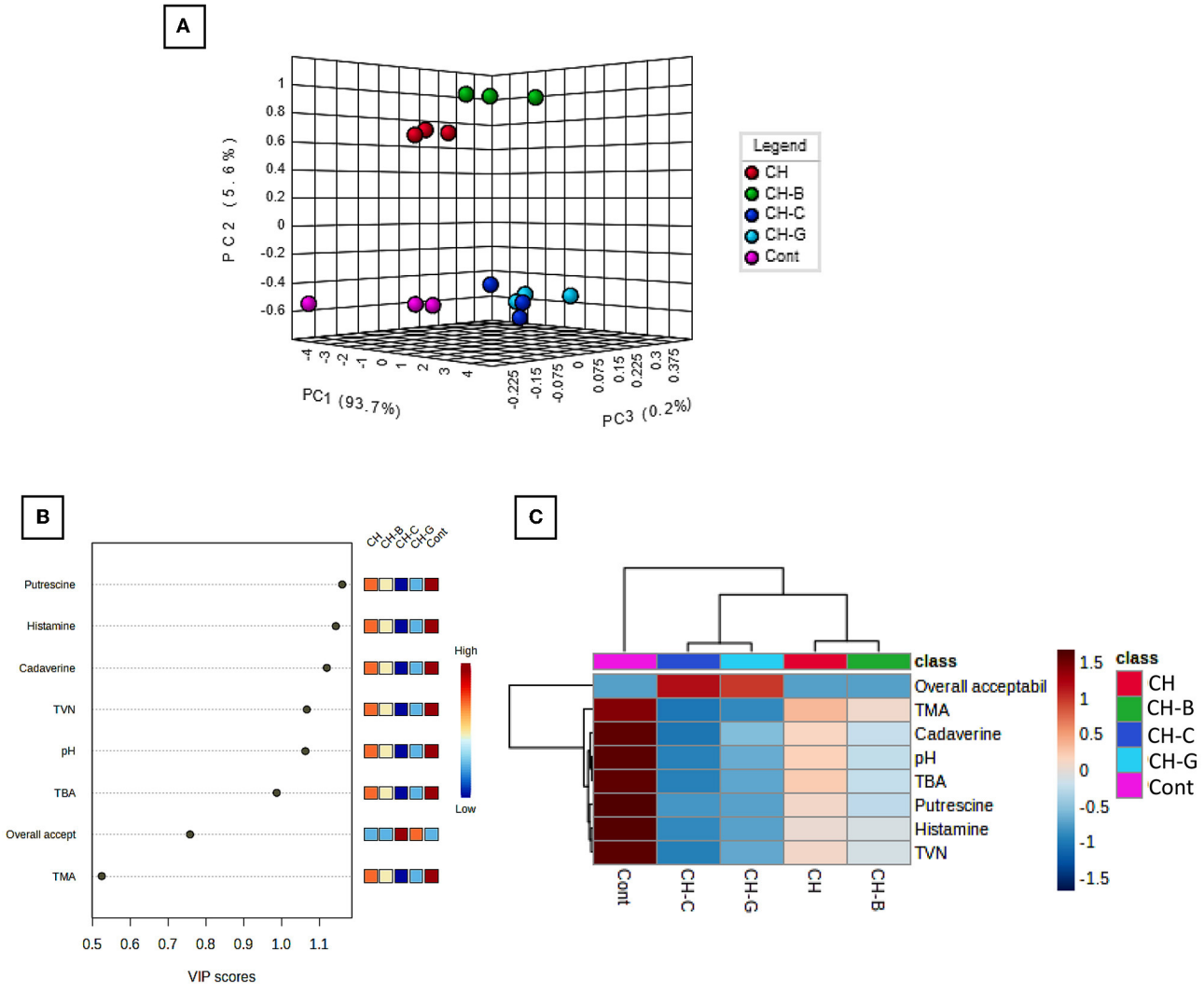

FIGURE 5
Principal components analysis (PCA) and data clustering analysis of control (Cont) and chitosan-treated samples (CH, CH-B, CH-C, and CH-G). **(A)** 3D score plot of PCA for discerning the five treatments. Percentage values specified on the axes depict the contribution rate of PC1 (93.7%), PC2 (5.6%), and PC3 (0.2%) to the overall number of variations. **(B)** Variable importance in projection (VIP): the colored boxes on the right display the relative concentrations of the relevant measured parameters in each study group, while, the contribution intensity is illustrated with colors ranging from highest (red) to lowest (blue). **(C)** All datasets can be visually represented intuitively thanks to hierarchical clustering heatmap. With varied averages in rows and various treatment sets in columns, the concentration levels are indicated by each colored cell on the map. On the gradation scale, dark red is the highest value and blue is the lowest.

resulted in a reduction of BA formation in the treated samples. Furthermore, decreases in pH, TBA index, TMA, and TVB were observed during the storage period. Beetroot, garlic, and curcumin extracts could be used in pharmaceutical and food applications as replacers for synthetic antioxidants and chemical preservatives to slow down lipid oxidation and prevent the growth of microorganisms due to their potential antioxidant activity and high concentrations of phenolic compounds.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study protocol was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Benha University (Approval no. BUFVTM 32-10-22).

Author contributions

Conceptualization: RE, SSI, EA-E, AA, and MM. Methodology: RE, SSI, EA-E, AA, AE-S, and MM. Software: RE, SSI, LŞ, RA, and AE-S. Validation: MA, SFI, RA, and AE-S. Formal analysis: RE, SSI, AA, AR, LŞ, and MM. Investigation: RE, SSI, AR, AE-S, and MM. Resources: AA, SFI, FI, and LŞ. Data curation: RE, SSI, AA, MA, RA, and AE-S. Writing—review and editing: RE, AA, AR, RA, and FI. Visualization: SFI, MA, RA, and FI. Supervision:

AA and AR. Project administration: RE. Funding acquisition: SFI and FI. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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