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Research Paper

Influence of chitosan-gelatin edible coating incorporated with longkong pericarp extract on refrigerated black tiger Shrimp (*Penaeus monodon*)

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ABSTRACT

Chitosan-Gelatin (CHI-Gel) based edible coating incorporated with longkong pericarp extract (LPE) was developed and investigated for its impact on the quality of black tiger shrimp (*Penaeus monodon*) during refrigerated storage (4 °C) for 20 days. Shrimp coated with CHI-Gel-LPE (1.5%) had better quality indices than control (no coating), those coated with CHI, CHI-Gel, and CHI-Gel-LPE at lower concentrations (0.5 and 1%). The CHI-Gel-LPE inhibited melanosis and polyphenol oxidase (PPO) and controlled the pH changes in a dose-dependent manner. Lipid oxidation indices such as TBARS, PV, *p*-anisidine, and totox values were significantly controlled by the treatments throughout the storage. The CHI-GEL-LPE-1.5% coated sample had the lowest protein oxidation, and it's ascertained by the lowest loss of sulfhydryl groups, with the lowest carbonyl content throughout the storage ($P < 0.05$). CHI-Gel-LPE (0.5–1.5%) coated samples had the lowest microbial growth (total viable count, lactic acid bacteria, *Enterobacteriaceae*, and *Psychrotrophic* bacteria) relative to the other treatments. Efficacy in quality maintenance of shrimp by LPE incorporated coating was improved with augmenting concentration used. Overall, LPE in the CHI-Gel edible coating served as a natural antioxidant, with antimicrobial activity and inhibiting melanosis, thus retain the quality and extend the shelf-life of shrimp stored at a refrigerated temperature.

1. Introduction

Penaeid shrimp is an economically important aquaculture species in several countries, including Thailand (Jescovitch et al., 2018). Shrimp is highly perishable, mainly due to melanosis in association with rapid microbial and chemical deterioration, which leads to a limited shelf-life. Even though melanosis is harmless, black spots on the shrimp surfaces could reduce the consumer acceptability and market value of the product (Sae-leaw & Benjakul, 2019). Apart from melanosis, lipid and protein oxidation are other deteriorative reactions that worsen the shrimp's quality. During post-mortem storage, autoxidation, microbial actions, and enzymatic reactions occur in shrimp and are accelerated by lipoxygenase and peroxidase activities (Nirmal and Benjakul, 2009). Worldwide, the predominant issue in food research is to maintain the freshness and quality of fresh products. Several methods such as low temperature storage, high-pressure processing, modified atmosphere packaging, irradiation, biochemical preservation, and ozone preservation have been developed to keep the freshness in the aquatic products (Wu et al., 2014). Among these, chemical preservation is one of the most universal and reliable approaches for preserving shrimp quality and extending its shelf life during storage (Zhang et al., 2019). However, the application of synthetic compounds such as 4-hexyl-1,3-benzenediol (4-hexylresorcinol), sulfite-based compounds, and phosphates in seafood is restricted due to their potential toxicity and increasing regulatory attention. Consumer awareness about the risk associated with chemical additives has created the demand for a safe and effective alternative. Besides the safety aspects of edible coating, it can serve as carriers for natural preservatives such as antioxidants and antimicrobial agents. Recent studies have found that edible coatings are a promising method that extends food's shelf life, with easy handling, eco-friendliness,

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effectiveness, biocompatibility, and wide availability.

For preservation, protein and polysaccharides-based coatings are widely used. Among different edible coatings, chitosan is a commonly used material due to its hydrophilic nature with potent antioxidant and antimicrobial activities against free radicals and food-borne pathogens (Nowzari et al., 2013). Chitosan is a polysaccharide and key component of the crustacean shell obtained from the alkaline hydrolysis of the N-acetyl group of chitin. Several functional properties have been identified, including film-forming ability, excellent oxygen barrier properties, antimicrobial and antioxidant activities (Yuan et al., 2016). Chitosan-based edible coatings may be used as carriers of active ingredients, whereas gelatin has an excellent film-forming capacity and wide applicability (Gómez-Guillén et al., 2009). It has been documented in several studies that chitosan extends the shelf-life of food. However, chitosan alone shows limited protection against autoxidation and microbial spoilage, so there is a need to incorporate novel natural active agents or phytochemicals into the edible coating (Yu et al., 2017). To ease the melanosis and oxidation in shrimp, sulfiting agents and synthetic antioxidants are commonly used. However, increased regulatory attention and consumer awareness urge discovering novel and safer food preservation techniques (Sae-leaw & Benjakul, 2019).

Phenolic compounds are known as effective natural additives with antioxidant and antimicrobial properties and potent alternatives to synthetic agents (Banerjee, 2006). Longkong belongs to the *Meliaceae* family and is a unique tropical and non-climacteric fruit with rich health benefits. It is mainly grown in peninsular Thailand and mostly in its southern part (Venkatachalam, 2018). Longkong fruit is also widely grown in other countries, including the Philippines, Vietnam, Myanmar, India, Sri Lanka, Australia, Surinam, and Puerto Rico. Longkong fruit pericarp has abundant primary and secondary antioxidant activities (Venkatachalam and Meenune, 2012). As per the literature review carried out for this study, it was understood that no prior information on the use of longkong pericarp extracts to maintain the freshness of shrimp. Thus, this investigation aimed to study *Penaeus monodon* shrimp's quality changes when coated with a chitosan-gelatin edible coating incorporated with longkong pericarp extract and stored under refrigerated conditions for a prolonged period.

2. Materials and methods

2.1. Raw material collection and preparation

For this study, the fresh black tiger shrimp (*Penaeus monodon*) samples (approximately 6–8 cm length) were collected from a local farm. The collected shrimps were placed in ice with a ratio of 1:2 (shrimp/ice, w/w) and then brought to the laboratory within 1 h. Upon arrival, the shrimps were cleaned of any dirt and thoroughly washed using cold water, and after that, the cleaned shrimps were kept in ice until further use (within 5 h). Before submerged in the coating solution, the freshly harvested shrimp samples were manually beheaded, deshelled, and deveined and followed by thorough washing in cold filtered water and proceeded further for coating as described in section 2.4.

2.2. Preparation of longkong pericarp extract (LPE)

Fully matured longkong fruits (*Aglaia dookoo Griff.*) were freshly harvested from a local garden and brought to the laboratory. After that, the pericarp was separated from fruit flesh and washed under cold water that contained 2% ascorbic acid. After washing, the fruit's pericarp was kept in a hot air oven (Memmert, Schwabach, Germany) and thoroughly dried at 40 °C until constant weight. Then the pericarp was ground into a fine powder. 1 g pericarp powder was mixed with 20 mL of absolute ethanol, and the extraction was conducted at 40 °C in a water bath (W350; Memmert, Schwabach, Germany) coupled with a shaker (210 Vib/min) for 4 h. The mixtures were then left at ambient temperature overnight and then filtered; after that, the filtrate was collected and used in a rotary evaporator (EYELA, Tokyo, Japan) to remove the solvent at 40 °C under low pressure. After complete removal of the solvent, the longkong pericarp extract (LPE) was collected and kept at −20 °C for further use.

2.3. Preparation of coating materials

Chitosan served as the basis of coating in all the treatments. The food-grade chitosan (2%) was dissolved in acetic acid (1.5%) to obtain a homogenous CHI coating solution. Then, the CHI solution was warmed to 40 °C, followed by the addition of gelatin (Gel) powder (2%) and thorough mixing to obtain a homogenous CHI-Gel coating solution. After that, the CHI-Gel coating mixture was continuously stirred for 10 min at 70 °C and then cooled down to the ambient temperature. Into the CHI-Gel coating solution, longkong pericarp extract (LPE) was added separately at three concentrations (0.5, 1.0, and 1.5%) and mixed thoroughly to obtain homogenous coating solutions. There were five treatments (Table 1), namely CHI, CHI-Gel, CHI-Gel-LPE 0.5%, CHI-Gel-LPE 1.0%, and CHI-Gel-LPE 1.5%, and dipping in distilled water served as the negative control treatment. All the coating materials were used within an hour after preparation.

2.4. Coating of shrimp and storage

After the coating solutions were prepared, shrimps were wholly submerged in a coating material and positioned on a wire rack for a min to drip off any excess coating. At the same time, it was exposed to air blown by using an electric fan to help speed up the process to dry up the coating material on the surface of the shrimp. After that, the shrimps were placed in polystyrene trays (12 shrimps/tray) and wrapped with polyolefin wrap film and were then kept at 4 °C for up to 20 days. During this storage, samples were collected randomly at every 5 days of an interval for various physicochemical, microbiological, and sensory analyses.

2.5. Physicochemical analysis

2.5.1. pH

Shrimps (20 g) were homogenized using 100 mL of distilled water, and the homogenate was checked of pH using a table top digital pH meter (Nirmal and Benjakul, 2009).

2.5.2. Melanosis score

Melanosis scores of the shrimps were analyzed by visual inspection by following the method of Montero et al. (2004) with slight modifications. Fifteen trained panelists were used in this study. Panelists were requested to score the shrimps based on a 1–10 scale, where 1 represents the absence of black spots on the shrimp, and 10 represents substantial brown spots on the entire shrimp.

Table 1
Treatments with coating solutions for black tiger shrimp.

Treatment	Description
Control	Shrimps were not coated with any coating solution.
CHI	Shrimps were coated with the coating solution containing 2% chitosan.
CHI-Gel	Shrimps were coated with the coating solution containing 2% chitosan and 2% gelatin.
CHI-Gel-LPE 0.5%	Shrimps were coated with the coating solution containing 2% chitosan, 2% gelatin and 0.5% longkong pericarp extract.
CHI-Gel-LPE 1.0%	Shrimps were coated with the coating solution containing 2% chitosan, 2% gelatin and 1.0% longkong pericarp extract.
CHI-Gel- LPE 1.5%	Shrimps were coated with the coating solution containing 2% chitosan, 2% gelatin and 1.5% longkong pericarp extract.

2.5.3. Extraction and determination of polyphenol oxidase (PPO)

The extraction and determination of PPO were conducted for the shrimp meats by following the method of Basiri et al. (2015). PPO activity was measured using the *L*-3, 4-dihydroxylphenyl alanine (*L*-DOPA) substrate. One unit of PPO activity was described as an increase in absorbance by 0.001/min. The results are presented in percentages of relative activity in shrimp meat.

2.5.4. Extraction of shrimp protein isolate

Shrimp protein isolate was extracted by the method of Liu and Xiong (1996), and then the extraction was measured of the carbonyl and sulfhydryl contents. Carbonyl groups in the shrimp protein isolate were measured by following the method of Xia et al. (2009). The results are given in nmol/mg protein. Total sulfhydryl content was determined by following the method of Ellman (1959). The protein concentration in the isolate was determined using Biuret method. The results are expressed in μmol sulfhydryl/g protein. Reactive sulfhydryl content was determined in the shrimp protein isolate by using the method of Xia et al. (2009). The reactive sulfhydryl in the shrimp protein isolate was calculated as follows:

Reactive sulfhydryl content (μmol/g) = 73.53 x (A_{412} – 1.6934 x A_{532} + 0.009932).

2.5.5. Determination of thiobarbituric acid reactive substances (TBARS)

Lipid oxidation in the shrimp was assessed using TBARS by following Sørensen and Jørgensen (1996) method. A standard curve of malondialdehyde (0–5 μM) was used to determine the sample's malondialdehyde equivalent content. The results are expressed in mg MDA/kg shrimp.

2.5.6. Determination of peroxide value (PV)

The shrimp sample's PV was measured by following the method of Okpala (2014). The results are expressed in mEq O_2/kg shrimp.

2.5.7. Determination of p-anisidine value (p-AnV)

The *p*-AnV in the samples was measured by following the method of Okpala (2014). The *p*-AnV was calculated as follows:

$$p\text{-AnV}\ (\%) = [25 \times \{1.2\ A_2 - A_1\}]/\ M$$

Where A_1 and A_2 are the absorbances, read before and after the addition of *p*-anisidine, and *M* represents the mass of the sample (g).

2.5.8. Determination of totox value

The totox values of the shrimp samples were determined from their PV and *p*-AnV by calculating the following equation proposed by Sun-Waterhouse et al. (2011).

$$\text{Totox value} = 2\ PV + p\text{-AnV}$$

2.6. Microbiological analysis

Aseptically, the shrimp meat samples (25 g) were measured and placed in sterile bags, followed by the addition of 225 mL of ringer water, and then homogenized by using a stomacher for 2 min at room temperature. The homogenate was subjected to appropriate serial dilution. The following microbial analysis included total viable count, lactic acid bacteria, *Enterobacteriaceae,* and *psychrotrophic* bacteria. They were observed in the homogenate by using suitable media, and all the microbial analysis was conducted by the method of Carrión-Granda et al. (2016). All the microbial analyses were visually examined for the count of colonies and their morphological appearances. The results are expressed in logs of colony-forming units (CFU) per gram of sample.

2.7. Statistical analysis

All the experiments were done in triplicates. The results are shown as mean ± standard deviation, and the probability value of $P < 0.05$ was considered significant. One-way analysis of variance (ANOVA) was applied in this study, and Duncan's multiple range test was used to compare the mean differences. All the statistical assessment was conducted using Statistical Package for Social Sciences (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. pH

The changes in pH of control and coated black tiger shrimps stored at refrigerated temperature are depicted in Fig. 1A. During extended storage, the pH of the control sample decreased more than the others. With storage time, the pH of all treatments decreased significantly ($P < 0.05$). Generally, the release of inorganic phosphate and the production of lactic acid might occur during storage, which could be the reason for the reduction of sample pH (Li et al., 2020). Furthermore, Qiu et al. (2014) reported that lactic acid production in samples could happen through glycolysis and ATP degradation that reduced the pH. The lower level of pH was observed in control and followed by the CHI, CHI-Gel, and CHI-Gel-LPE treated samples during storage ($P < 0.05$). A lower pH in the control samples indicated the gradual increment of spoilage by the microorganism during storage and whereas, in the treated samples were slightly reduced of this incidence. Irrespective of the LPE concentrations, the pH remained stable throughout the storage in CHI-Gel-LPE coated shrimps. It could be due to the antimicrobial effect of LPE, which probably controlled the microbial growth and reduced the degradation of ATP during storage.

3.2. Melanosis score

The melanosis scores of control and coated black tiger shrimps during storage under refrigerated conditions are shown in Fig. 1B. At the beginning of the storage, there was no melanosis observed in any of the shrimp samples. Black spot formation appeared on 5th day of storage, and control treatment recorded the most pigment formation ($P < 0.05$), which continued throughout the storage period. After 5 days, all samples had melanosis scores that increased with the storage period ($P < 0.05$). However, shrimp coated with CHI-Gel-LPE at 1 and 1.5% concentrations showed no significant differences ($P > 0.00.5$) in melanosis scores during the storage. Shrimps coated with LPE at different concentrations showed lower melanosis scores than control, CHI, and CHI-Gel coated samples ($P < 0.05$). The reason might be due to phenolic compounds in LPE to inhibit PPO; thus, it reduced melanosis in shrimp. Melanosis in shrimp might be inhibited by bisulfite through interacting with intermediate quinine or producing sulfoquinone (Sae-leaw & Benjakul, 2019). Gokoglu and Yerlikaya (2008) stated that shrimp (*Parapenaeus longirostris*) treated with grape seed extract (1.5%) showed lower melanosis scores than control, and all those samples treated with lower grape seed extract concentration when stored at 0 °C. These results indicated that organic extracts obtained from parts of fruits could control melanosis that the use of LPE could effectively delay the melanosis in shrimp during extended refrigerated storage.

3.3. Polyphenol oxidase (PPO)

PPO activities of control and coated black tiger shrimp during storage under refrigerated conditions are given in Fig. 1C. The control sample showed the highest PPO activity, while the lowest was recorded in coated

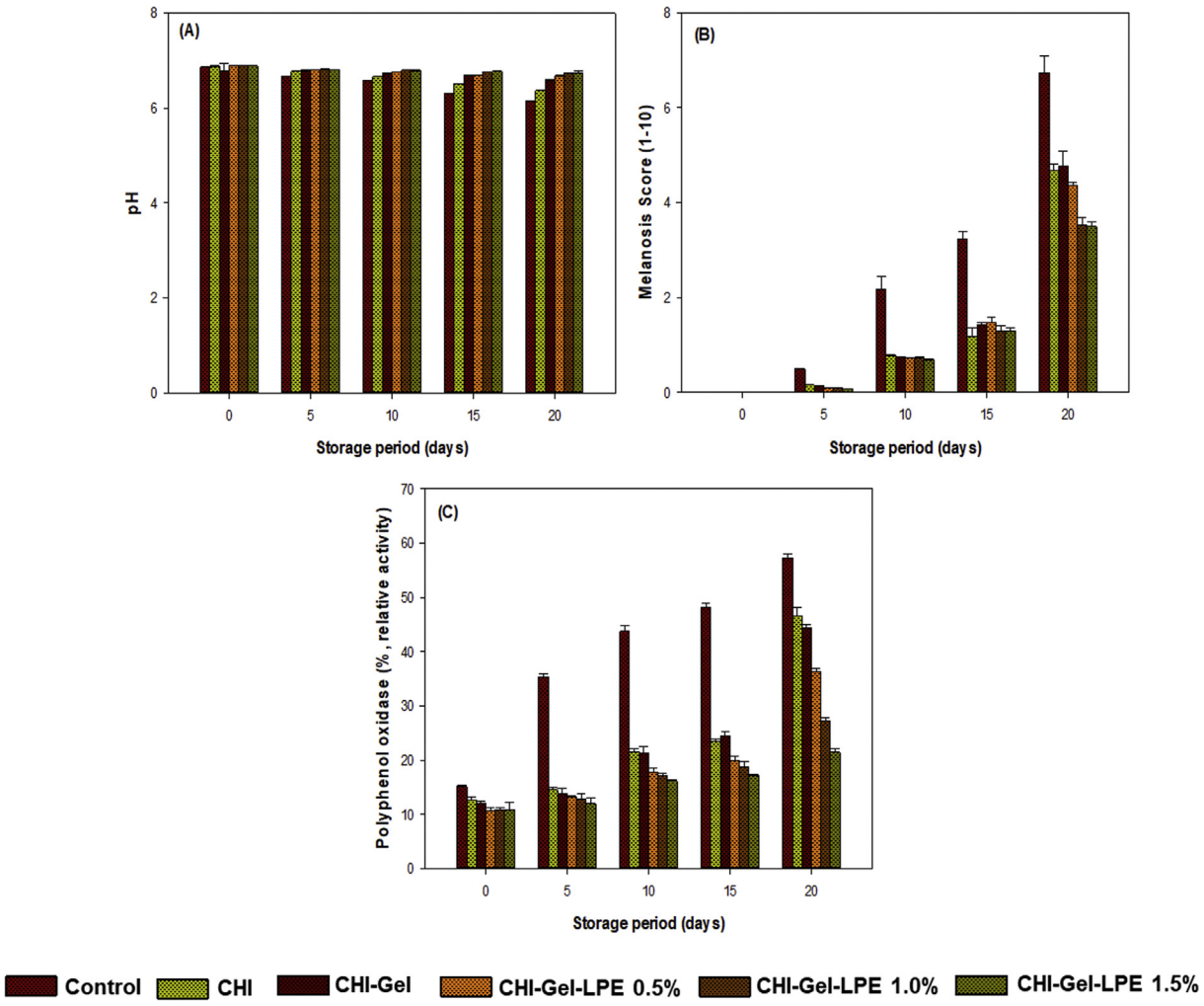

Fig. 1. Time profiles of pH (A), melanosis score (B), and polyphenol oxidase activity (C) in black tiger shrimp samples stored at a refrigerated temperature for up to 20 days.

groups ($P < 0.05$) all through the study period. However, LPE incorporated in edible coating at high concentration (1.5%) had maximum PPO inhibitory effect against all other treatments ($P < 0.05$). PPO decreased as the concentration of LPE increased. This might be due to the phenolic compounds in LPE extract that likely acted as PPO inhibitors by reducing quinone or interacting with enzymes' active sites (Nirmal and Benjakul, 2012). Sae-Leaw et al. (2017) stated that catechin and its metabolites inhibited pacific white shrimp PPO activity. This result confirms that LPE might be used effectively as a natural inhibitor of PPO activity in black tiger shrimp.

3.4. Carbonyl content

Carbonyl content in control and coated shrimp throughout storage in refrigerated temperature is shown in Fig. 2A. The degree of protein oxidation has been determined by estimating carbonyl content by derivatization with DNPH (2,4-Dinitrophenylhydrazine) in different protein systems and has been commonly used as a general measure (Lund et al., 2007). The carbonyl compounds' formation results in oxidative deterioration of side chains of lysine, proline, arginine, and histidine residues (Bazargani-Gilani et al., 2015). The number of protein carbonyls in control and coated samples was found significantly ($P < 0.05$) increased. During the extended storage period, the carbonyl content of the samples, particularly for the control, was prominently increased ($P < 0.05$). On the other hand, the carbonyl content exhibited no significant differences amongst treated samples until 10 days of the storage ($P > 0.05$). After that, a significant change in carbonyl content was observed for the treated samples, and though, it was still lesser than the control samples ($P < 0.05$). A similar increasing pattern in carbonyl groups has also been reported in horse mackerel mince during cold storage (Eymard et al., 2009). Increases in the overall carbonyl content suggest that shrimp had oxidative changes during refrigerated storage, as the key products of autoxidation are carbonyls. An increase of carbonyls in marine sources could also be induced by oxidative peptide scission. During protein oxidation, protein carbonyl content increases because these groups are converted into carbonyl groups (Xia et al., 2009). Among coated samples, LPE incorporated coatings controlled the protein oxidation more effectively than the other coatings, which could be due to the phenolics in the LPE extract, which express significant antioxidant activities (Venkatachalam, 2020).

3.5. Total and reactive sulfhydryl groups

The total and reactive sulfhydryl groups of control and coated shrimp samples during storage under refrigerated temperature are depicted in Fig. 2B and C. Sulfhydryl, a highly active group in myofibrillar protein, possesses weak secondary bonds and maintains its tertiary structure. Protein oxidation is linked to reduced sulfhydryl groups transformed into disulfides (Soyer et al., 2010). Wu et al. (2019) stated that denaturation and aggregation of muscle proteins are correlated with the disulfide

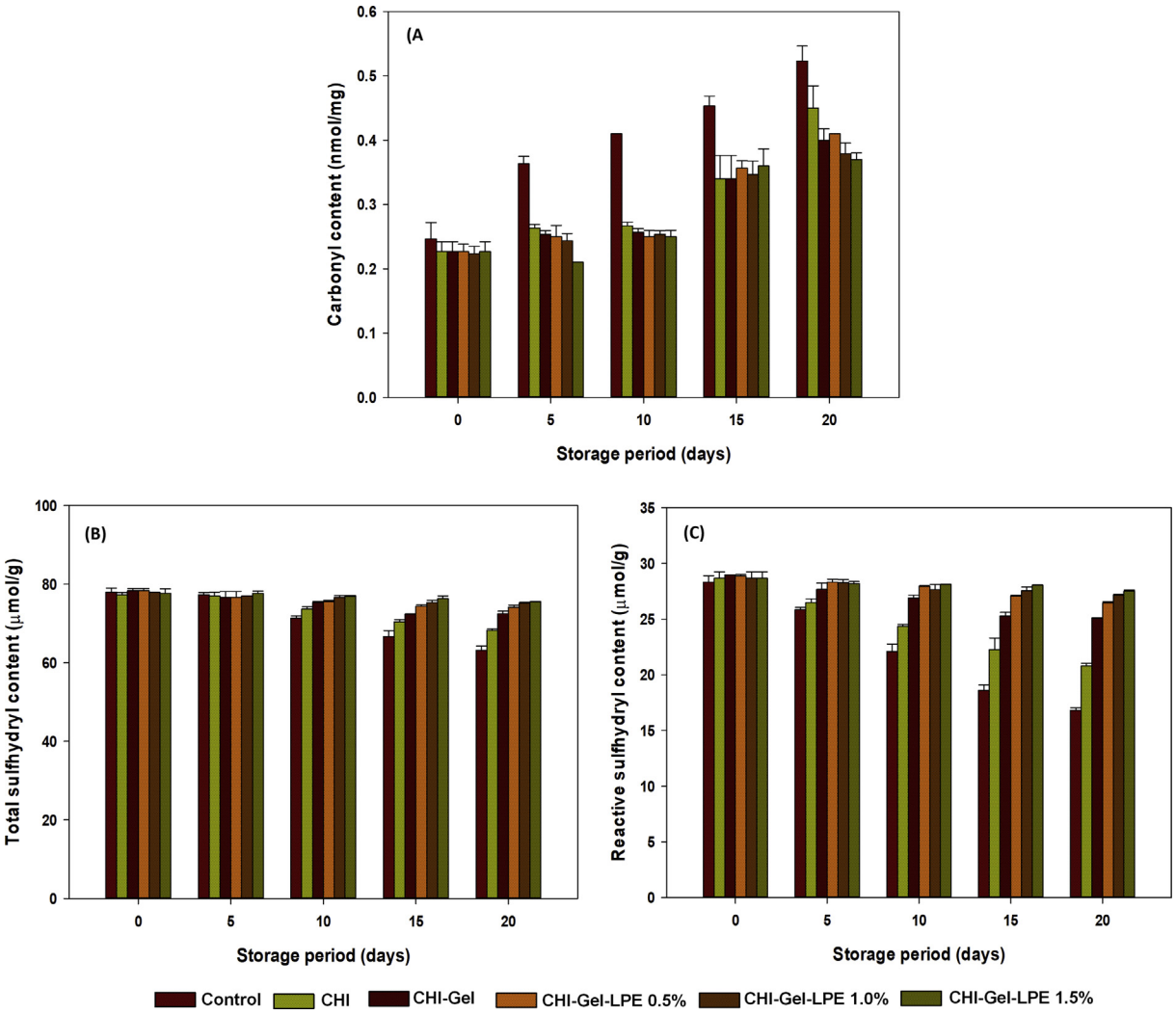

Fig. 2. Time profiles of carbonyl content (A), total sulfhydryl content (B), and reactive sulfhydryl content (C) in black tiger shrimp samples stored at a refrigerated temperature for up to 20 days.

bonds. Refrigerated temperature and storage duration show a significant ($P < 0.05$) impact on the control samples' total and reactive sulfhydryl contents. The mean contents decrease from 80 to 65 μmol/g and from 28 to 18 μmol/g protein for total and reactive sulfhydryl contents, respectively. Among all the coated samples, the largest loss was found in the CHI-coated samples, while the least loss was observed for LPE incorporated samples, irrespective of the concentration. However, total sulfhydryl content showed no significant ($P > 0.05$) difference, but reactive sulfhydryl content varied significantly across the treatments ($P < 0.05$). Eymard et al. (2009) reported that minced fish kept in refrigerated storage showed lower sulfhydryl content. The production of disulfide bonds within polypeptides or between polypeptides might be the reason for losing sulfhydryl groups. The reactive oxygen species could adversely influence the protein content and induced the carbonyl compounds' production and sulfhydryl groups' reduction from the protein. Primary (hydroperoxides) and secondary products (aldehydes) of lipid oxidation might interact with proteins, thus leading to the oxidation of protein (Soyer et al., 2010). The present study showed similar patterns in protein carbonyls and lipid hydroperoxides during the storage period. It has been reported that protein oxidation is connected to lipid oxidation in marine species (Hematyar et al., 2019).

3.6. Lipid oxidation

3.6.1. TBARS

TBARS of the control and coated shrimp samples during storage under refrigerated temperature is represented in Fig. 3A. A rise in TBARS was related to the partial dehydration and oxidation of unsaturated fatty acids (Nowzari et al., 2013). Chitosan coating is an excellent barrier to oxygen permeation, thereby slowing oxygen diffusion to the exterior and reducing lipid oxidation (Li et al., 2013). The control sample showed higher TBARS than that of the coated sample ($P < 0.05$), which is in accordance with the result reported by Souza et al. (2010) for salmon. TBARS in shrimp samples coated with CHI-Gel were minimal compared with control and CHI-coated samples. The reason might be due to the excellent gelatin's oxygen barrier properties. In all samples, the TBARS stayed below the maximum limit of 1–2 mg malonaldehyde/kg that is known as the limit for developing objectionable odor and taste (Fan et al., 2009). Shrimp coated with a solution having LPE at the highest tested concentration (1.5%) showed lesser TBARS than the other CHI-Gel-LPE coated samples. The results indicate that LPE could interact with CHI and Gel to form a strong barrier coating on the shrimp's surface and controlled lipid oxidation. Furthermore, LPE is known to have strong antioxidant activity, enabling it to act as a key scavenger inhibiting lipid peroxidation.

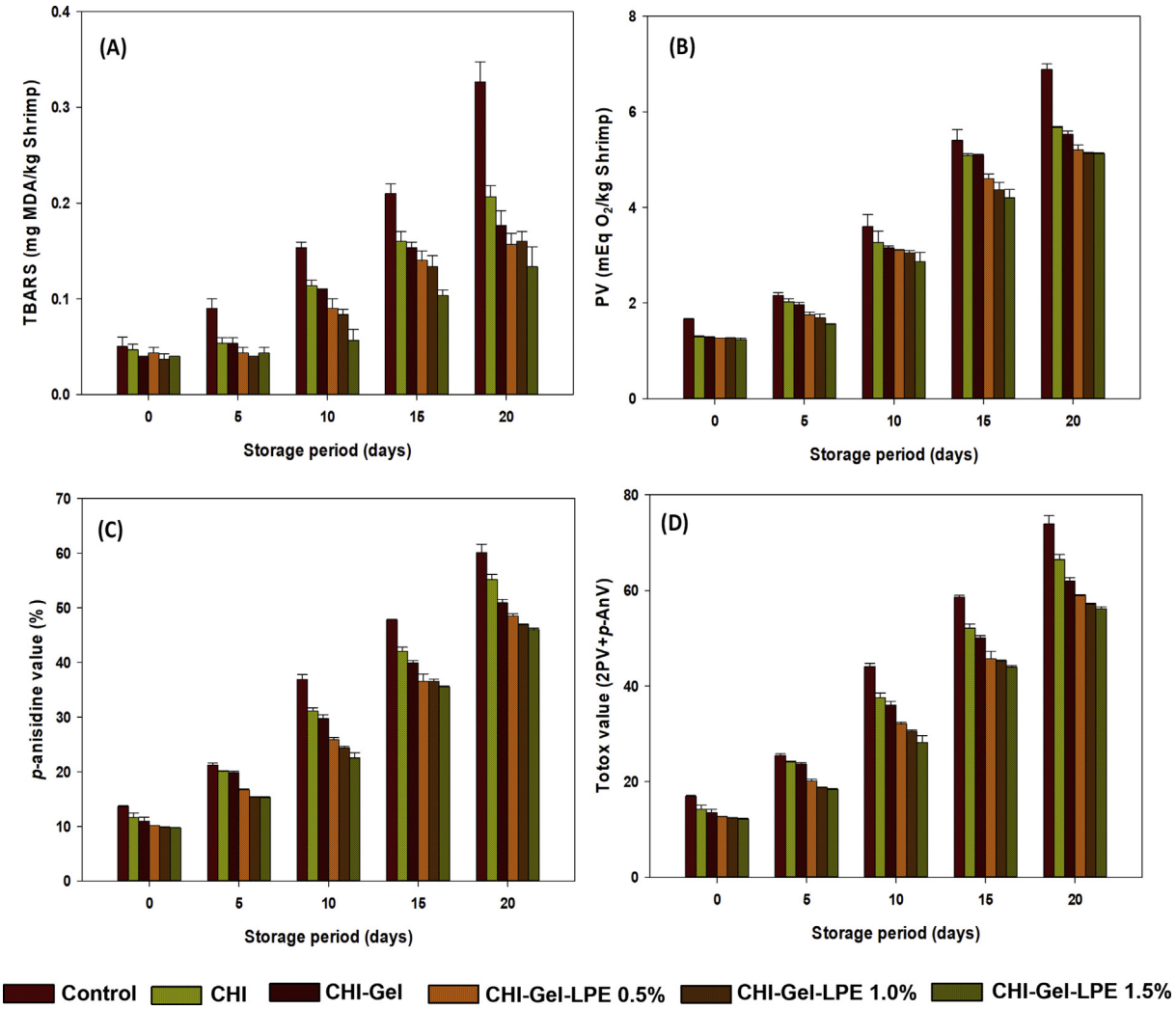

Fig. 3. Time profiles of TBARS (A), PV (B), *p*-anisidine (C), and totox values (D) in black tiger shrimp samples stored at a refrigerated temperature for up to 20 days.

3.6.2. PV

PV of control and coated shrimp samples during storage under refrigerated temperature is given in Fig. 3B. The extent of lipid oxidation in the initial stages was determined by measuring hydroperoxides that are the main oxidation products (Soyer et al., 2010). In all cases, the PV increased continuously during the storage and showed primary product formation due to lipid oxidation. A significantly ($P < 0.05$) greater rise of PV was found in control than for the other treatments during the storage. The rise in PV was reduced by CHI-Gel coating relative to coating with CHI alone at the 20th of the storage ($P < 0.05$). The reason might be due to gelatin functioning as oxygen barrier and reducing lipid oxidation (Gómez-Estaca et al., 2014). Sample coated with CHI-Gel-LPE showed lower PV ($P < 0.05$). However, no significant ($P > 0.05$) differences were recorded among the different CHI-Gel-LPE coatings of shrimps. The PV did not exceed the acceptability limit (18–20 meq/kg) suggested by Reesha et al. (2015) with any of the treatments. PV analysis shows the potential free-radical-scavenging ability of LPE and the ability of chitosan to hinder the development of secondary lipid oxidation products. Thus, a slow increase in the PV confirms that LPE enhances the edible coating's antioxidant effects, irrespective of its concentration. Therefore, incorporating LPE in CHI-Gel edible coating played a role in reducing the PV during the study period.

3.6.3. p-Anisidine value

p-AnV of control and coated shrimp samples under prolonged refrigerated storage is depicted in Fig. 3C. The secondary oxidation products were measured by *p*-AnV, including aldehydes, ketones, and numerous other substances. In general, *p*-AnV increased continuously for all samples throughout storage ($P < 0.05$). The rise in *p*-AnV level indicates a sequence of the reaction occurred between unsaturated fatty alkyl groups and oxygen, thus generating various lipid oxidative products. An extreme rise in *p*-AnV was noted after 15 days of storage, indicating that prolonged storage loosened the shrimp coating. Furthermore, lipid oxidation might be triggered and sped up by various processes, such as the singlet oxygen production, enzymatic and non-enzymatic production of free radicals, and active oxygen (Papuc et al., 2016). The control sample showed the highest *p*-AnV ($P < 0.05$), while lower *p*-AnV was observed for samples coated with CHI-Gel, regardless of incorporation of LPE. Among CHI-Gel-LPE coated samples, those with the highest tested LPE concentration (1.5%) showed the least p-AnV.

3.6.4. Totox value

Totox value of control and coated shrimp samples stored under refrigerated temperature is given in Fig. 3D. The entire oxidation status of the stored sample was measured by totox value, and it can be calculated from PV and *p*-AV. In all cases, the totox value increased throughout the storage time. It was found that a higher totox value was recorded for the control samples than for the others ($P < 0.05$), whereas shrimp samples coated with CHI-Gel, followed by CHI coated samples ($P < 0.05$), showed a lower totox value. A lower totox value indicates better shrimp

quality (O'Connor et al., 2007). LPE incorporated at high concentrations in the coated sample showed comparatively lower totox values than at lower concentrations ($P < 0.05$). This result indicates greater stability against lipid oxidation, and the antioxidative effect of LPE might support this. In the autoxidation process, free radicals formation might absorb or react with oxygen, inhibiting LPE antioxidants, thus delaying the onset of autooxidation (Turhan et al., 2009).

3.7. Microbial analysis

3.7.1. TVC

The total viable count (TVC) of control and coated shrimps throughout the storage under refrigerated temperature is given in Fig. 4A. On day 0, TVC was around 2 log CFU/g for all cases, suggesting the existence of certain microorganisms, possibly from contamination while handling, packing, etc. TVC of the control group exceeded 7 log CFU/g at day 20, which is regarded as a higher acceptability level for inland and coastal species according to ICMSF (2002). During storage, the control samples had gradually increased TVC exceeding those in chitosan-coated samples ($P < 0.05$). Rabea et al. (2003) stated that chitosan possesses excellent film-forming properties and wide antimicrobial properties against several microbes. The antimicrobial properties of chitosan are attributed to the interactions between positively charged chitosan and negative charged microbial cell membrane resulting in the release of cellular proteins and other intracellular components, chelation of vital nutrients, spore constituents, and penetration into the nuclei of microorganism and disruption of the DNA (Pereda et al., 2011). Among the coated samples, the LPE incorporated samples showed lesser TVC than the other types of coated samples in this analysis ($P < 0.05$). The greater inhibitory effect was recorded in CHI-Gel-LPE (1.5%) among all treatments. This result confirms that the antimicrobial activity of LPE increased with its concentration in the coating solution. The findings were in line with lower microbial counts in rainbow trout in chitosan film wrapped samples incorporated with essential oils (Mexis et al., 2009) and bologna slices (Zivanovic et al., 2005).

3.7.2. Lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) of control and coated shrimp samples while storage under refrigerated temperature are depicted in Fig. 4B. LAB gradually raised with storage time ($P < 0.05$). A continuous increment in LAB level was found in the control samples than other treatments during the storage. LAB is facultative anaerobes that can grow under elevated

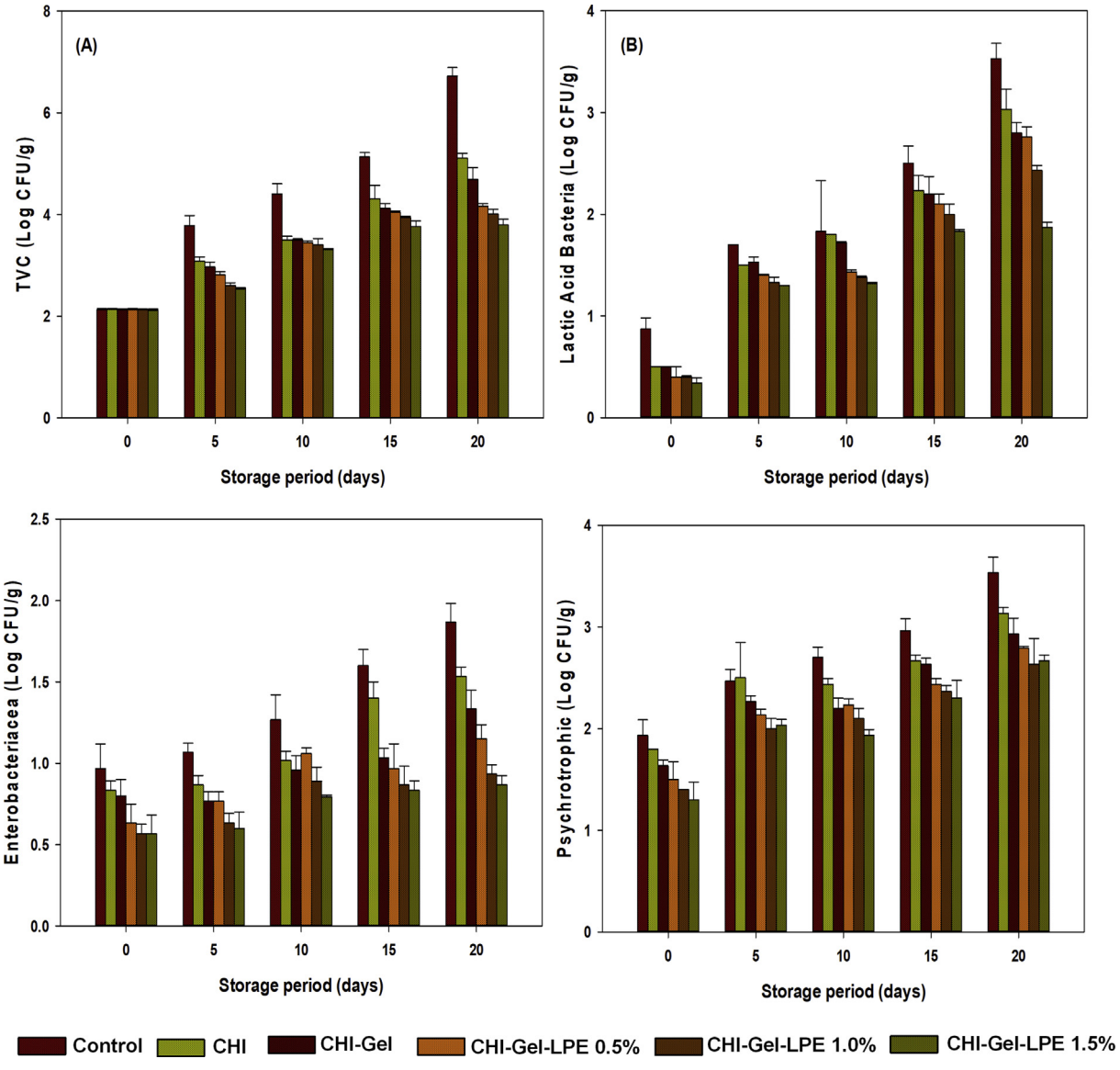

Fig. 4. Microbial growth in the black tiger shrimp samples stored at a refrigerated temperature for up to 20 days.

CO_2 concentration and represent a significant part of the natural microbiota in anaerobically stored food (Arfat et al., 2015). A better reduction in LAB count was observed in CHI-Gel coated sample compared to the CHI treated samples, and it could be due to the antimicrobial properties of gelatin. Gelatin contains antimicrobial peptides that might be discharged to the exterior of the coated shrimps. Gelatin peptides show antimicrobial properties against many microorganisms (Gómez-Guillén et al., 2009). The chitosan coatings also act as oxygen barriers and hinder aerobic bacteria's proliferation (Devlieghere et al., 2004). In addition to CHI and Gel's antimicrobial activities, the incorporation of LPE at a high concentration (1.5%) in coatings also improved, inhibiting the growth of LAB effectively throughout the storage. A similar result was observed for seabass fillet and pork sausage by adding thyme and rosemary essential oil that reduced LAB counts (Kostaki et al., 2009; Georgantelis et al., 2007).

3.7.3. Enterobacteriaceae

Enterobacteriaceae of control and coated shrimp samples under refrigerated storage are included in Fig. 4C. *Enterobacteriaceae* are considered a hygiene indicator (Arfat*et al.*, 2015), and on day 0, its initial count was 1 log CFU/g, and it gradually attained 2 log CFU/g as in control samples on day 20. The LPE incorporated CHI-Gel coated samples had a lower count (<1.3 Log CFU/g) than all the other samples with day 20 of storage. The lower count observed in LPE coated samples was attributed to the defensive actions of LPE towards spoilage microbial species ($P < 0.05$). Venkatachalam (2020) stated that LPE exhibited a strong antimicrobial activity, especially against the pathogenic bacteria and *Enterobacteriaceae*. CHI-Gel coating showed inhibition of *Enterobacteriaceae*. This might be due to the gelatin's excellent oxygen barrier properties. When shrimp's exterior was covered with gelatin, that led to retarded proliferation of aerobic microbes (Chiou*et al.*, 2008). The chitosan's inhibitory effects incorporated with essential oils against *Enterobacteriaceae* in fresh pork patties at 4 °C have also been reported by Venkatachalam and Lekjing (2020).

3.7.4. Psychrotrophic bacterial count

The psychrotrophic bacterial counts of control and coated shrimp samples all through the storage at refrigerated temperature are seen in Fig. 4D. Gram-negative bacteria are the main psychrotrophic spoilage bacteria under cold storage conditions (Raeisi et al., 2015). Until the end of storage, the psychrotrophic bacterial counts gradually increased in all samples ($P < 0.05$). However, a dramatic rise in the psychrotrophic counts was noticed in the control sample, whereas samples coated with CHI-Gel-LPE had the lowest psychrotrophic counts below those of the control, CHI, and CHI-Gel ($P < 0.05$). CHI-Gel-LPE coatings effectively inhibited microbial growth. The lowest psychrotrophic count was observed in samples coated with a high concentration of LPE ($P < 0.05$). The phenolic compounds would perhaps form complexes with proteins in microorganisms' cell membranes, leading to lysis of the cell wall and leakage of intracellular electrolytes and proteinaceous constituents (Venkatachalam and Lekjing, 2020). Numerous studies have reported that the plant extract's antimicrobial activity depends mainly on their inhibitory power on the amylase and proteases in the cell wall (Venkatachalam and Lekjing, 2020). The present study results revealed that the addition of LPE in the CHI-Gel coating strongly controlled the psychrotrophic microorganisms.

4. Conclusion

CHI-Gel edible coating incorporated with LPE had positive effects on melanosis, chemical, microbiological, and sensory properties of black tiger shrimp stored refrigerated for 20 days. The CHI-Gel-LPE coating on the surface of shrimp delayed lipid and protein oxidation. It also inhibited melanosis and bacterial growth. LPE in the coating may enhance the sensory attributes of shrimp and prolong its freshness. The best quality was recorded for shrimp coated with LPE incorporated at the highest tested concentration among the coated samples. Therefore, LPE may serve as an effective melanosis inhibitor, an alternative to sulfiting agents, and function as a natural preservative for shrimp during refrigerated storage.

CRediT authorship contribution statement

Muralidharan Nagarajan: Conceptualization, Methodology, Software, Formal analysis, Writing – original draft. **Bharathipriya Rajasekaran:** Formal analysis, Investigation, Data curation, Writing – original draft. **Soottawat Benjakul:** Validation, Resources, Writing – review & editing. **Karthikeyan Venkatachalam:** Conceptualization, Resources, Writing – review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare there is no conflict of interest.

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