Shelf-life Extension of Refrigerated Hybrid Catfish (*Clarias macrocephalus* × *Clarias gariepinus*) Fillet by Chitosan- and Alginate-Based Edible Coatings

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ABSTRACT

Shelf-life of refrigerated catfish fillets is normally less than four days. In this study, the effects of different edible coatings derived from chitosan and alginate on the quality of refrigerated hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets were investigated for 21 days. Edible coatings studied were chitosan (CS), alginate (AL), chitosan and alginate layer-by-layer (LbL) electrostatic deposition coatings (CSAL and ALCS), and chitosan-alginate polyelectrolyte complex (PEC). All edible coatings showed the benefits of reducing bacterial growth and biochemical reactions of refrigerated catfish fillets during storage ($p<0.05$). The total psychrotrophic bacteria counts for CS- and CSAL-treated samples exceeded 6 log CFU·g^{-1} by 18 days ($p<0.05$), suggesting shelf-life extension of 12 days. CSAL LbL coating was more effective in control of microbial growth and lipid oxidation than ALCS LbL coating ($p<0.05$). The evolutions of total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) of coated catfish fillets were below the recommended thresholds of 35 mg·100 g^{-1} and 5 mg MDA·kg^{-1}, respectively, during storage ($p<0.05$). The overall sensory acceptability scores for CS-, AL-, and CSAL-treated samples were below the rejection criteria by day 15 ($p<0.05$). Based on the results from this study, edible CS and CSAL coatings are recommended for refrigerated storage of catfish fillets.

Keywords: Alginate, Chitosan, Edible coating, Hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*), Shelf-life extension

INTRODUCTION

Hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*), one of the most successful among the interspecific hybrids, has been cultured for more than 30 years in Thailand (Senanan *et al*., 2004; FAO, 2020). Hybrid catfish production in Thailand for 2018 was estimated to be 112,101 tonnes, with a value of 152.9 million USD (FAO, 2020). The majority of cultured hybrid catfish in Thailand were either sold domestically in raw form or exported as chilled and frozen products, with 6,337 tonnes of exports valued at 7.2 million USD in 2017 (FAO, 2019).

It is known that the shelf-life of refrigerated catfish fillets generally is less than four days (Bonilla *et al*., 2019). Although chilled storage of freshly caught fish is normally used to slow microbial growth and biochemical changes, these processes continue to occur (Yu *et al*., 2019; Duarte *et al*., 2020). Among several methods proposed to extend shelf-life of raw fish, bio-based edible coatings have been considered as an effective and environmentally friendly method to assure its safety and quality from catch to consumer (Dehghani *et al*., 2018; Socaciu *et al*., 2018; Sahraee *et al*., 2019; Yu *et al*., 2019; Bharti *et al*., 2020; Umaraw *et al*.,

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2020). Chitosan, a polycationic polysaccharide that is soluble in weak acid, leading to its film-forming property, is the most widely used edible coating material for fishery products due to its strong antibacterial activity, biocompatibility, gas-selective permeability, good adhesion ability, and film-forming characteristics (Wang *et al*., 2018; Ahmed *et al*., 2019; Kulawik *et al*., 2019; Yu *et al*., 2019). In contrast to chitosan, alginate is a polyanionic copolymer, and is also a widely used edible coating. In aqueous solution, the gelling characteristic of alginate is usually induced by calcium ions obtained from calcium chloride (Zhao *et al*., 2010).

Shelf-life extension efficacy of chitosan-based edible coatings on refrigerated fishery products has been reported in a range of three days to more than 10 days, or extended significantly without specific values, depending upon fish species, product variety, coating formulation, and coating method (Bonilla *et al*., 2018; Wang *et al*., 2018; Ahmed *et al*., 2019; Bonilla *et al*., 2019; Kulawik *et al*., 2019; Yu *et al*., 2019). Recent publications on edible chitosan coatings showed effective shelf-life extension of refrigerated catfish (*Ictalurus punctatus*) fillets, and compared aspects of coating method (Bonilla *et al*., 2018) and chitosan type (Bonilla *et al*., 2019). Problems arising from inadequate single coating have been addressed by using multicomponent coatings such as layer-by-layer (LbL) electrostatic deposition coating, which is based on the electrostatic interaction between oppositely charged layers (Poverenov *et al*., 2013; Kim *et al*., 2018; Xie *et al*., 2019). Previous studies on chitosan-alginate LbL electrostatic deposition coating demonstrated effective shelf-life extension of refrigerated shrimp (Kim *et al*., 2018) and fresh cut melon (Poverenov *et al*., 2013). Moreover, chitosan-alginate polyelectrolyte complex (PEC) is formed by an interaction between chitosan's positively charged amino groups and alginate's negatively charged carboxylate groups in aqueous solution (Meng *et al*., 2010; Castel-Molieres *et al*., 2017; Boni *et al*., 2021). Prolonged shelf-life by edible chitosan-alginate PEC coating for raw and cooked pork has been reported (Kulig *et al*., 2017).

In this study, we have employed a combination of chitosan and alginate in the form of LbL electrostatic deposition and PEC coatings in comparison with single chitosan and alginate coatings. To date, these combinations have not been applied for refrigerated hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillet preservation. The objective of this study was to evaluate the shelf-life of refrigerated hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets by a combination of chitosan- and alginate-based edible coatings.

MATERIALS AND METHODS

Materials

Dried shrimp shell was obtained from a food processing plant in Samut Sakhon province, Thailand. High viscosity food grade sodium alginate (Batch no. H051805412) was obtained from Qingdao Bright Moon Seaweed Group Co., Ltd., (Qingdao, China). Plate count agar media was purchased from HiMedia Laboratories (Mumbai, India). Potassium carbonate, boric acid, 2-Thiobarbituric acid, methyl red, and bromocresol green were obtained from Merck KGaA (Darmstadt, Germany). Lactic acid and trichloroacetic acid were obtained from Ajax Finechem (North Ryde, NSW, Australia). Sodium hydroxide, calcium chloride, hydrochloric acid and ethyl alcohol were purchased from VWR (Radnor, PA, USA). All chemicals used in this study were of analytical grade.

Preparation of coating solutions

Chitosan was prepared from shrimp shell chitin by the method of Jampafuang *et al*. (2019) and Basarah *et al*. (2021). Degree of deacetylation was determined to be 93.12 % by the first derivative UV spectrophotometry method (Kiang *et al*., 2004). The MW of chitosan was determined to be 1.04×10^3 kDa by the method described by Jung and Zhao (2012). To obtain 1.5 % w/v chitosan coating solution, 1.5 g shrimp chitosan was dissolved in 1 % v/v lactic acid solution with agitation until complete dissolution.

Sodium alginate coating solution was prepared using the method described by Kim *et al*. (2018) with slight modifications. To obtain 2 % w/v alginate coating solution, 2 g sodium alginate was dissolved in deionized water with agitation at 70 °C for 2 h. To obtain 3 % w/v calcium chloride solution, 3 g calcium chloride was dissolved in deionized water with agitation until complete dissolution.

Chitosan-alginate polyelectrolyte complex (PEC) was prepared by the method described by Wasupalli and Verma (2018) with some modifications. In brief, chitosan solution was mixed with sodium alginate solution at a ratio of 1:1 by adding chitosan dropwise into sodium alginate solution using an ultrasonic processor (VCX130, Sonics and Materials Inc., CT, USA) under continuous stirring for 2 h.

All coating solutions were autoclaved at 121 ºC for 15 min. The zeta potential of coating solutions was determined based on dynamic light scattering principle at 25±0.5 °C using a Zetasizer (Nano-ZS, Malvern Instruments, Malvern, UK).

Fish sample preparation and coating study

Fish were obtained alive from a local market in Bangkok, Thailand. Live fish were chilled in baths of ice. Thereafter fish were filleted, skinned, eviscerated, and packed in a cooler box filled with crushed ice, and transported to the laboratory within 2 h. The catfish fillets were equally divided into eight groups, which were then subjected one of eight soaking treatments: 1) uncoated control (C); 2) sterilized distilled water for 2 min (DW); 3) 1 % v/v lactic acid for 2 min (LA); 4) 1.5 % w/v chitosan for 2 min (CS); 5) 2 % w/v sodium alginate for 2 min, followed by 3 % w/v calcium chloride for 2 min (AL); 6) 1.5 % w/v chitosan for 2 min, followed by 2 % w/v sodium alginate for 2 min and 3 % w/v calcium chloride for 2 min (CSAL); 7) 2 % w/v sodium alginate for 2 min and 3 % w/v calcium chloride for 2 min, followed by 1.5 % w/v chitosan for 2 min (ALCS); and 8) chitosan-alginate polyelectrolyte complex (PEC) for 2 min. All samples were then drained for 2 min and air dried for 2 min, then packed in polyethylene bags and vacuum sealed. Fish samples were taken for quality analysis at 3-day intervals during 21 days of storage at 4±0.5 °C.

Microbial analyses

Microbial characteristics of fish samples were determined in terms of total aerobic mesophilic bacteria count (TMBC) and total psychrotrophic bacteria count (TPBC) by the method described by Kim *et al*. (2018). Briefly, a 5-g fish sample was shaken in 225 mL sterile 0.85 % w/v sodium chloride using a stomacher blender (Stomacher 400 laboratory blender, Seward, UK) for 30 s. Serial dilutions were adjusted, and the pour-plate method was carried out using plate count agar. The inoculated agar plates were incubated at 35 °C for 2 days for TMBC, and at 7 °C for 10 days for TPBC. Microbiological counts were carried out in triplicate.

Physicochemical analyses

Water activity (a_w)

Water activity of the fish samples was determined by benchtop water activity meter (4TEV, Aqualab, Pullman, WA, USA).

pH

To measure pH, a 10-g sample of fish was homogenized in 90 mL deionized water using a disperser (T25 digital Ultra-Turrax®, IKA, Staufen, Germany). Then, pH of the sample was measured using a pH meter (MM-60R TOA DKK, Tokyo, Japan) at room temperature.

Biochemical analyses

Thiobarbituric acid reactive substances (TBARS)

TBARS was quantified by the method described by Zarandona *et al*. (2021) with some modifications. In brief, a 1-g fish sample was mixed with 9 mL of 0.25 N hydrochloric acid solution containing 0.375 % w/v thiobarbituric acid and 15 % w/v trichloroacetic acid. The mixture was incubated at 90 ºC for 10 min, cooled, and then centrifuged at 4,000 g for 20 min. The intensity of supernatant was measured using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) at 532 nm. The value of TBARS was calculated from the malonaldehyde (MDA) standard curve and expressed as mg MDA·kg^{-1} of fish sample.

Total volatile basic nitrogen (TVBN)

TVBN was evaluated by the method described by Conway and Byrne (1936). In brief, a 2-g sample was mixed with 8 mL 4 % trichloroacetic acid and incubated at room temperature for 30 min. Then the mixture was filtered and used for TVBN analysis. TVBN was released after saturated potassium carbonate solution addition and diffused into boric acid solution. The sample was further titrated with 0.02 N hydrochloric acid until the color changed to pink. The value of TVBN was calculated as mg·100 g^{-1} of fish sample.

Sensory evaluation

Sensory evaluation was conducted by seven trained panelists (five females and two males between 22 and 26 years old). The fish fillets were cut into 4×4 cm portions and randomly evaluated for overall acceptability based on a nine-point hedonic scale, ranging from 1 (dislike extremely) to 9 (like extremely). The overall acceptability of fresh samples on day 0 was regarded as the best quality. It was assumed that rejection criteria for shelf-life would be represented by sensory scores below 6.

Statistical analysis

One-way analysis of variance (ANOVA) was performed and Duncan′s multiple range test was used for mean comparison at a significance level of 0.05.

RESULTS AND DISCUSSION

Microbiological analyses

Table 1 summarizes the evolution of mesophilic bacteria of catfish fillets during refrigerated storage. The mean initial load of mesophilic bacteria was 4.03±0.07 log CFU·g^{-1}, similar to previous reports for catfish (Bonilla *et al*., 2018), sea bream (*Sparus aurata*) (Calanche *et al*., 2020), and horse mackerel (*Trachurus trachurus*) fillets (Zarandona *et al*., 2021). On day 6 of storage, TMBC of C and

Table 1. Total mesophilic bacteria count (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	Mesophilic Bacteria (log CFU·g^{-1} sample)							
	0 Day	3 Day	6 Day	9 Day	12 Day	15 Day	18 Day	21 Day
C	3.99±0.00^{bH}	4.79±0.01^{aG}	6.00±0.00^{aF}	6.34±0.01^{aE}	7.18±0.01^{aD}	8.02±0.01^{aC}	8.17±0.02^{aB}	8.35±0.01^{aA}
DW	3.97±0.02^{bH}	4.72±0.01^{bG}	6.00±0.00^{aF}	6.32±0.02^{aE}	7.09±0.02^{bD}	7.38±0.00^{bC}	7.80±0.01^{bB}	8.05±0.01^{bA}
LA	4.06±0.06^{aG}	4.09±0.00^{eG}	4.77±0.01^{dF}	4.94±0.01^{cdE}	5.93±0.01^{fD}	6.23±0.01^{fC}	6.33±0.01^{fB}	6.73±0.01^{dA}
CS	3.94±0.02^{bH}	4.09±0.00^{eG}	4.46±0.01^{gF}	4.72±0.01^{eE}	5.84±0.01^{gD}	6.01±0.01^{hC}	6.08±0.01^{hB}	6.16±0.01^{gA}
AL	4.11±0.08^{aG}	4.32±0.00^{cF}	4.69±0.02^{eE}	4.93±0.01^{cdD}	5.98±0.01^{dC}	6.11±0.01^{gB}	6.29±0.01^{gA}	6.27±0.07^{fgA}
CSAL	4.09±0.01^{aH}	4.31±0.00^{dG}	4.68±0.01^{fF}	4.95±0.01^{cE}	5.98±0.01^{dD}	6.32±0.01^{eC}	6.36±0.01^{eB}	6.56±0.02^{deA}
ALCS	4.09±0.01^{aG}	4.31±0.00^{dF}	4.85±0.01^{cE}	4.93±0.01^{dD}	5.96±0.01^{eC}	6.36±0.01^{dB}	6.37±0.01^{dB}	6.43±0.03^{efA}
PEC	3.96±0.03^{bE}	4.79±0.01^{aD}	5.22±0.00^{bC}	5.35±0.01^{bC}	6.99±0.01^{cB}	7.35±0.01^{cA}	7.37±0.01^{cA}	7.35±0.27^{cA}

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

DW samples reached 6 log CFU·g^{-1}, which is the suggested maximum limit provided by regulations (Calanche *et al*., 2020; Duarte *et al*., 2020). After 15 days, the TMBC of CS-, AL-, CSAL-, and ALCS-treated samples reached beyond 6 log CFU·g^{-1}, but never reached 7 log CFU·g^{-1} during the trial period, which is considered as the acceptance quality limit (Calanche *et al*., 2020; Esteves and Anibal, 2021) during storage. The TMBC of PEC, however, reached 7 log CFU·g^{-1} by day 12. The TMBC of the CS-treated sample was lowest ($p<0.05$), suggesting strong antibacterial activity of chitosan, which has been widely described (Wang *et al*., 2018; Ahmed *et al*., 2019; Kulawik *et al*., 2019; Yu *et al*., 2019; Li and Zhuang, 2020). It is known that antibacterial activity of chitosan and its nanoparticle are affected by several factors such as type of bacteria, zeta potential, concentration, pH, and chitosan physicochemical properties (Chandrasekaran *et al*., 2020). Chitosan with higher zeta potential values has higher bacterial inhibition efficacy (Chang *et al*., 2015; Caetano *et al*., 2016; Chandrasekaran *et al*., 2020). In this study, the zeta potentials of CS and PEC coating solutions were determined to be 69.70±7.42 and 65.04±5.42, respectively. Zeta potential of PEC nanoparticles depends on the total protonated amino groups on chitosan. The magnitude of zeta potential of chitosan and PEC coating solutions was found to be higher than 50 mV, indicating significant antibacterial effect (Yilmaz, 2019; Spirescu *et al*., 2021). The magnitude of zeta potential of chitosan was higher than for PEC, which may explain why CS was more effective than PEC. Similarly, another study on antimicrobial activity of PEC revealed that the antimicrobial activity of PEC was because of chitosan, not alginate (Friedman *et al*., 2013; Kulig *et al*., 2017).

The evolutions of psychrotrophic bacteria of catfish fillets during chilled storage are presented in Table 2. Mean initial load of psychrotrophic bacteria was 3.34±0.31 log CFU·g^{-1}, and gradually increased in a similar manner to the evolution of mesophilic bacteria. TPBC of C and DW samples reached 6 log CFU·g^{-1} within the first six days. After 15 days of storage, the TPBC of AL, ALCS and PEC samples reached beyond 6 log CFU·g^{-1}, but values were still less than 7 log CFU·g^{-1}, suggesting shelf-life extension of at least nine days, while the TPBC of CS and CSAL samples reached beyond 6 log CFU·g^{-1} by 18 days, indicating shelf-life extension of 12 days ($p<0.05$). In this study, CSAL showed better efficacy in growth inhibition of psychotropic bacteria than ALCS ($p<0.05$), which was in good agreement with a previous study

Table 2. Total psychrotrophic bacteria count (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	Psychrotrophic Bacteria (log CFU·g^{-1} sample)							
	0 Day	**3 Day**	**6 Day**	**9 Day**	**12 Day**	**15 Day**	**18 Day**	**21 Day**
C	3.63±0.01^{cG}	4.94±0.03^{aF}	6.07±0.02^{aE}	6.20±0.01^{aD}	6.76±0.03^{aC}	7.10±0.01^{aB}	7.12±0.02^{aB}	7.33±0.01^{aA}
DW	3.03±0.02^{fG}	4.92±0.01^{aF}	6.08±0.01^{aE}	6.40±0.34^{aD}	6.65±0.02^{aC}	6.75±0.03^{bC}	6.98±0.01^{bB}	7.30±0.01^{bA}
LA	3.06±0.02^{eH}	4.35±0.01^{dG}	5.00±0.02^{cdF}	5.07±0.02^{cE}	5.22±0.01^{cdD}	5.81±0.02^{fC}	6.36±0.00^{dB}	6.84±0.01^{dA}
CS	3.06±0.02^{eH}	4.18±0.01^{eG}	4.79±0.03^{fF}	4.88±0.02^{cE}	5.07±0.01^{dD}	5.21±0.01^{gC}	6.18±0.02^{gB}	6.32±0.01^{hA}
AL	3.07±0.01^{eH}	4.64±0.03^{cG}	4.96±0.02^{eF}	5.00±0.01^{cE}	5.35±0.01^{bcD}	6.05±0.01^{dC}	6.30±0.01^{efB}	6.38±0.01^{fA}
CSAL	3.18±0.01^{dG}	4.71±0.03^{bF}	4.98±0.03^{deE}	5.08±0.01^{cD}	5.25±0.14^{cdC}	5.98±0.01^{eB}	6.28±0.01^{fA}	6.36±0.01^{gA}
ALCS	3.76±0.02^{bH}	4.68±0.04^{bG}	5.02±0.01^{cF}	5.08±0.01^{cE}	5.35±0.01^{bcD}	6.10±0.01^{cC}	6.32±0.01^{eB}	6.40±0.00^{eA}
PEC	3.93±0.02^{aF}	4.20±0.02^{eE}	5.18±0.01^{bD}	5.36±0.01^{bCD}	5.53±0.31^{bC}	6.09±0.02^{cB}	6.76±0.03^{cA}	6.92±0.02^{cA}

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

on shrimp (*Litopenaeus vannamei*) stored at 4 °C (Kim *et al*., 2018). The mechanism of action on bacterial growth between bilayer CSAL and ALCS is different. Chitosan more effectively inhibits microbial growth and retards lipid oxidation when compared with alginate (Bonilla *et al*., 2018; Kim *et al*., 2018).

Physicochemical analyses

Water activity is used as an indicator of deterioration susceptibility and shelf-life prediction (Duarte *et al*., 2020). Table 3 presents the effects of different coatings on water activity of hybrid catfish fillets stored at 4 °C. The water activity values of catfish fillets treated with different coatings gradually increased from an average initial value of 0.9916±0.0003 to the maximum value of 0.9941±0.0001 for the control sample ($p<0.05$). The water activity of C- and DW-treated samples increased to a higher extent than those with other coatings ($p<0.05$), indicating the control of moisture migration by edible coatings on the fish surface (Dehghani *et al*., 2018; Socaciu *et al*., 2018; Sahraee *et al*., 2019; Yu *et al*., 2019; Bharti *et al*., 2020; Umaraw *et al*., 2020).

The pH value of fish samples can be used as an indicator of fish freshness, and pH 7 is considered the upper limit for acceptable quality (Abbas *et al*., 2008; Reyes *et al*., 2015; Duarte *et al*., 2020). The pH of fresh meat is neutral in the range 6.6-7.5, which favors growth of most microorganisms (Umaraw *et al*., 2020). The effects of different coatings on pH values of refrigerated hybrid catfish fillets are presented in Table 4. The pH values of all refrigerated catfish fillets gradually increased with an increase in storage period ($p<0.05$). During the 21-day experiment, pH of the control group gradually increased from an average initial value of 6.35±0.03 to a maximum value of 6.67±0.01 ($p<0.05$). The pH of C and DW samples increased to a higher value than those with coatings ($p<0.05$), indicating more non-desirable alkaline compounds generated from endogenous enzymes and spoilage bacteria such as ammonia and trimethylamine (Bonilla *et al*., 2019; Zarandona *et al*., 2021). In the present study, the pH values of lactic acid, chitosan, alginate, and PEC coating solutions were 1.46, 5.12, 3.41, and 3.96, respectively. A decline in pH of catfish fillets due to acidic coating treatments was observed, similar to previous findings, indicating a penetration of soaking solutions into catfish fillet tissues (Bonilla *et al*., 2018; 2019). The pH of chitosan-coated samples has been reported in the range of 6.1-6.7 (Bonilla *et al*., 2018; 2019). The pH of all of our samples during refrigerated storage

Table 3. Water activity values (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	Water activity (a_w)							
	0 Day	3 Day	6 Day	9 Day	12 Day	15 Day	18 Day	21 Day
C	$0.9921±0.00^{aF}$	$0.9922±0.00^{aF}$	$0.9929±0.00^{aE}$	$0.9933±0.00^{aD}$	$0.9935±0.00^{aCD}$	$0.9938±0.00^{aBC}$	$0.9939±0.00^{aAB}$	$0.9941±0.00^{aA}$
DW	$0.9916±0.00^{bcF}$	$0.9918±0.00^{abF}$	$0.9927±0.00^{aE}$	$0.9929±0.00^{bD}$	$0.9932±0.00^{bC}$	$0.9935±0.00^{aB}$	$0.9939±0.00^{aA}$	$0.9940±0.00^{aA}$
LA	$0.9914±0.00^{cdDE}$	$0.9911±0.00^{cE}$	$0.9916±0.00^{bCD}$	$0.9918±0.00^{eC}$	$0.9922±0.00^{eB}$	$0.9925±0.00^{dAB}$	$0.9926±0.00^{dA}$	$0.9927±0.00^{eA}$
CS	$0.9919±0.00^{abE}$	$0.9919±0.00^{abE}$	$0.9919±0.00^{bE}$	$0.9921±0.00^{deD}$	$0.9923±0.00^{eC}$	$0.9926±0.00^{cdB}$	$0.9928±0.00^{cdA}$	$0.9929±0.00^{dA}$
AL	$0.9911±0.00^{dE}$	$0.9912±0.00^{cE}$	$0.9918±0.00^{bD}$	$0.9923±0.00^{cdC}$	$0.9926±0.00^{dBC}$	$0.9929±0.00^{bAB}$	$0.9929±0.00^{bcAB}$	$0.9931±0.00^{cdA}$
CSAL	$0.9913±0.00^{cdC}$	$0.9915±0.00^{bcC}$	$0.9916±0.00^{bC}$	$0.9925±0.00^{cB}$	$0.9929±0.00^{bcA}$	$0.9929±0.00^{bcA}$	$0.9930±0.00^{bcA}$	$0.9932±0.00^{bcA}$
ALCS	$0.9919±0.00^{abBC}$	$0.9912±0.00^{cD}$	$0.9917±0.00^{bC}$	$0.9923±0.00^{cdB}$	$0.9928±0.00^{cdA}$	$0.9929±0.00^{bA}$	$0.9931±0.00^{bA}$	$0.9932±0.00^{bcA}$
PEC	$0.9915±0.00^{bcdE}$	$0.9918±0.00^{abE}$	$0.9919±0.00^{bE}$	$0.9922±0.00^{cdD}$	$0.9926±0.00^{dC}$	$0.9929±0.00^{bcBC}$	$0.9931±0.00^{bAB}$	$0.9933±0.00^{bA}$

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

did not exceed the upper limit (pH 7) for acceptable quality during 21 days. This is consistent with previous reports on chitosan coatings for refrigerated salmon fillets (Xiong *et al*., 2021) and refrigerated catfish fillets (Bonilla *et al*., 2018; 2019).

Biochemical analyses

TBARS is used to evaluate secondary lipid oxidation components, such as aldehydes and ketones (Duarte *et al*., 2020). Table 5 presents the effects of different coatings on TBARS of refrigerated hybrid catfish fillets. TBARS in the control sample rapidly increased during the first six days of storage, and gradually increased thereafter, ranging from a mean initial value of 0.49±0.06 mg MDA·kg^{-1} of fish sample to the maximum value of 1.31 mg MDA·kg^{-1} ($p<0.05$). The TBARS of C, DW, and LA samples were higher than those with bio-edible coatings ($p<0.05$). The TBARS values for all fish samples were found to be far below the acceptance limit of 5 mg MDA·kg^{-1} sample, suggesting the control of lipid oxidation by edible coatings on the surface of fish sample (Dehghani *et al*., 2018; Socaciu *et al*., 2018; Sahraee *et al*., 2019; Yu *et al*., 2019; Duarte *et al*., 2020). In a previous study, the stability of catfish lipid was found to be more stable than lipids of other fatty fish, such as sardines, mackerel, and rainbow trout, resulting in a smaller increase in TBARS (Bonilla *et al*., 2019).

Table 6 demonstrates the effects of different coatings on TVBN of chilled hybrid catfish fillets. TVBN of catfish fillets in the control treatment gradually increased during the first nine days, rapidly increasing from an initial value of 7.80± 0.08 mg·100 g^{-1} fish sample to the maximum value of 43.75±0.01 mg·100 g^{-1} ($p<0.05$). In a similar manner to TBARS, the TVBN values for C, DW, and LA samples were higher than those with bio-edible coatings ($p<0.05$). By day 15 for C-, and by day 18 for DW-treated samples, TVBN reached the shelf-life limit of >35 mg·100 g^{-1}, which is usually regarded as spoiled (Reyes *et al*., 2015; Calanche *et al*., 2020; Prabhakar *et al*., 2020). TVBN was below the acceptance limit (35 mg·100 g^{-1} sample) for CS, AL, CSAL, ALCS, and PEC treatments, implying the control of microbial growth by bio-edible coatings on the fish surface (Dehghani *et al*., 2018; Socaciu *et al*., 2018; Sahraee *et al*., 2019; Yu *et al*., 2019; Duarte *et al*., 2020). TVBN results from degradation of protein and formation of non-protein nitrogenous components by microorganisms (Wu and Bechtel, 2008). The increase of TVBN observed during storage presented a similar profile to that pH value evolution shown in Table 4.

Table 4. pH values (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	pH							
	0 Day	3 Day	6 Day	9 Day	12 Day	15 Day	18 Day	21 Day
C	6.37±0.01^{aG}	6.40±0.01^{bF}	6.50±0.00^{abE}	6.56±0.01^{aD}	6.60±0.00^{aC}	6.63±0.01^{aB}	6.66±0.00^{aA}	6.67±0.00^{aA}
DW	6.36±0.00^{aE}	6.45±0.00^{aD}	6.53±0.04^{aC}	6.55±0.00^{abC}	6.55±0.01^{aC}	6.61±0.01^{aB}	6.64±0.01^{aA}	6.65±0.00^{bA}
LA	6.38±0.04^{aC}	6.42±0.01^{abB}	6.44±0.01^{cB}	6.51±0.01^{abcA}	6.52±0.01^{bcA}	6.52±0.00^{bcA}	6.53±0.00^{bcA}	6.54±0.00^{deA}
CS	6.28±0.03^{bE}	6.30±0.00^{cE}	6.36±0.01^{dD}	6.41±0.01^{dC}	6.43±0.02^{dBC}	6.45±0.00^{dAB}	6.45±0.00^{dAB}	6.47±0.01^{fA}
AL	6.38±0.03^{aC}	6.43±0.04^{abBC}	6.43±0.04^{cBC}	6.48±0.04^{cAB}	6.48±0.04^{cAB}	6.49±0.03^{cAB}	6.52±0.02^{cA}	6.54±0.01^{eA}
CSAL	6.35±0.00^{aE}	6.41±0.01^{abD}	6.41±0.01^{cdD}	6.48±0.04^{cC}	6.51±0.01^{bcBC}	6.53±0.01^{bcAB}	6.54±0.01^{bcAB}	6.55±0.00^{dA}
ALCS	6.36±0.01^{aC}	6.42±0.01^{abB}	6.43±0.04^{cB}	6.50±0.00^{cA}	6.50±0.00^{bcA}	6.53±0.04^{bcA}	6.55±0.01^{bA}	6.55±0.01^{deA}
PEC	6.36±0.00^{aE}	6.43±0.04^{abD}	6.46±0.01^{bcD}	6.51±0.01^{bcC}	6.52±0.02^{bBC}	6.54±0.01^{bABC}	6.55±0.00^{bAB}	6.56±0.00^{cA}

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

Table 5. Thiobarbituric acid reactive substances (TBARS) evolutions (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	TBARS (mg MDA·kg^{-1} sample)							
	0 Day	**3 Day**	**6 Day**	**9 Day**	**12 Day**	**15 Day**	**18 Day**	**21 Day**
C	0.49±0.02^{bE}	0.74±0.01^{abcdD}	1.13± 0.03^{aC}	1.22± 0.03^{aB}	1.27±0.02^{aAB}	1.29± 0.01^{aA}	1.3± 0.01^{aA}	1.31±0.02^{aA}
DW	0.50±0.01^{bE}	0.82±0.02^{aD}	1.09±0.01^{aC}	1.19±0.01^{aB}	1.20±0.01^{bAB}	1.22±0.00^{bAB}	1.24±0.04^{aA}	1.25±0.01^{bA}
LA	0.64±0.04^{aF}	0.78±0.04^{abE}	1.14±0.01^{aD}	1.17±0.03^{aCD}	1.22±0.05^{abBC}	1.23±0.03^{bABC}	1.28±0.01^{aAB}	1.30±0.01^{aA}
CS	0.44±0.01^{bD}	0.78±0.01^{abC}	0.82±0.01^{bBC}	0.80± 0.01^{cC}	0.81± 0.00^{dC}	0.87± 0.04^{eB}	0.92± 0.04^{bA}	0.96±0.00^{eA}
AL	0.46±0.01^{bF}	0.69± 0.04^{dE}	0.86±0.02^{bCD}	0.81± 0.01^{cD}	0.89± 0.01^{cC}	0.90±0.02^{deBC}	0.94± 0.01^{bAB}	0.97±0.01^{eA}
CSAL	0.47±0.01^{bF}	0.77±0.05^{abcE}	0.82±0.04^{bDE}	0.85±0.01^{bcCD}	0.92±0.04^{cBC}	0.94± 0.04^{cdB}	0.96± 0.04^{bB}	1.06±0.01^{cA}
ALCS	0.48±0.04^{bF}	0.68± 0.04^{dE}	0.82± 0.01^{bD}	0.89± 0.02^{bC}	0.91±0.02^{cBC}	0.97± 0.01^{cAB}	0.96± 0.00^{bAB}	1.02±0.04^{dA}
PEC	0.45±0.03^{bE}	0.73±0.01^{bcdD}	0.81± 0.00^{bC}	0.88± 0.05^{bB}	0.89± 0.02^{cB}	0.89± 0.01^{deB}	0.93± 0.02^{bB}	0.99±0.01^{deA}

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

Table 6. Total volatile basic nitrogen (TVBN) evolutions (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	TVBN (mg·100 g^{-1} sample)							
	0 Day	**3 Day**	**6 Day**	**9 Day**	**12 Day**	**15 Day**	**18 Day**	**21 Day**
C	7.79± 0.03^{cdH}	10.38±0.01^{bG}	13.05±0.04^{bF}	15.81±0.05^{aE}	23.65±0.02^{aD}	36.90±0.03^{aC}	43.48±0.05^{aB}	43.75±0.01^{aA}
DW	7.76± 0.02^{deH}	10.34±0.06^{bG}	13.00±0.12^{bcF}	15.67±0.15^{aE}	23.27±0.04^{bD}	30.68±0.05^{bC}	39.57±0.04^{bB}	40.02±0.01^{bA}
LA	7.95±0.02^{aH}	10.67±0.01^{aG}	13.20±0.01^{aF}	15.47±0.04^{bE}	20.89±0.01^{cD}	25.87±0.05^{cC}	35.42±0.01^{cB}	39.62±0.06^{cA}
CS	7.69±0.04^{fH}	7.77±0.01^{eG}	10.25±0.01^{eF}	10.65±0.04^{dE}	12.94±0.03^{gD}	13.29±0.04^{hC}	17.22±0.01^{gB}	19.44±0.03^{gA}
AL	7.86±0.01^{bH}	7.95±0.01^{cG}	10.56±0.02^{dF}	10.67±0.01^{dE}	15.74±0.03^{fD}	18.00±0.04^{gC}	19.80±0.01^{eB}	23.33±0.03^{fA}
CSAL	7.71±0.04^{efF}	7.70±0.01^{fF}	10.53±0.02^{dE}	10.58±0.03^{dE}	15.93±0.03^{eD}	18.43±0.01^{eC}	19.81±0.02^{eB}	23.30±0.04^{fA}
ALCS	7.84±0.01^{bcG}	7.98±0.03^{cF}	10.57±0.03^{dE}	10.64±0.06^{dE}	15.77±0.08^{fD}	18.14±0.01^{fC}	19.61±0.04^{fB}	23.57±0.02^{eA}
PEC	7.82±0.02^{bcG}	7.85±0.03^{dG}	12.92±0.04^{cF}	13.14±0.02^{cE}	20.65±0.02^{dD}	23.74±0.01^{dC}	27.65±0.03^{dA}	27.57±0.03^{dB}

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

Sensory evaluation

Table 7 presents the effects of different coatings on sensory score, indicating the edible coatings lead to longer shelf-life of chilled hybrid catfish fillets. Results showed that sensory scores decreased with an increase in storage time. The overall acceptability scores of C, DW, and LA samples were below the rejection criteria (6 points) by day 6, day 9, and day 12, respectively ($p<0.05$). The overall acceptability scores of ALCS- and PEC-coated samples were below the rejection criteria by day 12 and day 15, respectively, whereas the overall acceptability scores of CS, AL, and CSAL

Table 7. Overall acceptability score (mean±SD) of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fillets stored at 4 °C.

Coatings	Overall acceptability							
	0 Day	3 Day	6 Day	9 Day	12 Day	15 Day	18 Day	21 Day
C	$8.29±0.49^{abA}$	$7.43±0.53^{abB}$	$5.71±0.49^{eC}$	$5.29±0.49^{bD}$	$4.71±0.49^{cE}$	$4.14±0.38^{cF}$	$4.00±0.00^{bF}$	$2.00±0.00^{cG}$
DW	$8.29±0.49^{abA}$	$7.14±0.38^{abcB}$	$6.14±0.38^{deC}$	$5.29±0.49^{bD}$	$4.86±0.38^{cD}$	$4.14±0.38^{cE}$	$4.14±0.38^{bE}$	$2.00±0.00^{cF}$
LA	$8.29±0.49^{abA}$	$7.71±0.49^{aB}$	$7.29±0.49^{aB}$	$6.71±0.49^{aC}$	$6.14±0.38^{abD}$	$5.14±0.38^{bE}$	$4.71±0.49^{aE}$	$3.00±0.00^{bF}$
CS	$8.43±0.53^{aA}$	$7.57±0.53^{abB}$	$7.14±0.38^{abB}$	$6.57±0.53^{aC}$	$6.29±0.49^{aCD}$	$6.00±0.00^{aD}$	$4.86±0.38^{aE}$	$3.86±0.38^{aF}$
AL	$8.14±0.38^{abcA}$	$6.71±0.49^{cB}$	$6.57±0.53^{cdB}$	$6.57±0.53^{aB}$	$6.29±0.49^{aBC}$	$6.00±0.00^{aC}$	$4.00±0.00^{bD}$	$3.86±0.38^{aD}$
CSAL	$8.14±0.38^{abcA}$	$7.00±0.58^{bcB}$	$6.71±0.49^{bcB}$	$6.57±0.53^{aBC}$	$6.14±0.38^{abCD}$	$6.00±0.00^{aD}$	$4.86±0.38^{aE}$	$4.00±0.00^{aF}$
ALCS	$7.57±0.79^{cA}$	$6.71±0.49^{cB}$	$6.71±0.49^{bcB}$	$6.57±0.53^{aB}$	$5.71±0.49^{bC}$	$5.14±0.38^{bD}$	$4.14±0.38^{bE}$	$3.00±0.00^{bF}$
PEC	$7.71±0.49^{bcA}$	$7.43±0.53^{abA}$	$6.71±0.49^{bcB}$	$6.43±0.53^{aB}$	$6.43±0.53^{aB}$	$5.29±0.49^{bC}$	$3.86±0.38^{bD}$	$3.00±0.00^{bE}$

Note: C = Control (uncoated); DW = distilled water; LA = lactic acid; CS = chitosan; AL = alginate; CSAL = layer-by-layer chitosan-alginate; ALCS = layer-by-layer alginate-chitosan; PEC = Chitosan-alginate polyelectrolyte complex; Different lowercase superscripts indicate significant difference between means in the same column; different uppercase superscripts indicate significant difference between means in the same row ($p<0.05$).

coated samples were at the rejection level (6 points) at day 15 ($p<0.05$). As shown in Table 7, CSAL coating was more acceptable than ALCS coating based on sensory evaluation ($p<0.05$). The results of the present study are in good agreement with a previous investigation of chitosan and alginate bilayer coatings on refrigerated shrimp, wherein CSAL coating was preferred over ALCS due to sour odor derived from the chitosan coating (Kim *et al*., 2018). Sensory analysis is commonly used for fish freshness evaluation (Calanche *et al*., 2020; Esteves and Anibal, 2021). The sensory data are corroborated by TVBN values, which indicate fish spoilage (Duarte *et al*., 2020).

CONCLUSION

Edible coating materials derived from chitosan and alginate showed effective control on the surface of refrigerated hybrid catfish fillets, resulting in less moisture movement, less microbial contamination, and less lipid oxidation. Psychrotrophic bacterial counts of coated samples were controlled below the maximum threshold of 7 log $CFU \cdot g^{-1}$ during storage ($p<0.05$). Chitosan and CSAL were the most effective treatments to control microbial growth and lipid oxidation, suggesting shelf-life extension of 12 days of chilled catfish fillets. CSAL LbL coating was more effective in control of microbial growth and lipid oxidation than ALCS LbL coating. Biochemical reactions in terms of TVBN and TBARS were reduced in coated samples, and remained below the recommended thresholds of 35 $mg \cdot 100\ g^{-1}$ and 5 $mg\ MDA \cdot kg^{-1}$, respectively, during storage ($p<0.05$). Different formulations of chitosan-based coatings demonstrated significant differences in control of microbial growth and lipid oxidation of refrigerated catfish fillets. Chitosan-alginate polyelectrolyte complex (PEC) showed less efficacy in control of microbial growth and lipid oxidation than other coatings. Based on the results from this study, edible CS and CSAL coatings are recommended for refrigerated catfish fillet storage.

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