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Original Article

Gelatin chitosan film incorporated with clove essential oil for retaining quality of silver pomfret fish fillet

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Abstract

Clove (*Syzgium aromaticum* L.) essential oil has been reported for its potent antioxidant and antimicrobial activities. In this study, clove essential oil was incorporated with gelatin-chitosan solution to develop an edible film (CEO film) for fish preservation. The objective was to determine the effectiveness of this edible film in controlling physical, biochemical and microbial changes in fillet of silver pomfret (*Pampus argenteus*). The effectiveness of this film was compared with uncoated fillet (control), and gelatin-chitosan film (GC film). The formulated film was tested for water solubility and antimicrobial activity against four selected microorganisms: *Escherichia coli*, *Pseudomonas aeroginosa*, *Salmonella enterica* and *Bacillus cereus*. The effectiveness of the formulated film on the silver pomfret fillet was evaluated based on weight loss, pH, firmness, total volatile basic nitrogen (TVB-N) and total plate count (TPC) of the fish fillet. The CEO films intermediately inhibit the growth of *E. coli* and *S. enterica*. Meanwhile, GC film did not show inhibition on the growth of tested microorganisms. CEO film had lower water solubility compared to GC film. CEO film was observed to reduce weight loss ($p<0.05$), lower pH on day 6 ($p<0.05$), and increase firmness of fish fillet when compared to the control ($p<0.05$). Fish fillet applied with CEO film also had lower TVB-N value and microbial count. This study shows that the CEO film has antimicrobial properties which can benefit fish preservation. Improvement for developing the edible film with acceptable properties is thus important to extend the shelf life of fish fillet.

Keywords: Clove, Essential oil, Edible film, Fish fillet, Shelf life

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Introduction

Silver pomfret (*Pampus argenteus*) is one of the important fish species with high market value (Zhao et al., 2010). The silver pomfret plays an important role in the fisheries sector of many countries in the world including Malaysia, China, India, Thailand, Japan, Korea, Kuwait and Iran (FAO, 1995). Silver pomfret is highly perishable during post-mortem aging. Studies have explored various approaches on extending shelf life of fish and fishery products, including packaging techniques, edible film and utilization of low storage

temperature (Lakshmanan et al., 2002; Fan et al., 2009; DeWitt and Oliveira, 2016).

Spices have been largely utilized in food as flavoring agents as well as food preservatives and this is attributed to its antioxidant and antimicrobial activities. Clove (*Syzygium aromaticum* L.) in particular has been reported for its potent antioxidant and antimicrobial activities (Shan et al., 2005). Their applications as preservatives in food or antiseptics and disinfectants have been widely investigated. Essential oil fractions are found to contain active compounds that provide valuable antimicrobial activity, against food-borne pathogens and spoilage bacteria (Gutierrez et al., 2008). Clove essential oil which contains mainly eugenol was reported as an effective inhibitor of pathogenic bacteria in previous studies (Cressy et al., 2003; Mytle et al., 2006). Edible films incorporated with various essential oils were shown to have antimicrobial property against foodborne pathogens (Fernández-Pan et al., 2012).

This study aims to identify the effect of gelatin-chitosan films incorporated with clove essential oil in controlling physical, biochemical and microbial changes of silver pomfret fillet.

Material and Methods

Film formulation

Gelatin from bovine-hide in combination with chitosan (Guinama, Valencia) were used to develop the film forming solution as previously described in Gomez-Estaca et al. (2010). For gelatin-chitosan film forming solutions, 6 g of gelatin (dissolved in distilled water) and 1 g of chitosan (dissolved in 0.15 M acetic acid) per 100 ml of film forming solution were used. Sorbitol and glycerol (0.15g/g biopolymer, gelatin plus chitosan) were added as plasticizers, then warmed and stirred at 45°C for 15 min. In preparation of the clove-added film forming solution, food grade clove essential oil was added at the proportion of 0.75 ml/g biopolymer. Soya lecithin (0.125 g/g biopolymer) was added as an emulsifying agent. The clove-added film forming solution was homogenized with an Ulraturrax blender (position 5, 1 min). 5ml of the solutions were casted in a petri dish and dried at 45°C in a forced-air oven for 15 h to yield a film with uniform thickness of 200 µm. Two different types of films were then obtained: a gelatin-chitosan film (GC) and a gelatin-chitosan clove-added film (CEO).

Water solubility

Films portions measuring 1 cm^2 was placed in aluminum capsules with 15 ml of distilled water and gently shaken at 22°C for 15 h. The solution was then filtered using Whatman no. 1 filter paper to recover the remaining undissolved film. The undissolved film was then desiccated at 105°C for 24 h. Film solubility is calculated according to Gómez-Estaca et al. (2010) using the following equation:

$$\text{Film solubility, FS}(\%) = \left(\frac{W_0 - W_f}{W_0}\right) \times 100$$

Where

W_0 = initial weight of the film expressed as dry matter

W_f = weight of the undissolved desicated film residue

Antimicrobial activity measurement

The formulated films were tested for antimicrobial activity over four microorganisms: *Eschericia coli*, *Pseudomonas aeroginosa*, *Bacillus cereus*, and *Salmonella enterica* according to Duan et al. (2010) with modifications. Spread plates of BHI Agar were inoculated with 100 µl of the respective bacteria and grown overnight (108 CFU/ml). Then, circular pieces of the each formulated film (1.5 cm in diameter) were laid onto the surface of the inoculated plate. After incubation, the observed inhibition zones-surrounding clear areas were measured for indication of its antimicrobial activity.

Fish storage trial

Silver pomfret (*P. argenteus*) were cut in fillets (5 cm x 4 cm) and divided into three groups which are uncoated, coated with gelatin-chitosan film (GC) and coated with the gelatin-chitosan films containing clove essential oil (CEO). All fillets were then vacuum-packed in HDPE and stored at 2°C for 8 days. All treatments were done in triplicate. The uncoated and coated fillets were then measured for its physical, chemical and microbiological changes.

Weight loss of fish fillet

The weight of uncoated, GC and CEO fillets were aseptically measured at an interval of two days until the end of storage period. The weight loss were then calculated and presented as percentage of weight loss.

pH of fish fillet

Approximately 5-10 g of fish fillet was homogenized with a double quantity of distilled water. After 5 min at ambient temperature, the pH was determined. pH

value was measured at an interval of two days until the end of storage period.

Firmness of fish fillet

Textural properties of the fish fillet were assessed using the puncture test with a TA-XT2 Texture Analyser. The maximum force required to puncture the fish fillets or films was recorded. Compression applied using P2N (needle) at a speed of 1.0 mms^{-1} was used for puncturing the plugs position over a 3-mm hole. Three replicates were analysed for each treatment. Firmness of all fillets was measured at an interval of two days until the end of storage period.

Total volatile basic nitrogen (TVB-N) of fish fillet

Total volatile basic nitrogen (TVB-N) was assessed according to Cobb III et al. (1973) with some modification. Fish fillet (1 g) was loaded into Kjeldahl-type distillation tube and added with 12 ml of concentrated sulphuric acid for protein digestion along with one tab of Kjeltabs Cu-3.5. After digestion, 40% sodium hydroxide (NaOH) solution (50 ml) was added and followed by 75 ml of distilled water. The distillation was performed and the distillate was collected into a conical flask filled with 3% boric acid solution and methyl red and bromocresol green indicator. The solution was then titrated with 0.05 N hydrochloric acid (HCl). TVB-N was presented as nitrogen per 100 g calculated with the following equation:

$$\text{Nitrogen} = \frac{\text{titre volume x } 0.05 \text{ x } 14}{\text{weight of sample in grams}}$$

TVB-N of fillet for uncoated, GC and CEO coated fillet were determined at the last two days of the storage period.

Microbiological count of fish fillet

Microbial count was determined through total plate count. A total amount of 10 g of fish fillet were placed in a sterile plastic bag with 90 ml of buffered 0.1% peptone water in a vertical laminar-flow cabinet. Appropriate dilutions were prepared and spread onto PCA agar, which was then incubated at 35°C for 24 h. All microbiological counts were expressed as the log of the colony-forming units per gram (log CFU/g) of sample. The analysis was performed at the end of the storage period. Analysis was done in triplicates.

Statistical analysis

Data analysis was carried out using SPSS version 20.0. The differences of mean among treatment were analyzed using one-way analysis of variance (ANOVA) and Tukey test, with a level of significance at $p<0.05$.

Results and Discussion

Water solubility of formulated fish film

Figure 1 shows the solubility of the two formulated edible films (gelatin-chitosan film, GC and gelatin-chitosan incorporated with clove essential oil, CEO). GC film showed a higher solubility value of (76.5%) than the CEO film (8.98%). This indicated that the CEO film is better in terms of potential protection for the fish fillet. Edible film with low solubility enhances product integrity and its water resistance (Perez-Gago and Krochta, 1999). Films solubility is often designed to be of low solubility to aid in protecting product from humidity and water loss (Gontard et al., 1993). The conjoint hydrophobic property of oil and gelatin could enhance the hydrophobicity of our CEO formulated film (Ahmad et al., 2012).

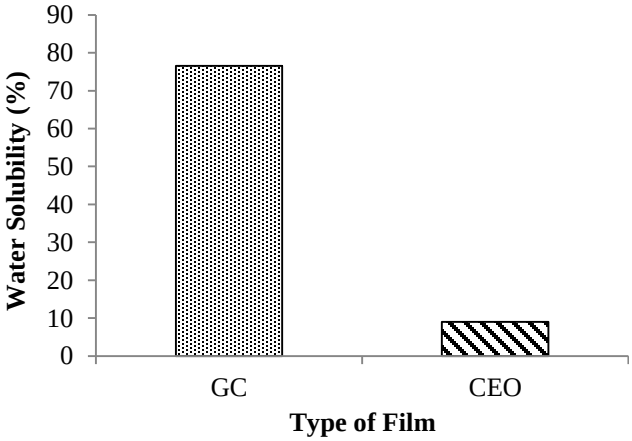

Fig. 1. Water solubility of formulated film

Antimicrobial activity of formulated fish film

Table 1 summarizes the results from antimicrobial test of formulated film against selected microorganism. GC film did not show any inhibition on growth of selected microorganism (*Eschericia coli* 2922, *Pseudomonas aeroginosa, Bacillus cereus*, and *Salmonella enterica*). CEO film showed an intermediate inhibition against growth of *E.coli* 2922 and *S. enterica*. Components of clove oil were reported to have efficiency in inhibiting growth of microorganisms (Burt, 2004). Clove oil has chemical

compositions characterized by terpenoids (eugenol) which are active against a broad range of microorganism (Dorman and Deans, 2000). Eugenol was reported to suppress the growth of *L.monocytogenes, C. jejuni, S. Enteritidis, E. coli* and *S. aureus* (Cressy et al., 2003).

Table 1. Antimicrobial activity of formulated film

Type of film	*Escherichia coli*	*Bacillus cereus*	*Salmonella enterica blue*	*Pseudomonas aeroginosa*
GC	X	X	X	X
CEO	✓	X	✓	X

* ✓ : inhibition, x: no inhibition zone

Effect of formulated films on weight loss of fish fillet

Figure 2 illustrates the percentage of weight loss in uncoated, GC and CEO coated fillet. Both GC and CEO coated fillet had a significantly reduced percentage of weight loss when compared to uncoated fillet ($p<0.05$). Moreover, addition of clove oil (CEO) showed a tendency for a lesser weight loss in the fish fillet when compared to GC, but there was no significant difference among the two treatment groups.

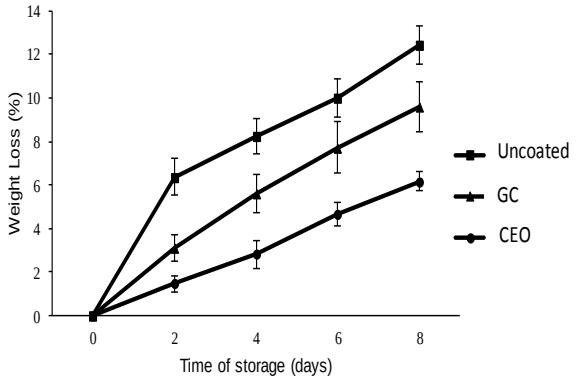

Fig. 2. Effect of different treatments on weight loss of silver pomfret fish fillet (data are presented as mean ± standard deviation)

Apart from storage temperature, postharvest treatment is known to influence the rate of weight loss (Hernández-Muñoz et al., 2006). Coating was previously reported to be effective in reducing water loss and in prolonging shelf life of fish fillets (Jeon et al., 2002). Incorporation of essential oil into coating such as chitosan-oregano oil coating was reported to be effective in lowering weight loss in fruit (Wu et al., 2016) which supported our observation in this study. Oregano oil with combination of oxygen absorber was also found to reduce weight loss in rainbow trout (Mexis et al., 2009). Coating may act as an effective barrier to oxygen, carbon dioxide and water vapour transmission which helps reduce the loss of weight in produce (de Aquino et al., 2015).

Effect of formulated films on pH of fish fillet

Figure 3 shows the effect of different treatments (uncoated, GC and CEO coating) on the pH of the fish fillets. There was a tendency for lower pH value in fillet coated with GC and CEO when compared to uncoated fillet from day 2 and 4 after treatment, but no significant difference were detected between the treatment groups. On day 6 and 8 after treatment, the CEO coated had a significantly lower pH value when compared to the uncoated fillet ($p<0.05$). The increase of pH could be a result of accumulation of alkaline compounds which includes ammonia and trimethylamine which are produced during spoilage of fish (Ruiz-Capillas and Moral, 2005; Özyurt et al., 2009). Therefore, the decrease in pH of CEO film coated fillet indicated an inhibition of fish spoilage.

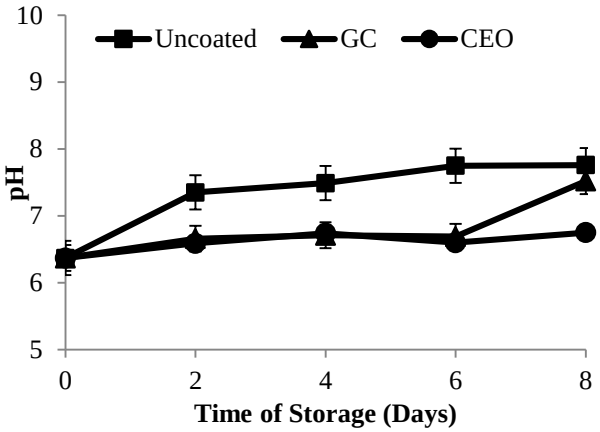

Fig. 3. Effect of different treatments on pH of silver pomfret fish fillet (data are presented as mean ± standard deviation)

Effect of formulated films on texture changes of fish fillet

Figure 4 shows the effect of different treatments (uncoated, GC and CEO coating) on the firmness of the fish fillets. No significant difference was observed

between treatments, although there was a slight tendency for a slower reduction in firmness of CEO coated compared to uncoated fish fillet.

Autolytic enzymes reduces textural quality during early stages of deterioration when spoilage characteristics such as off-odors and off-flavors are not yet prevalent (Hansen et al., 1996). Microbial action, proteolytic activity and water loss in the muscle could result in degradation in protein which thereafter affects the texture loss in the fish meat (Suárez-Mahecha et al., 2007; Pacheco-Aguilar et al., 2008). Softening of the fish meat is a critical quality deterioration of fish meat that would largely affect consumer's acceptance.

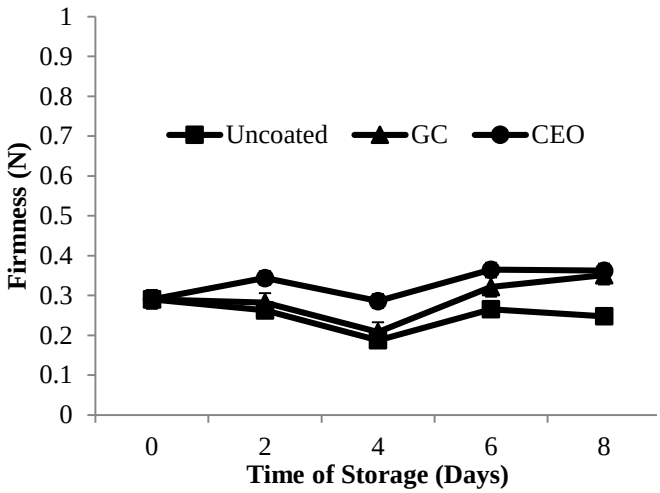

Fig. 4. Effects of different treatments on firmness of silver pomfret fish fillet (data are presented as mean ± standard deviation)

Effect of formulated films on TVB-N content of fish fillet

Table 2 shows the increase of TVB-N content in fish fillet during the last 2 days of storage with different treatments (uncoated, GC, CEO coating). CEO coated fish fillet showed the lowest TVB-N increase when compared to GC and uncoated fish fillet ($p<0.05$). Rotting fish has an enhanced level of TVB-N specifically its major constituents which are ammonia, dimethylamine and trimethylamine. During fish spoilage, bacterial deamination and autolytic breakdown of adenosine monophosphate occur which produces ammonia (Shakila et al., 2003). Antibacterial properties of clove essential oil may influence the lower TVB-N content in samples treated with CEO (Burt, 2004).

Ojagh et al. (2010) reported similar results on the effect of chitosan coating added with essential oil on rainbow trout meat.

Table 2. Increase of TVB-N content in silver pomfret fish fillet during the last 2 days of storage with different treatments

Treatments	Differences in TVB-N value from day 6 to day 8 (mg/100g)
Uncoated	4.2
GC	1.07
CEO	0.45

Effect of formulated films on total plate count of fish fillet

Table 3 shows the effect of different treatments (uncoated, GC, CEO coating) on total plate count of fish fillets. The result indicated a reduced microbial growth in fillet coated with CEO film compared to the GC film and uncoated fillet. In a previous study, incorporation of essential oil into gelatin-chitosan film was reported to reduce the growth of microorganism in fishes (Gomez-Estaca et al., 2010). In another study, a coating made up of chitosan and gelatin also showed an inhibitory effect on the gram-negative microbes of fish flesh (L´opez-Caballero et al., 2005), which supports our finding.

Table 3. Total plate count of silver pomfret fish fillet at the end of storage with different treatments

Treatment	Average
	Day 8 (cfu/g)
Uncoated	TMTC
GC	TMTC
CEO	1.91×10^9

* TMTC: too many to count

Conclusion

In conclusion, the addition of clove essential oil to formulation of gelatin-chitosan film allowed the attainment of edible films with antimicrobial properties and low water solubility. Gelatin-chitosan film incorporated with clove oil reduced the weight loss and the microbial growth of the fish fillet. The application of this edible film also delayed the increase of TVB-N value indicating a slower rate of spoilage. The effects provided by the formulated edible film can help in prolonging shelf life of the fish fillet.

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Contribution of Authors

Mubarak A: Design of work, data analysis and interpretation, drafting and critical revision of the article and final approval of the version to be published.
Othman ZS: Data collection, data analysis, data interpretation and drafting of the article.
Karim NU: Data analysis, data interpretation and drafting of the article.

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