Effects of chitosan and ascorbic acid coating on the chilled tilapia fish (Oreochromis niloticus) fillet

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Abstract. The effect of chitosan-based coating containing ascorbic acid (AA) for shelf-life extension of chilled (4 °C) tilapia fish fillet was evaluated over a 15-day duration of storage. A 3 X 3 Factorial Design comprising three concentrations of ascorbic acid (0, 2.5 and 5% w/v) and three concentrations of chitosan (1, 1.5 and 2% w/v) were used. The fish fillets were analyzed for aerobic plate count, lipid peroxidation, aw and pH changes throughout the duration of storage. The shelf-life of coated fillets (1.5 and 2%) was lengthened up to 15 days as compared to uncoated one (less than 6 days). The lipid oxidation of fillet with chitosan and AA (2C-5AA) was reported to be four times lower than that of the uncoated sample. The pH and aw of fish fillet coated with chitosan were lower than that of uncoated sample. The addition of ascorbic acid in chitosan coating further improved the oxidation inhibition by giving a lowered pH and aw changes for the duration of the storage. In conclusion, 2% chitosan coating added with 5% AA was the most effective coating to enhance the shelf life of chilled tilapia fish fillet.

1. Introduction

Fish are highly perishable due to the high amount of moisture content, as well as large quantities of free amino acids and volatile nitrogenous bases compared with other meats [1]. Chilled fish are extremely perishable food commodities which undergo rapid quality deterioration after harvesting. The spoilage of chilled fish is a complex process resulting from the composite activities of autolysis, spontaneous chemical reactions, bacterial attack and leaching by ice-melt water [2]. Enzymatic and chemical reactions are usually responsible for the initial loss of freshness, whereas microbial activity is responsible for the obvious spoilage and thereby establishes product shelf life [3].

Chitosan is a modified natural carbohydrate polymer derived by deacetylation of chitin from crustacean shells. It has attracted much attention as a natural food additive due to its non-toxic nature, antibacterial and anti-oxidative activity, film-forming ability, biocompatibility and biodegradability. Due to its intrinsic antimicrobial properties and good film-forming ability, chitosan has been used as active antimicrobial coatings and films. Chitosan-based film was reported to be effective to improve the shelf-life of fish [4-8]. Since fish contain large amount of polyunsaturated fatty acids, they are highly susceptible to oxidation and hence rancidity after prolonged storage [9]. The addition of antioxidant compounds in edible coating had been reported effective in reducing the lipid oxidation of fish [10].

Tilapia (Oreochromis niloticus) is one of the main farmed fresh water fish in Malaysia. Similar to other fish, rapid quality deterioration, short shelf life and potential food safety risk are the major challenges of the fish during retailing. Application of edible coating using natural additive on tilapia fillet offers the benefits of active packaging where tilapia can be marketed as minimally processed food, which is in high demand by today’s health-conscious consumers. Active edible coating also reduces the food waste and loss of profit to the industry. The objective of this study was to investigate the potential of chitosan and ascorbic acid as an edible coating in preservation of chilled tilapia fresh
2. Materials and Methods

2.1. Experimental design
Factorial Design (3 x 3) consisted of three levels of ascorbic acid (0, 2.5 and 5% w/v) and three levels of chitosan concentrations (1, 1.5 and 2% w/v) were used. The uncoated sample was used as a control. The assessments were carried out at 3-day intervals to determine the quality for 15 days at 4 °C. All analysis was performed in triplicate. The experiment was carried out from April to August, 2018.

2.2. Preparation of sample
The fish (*Oreochromis niloticus*) was purchased from the local market (average weight of 750 – 800 g) and transported to the laboratory (Microbiology Laboratory and Biochemistry Laboratory, Faculty of Food Science and Nutrition, University Malaysia Sabah) in an insulated box (covered with sufficient ice) within 2 hours post mortem. The fish was filleted using common household method to obtain two fillets from each fish. Chitosan solutions were prepared using low molecular weight chitosan (Sigma 448869) in 1% acetic acid [11]. L-Ascorbic acid (Sigma W210901) was added accordingly to achieve the required concentration. Fillet samples were randomly assigned into the treatment groups by dipping method for 5 min. After that, the fillets were allowed to drain on a pre-sterilized metal net to form the edible coatings and stored at 4°C for subsequent quality assessment.

2.3. Microbial analysis
1 g of sample was aseptically transferred into sterile stomacher bag and homogenized in 9 mL of sterile peptone for 1 min [12]. Tenfold serial dilution of fish homogenates were used for enumeration of bacterial using nutrient agar incubated at 37°C (48 hours) for Aerobic Plate Counts (APC).

2.4. Determination of thiobarbituric acid reactive substances (TBARS)
10 g of homogenised tilapia flesh was mixed with 2.5 mL of 4 M HCl and 97.5 mL of distilled water and then heated with steam distillation. After that, 5 mL of distillate was added to 5 mL of thiobarbituric reactive reagent (0.02 M TBA in 90% glacial acetic acid) and incubated in boiling water for 35 min. The mixture was cooled down and the absorbance of the supernatant was measured using a spectrophotometer (Perkin Elmer, Lambda 35) at 532 nm. The constant 7.8 was used to calculate the TBA number [13]. The TBA value was expressed as mg malonaldehyde equivalents/kg sample.

2.5. Determination of pH
5 g of sample was homogenized with 50 mL of distilled water and measured using pH meter (F20 Meter, Mettler-Toledo) at ambient temperature.

2.6. Determination of A_w
Measurement was carried out by using 2 g of sample with a water activity meter (HygroLab 3, Rotronic) at ambient temperature.

2.7. Statistical analysis
One-way Analysis of Variance (ANOVA) and Tukey Test were performed using Statistical Package for the Social Sciences (SPSS) 21 to compare the mean values at 5% confidence level.

3. Results and Discussion

3.1. Aerobic Plate Count (APC)
The initial microbial load of tilapia fillets ranged between 4.88 ± 0.04 to 5.52 ± 0.15, increase of varying degree of viable count was seen at the end of refrigeration storage (Table 1). The most rapid bacteria growth was observed in control with an increase of 3.2 log from 5.52 log cfu/g on day-1 to 8.72 log cfu/g on day-15, which exceeded the upper acceptability limit for fresh water and marine
species (7 log cfu/g) [14]. The control was found to have an acceptable level of up to 6 days of storage, whereas all the chitosan coated fillets were safe for up to 15 days except sample 1C (up to 12 days). This data indicated that the shelf life of chilled tilapia fillet can be doubled by using 1% chitosan coating alone. The antimicrobial activity of chitosan-based coating in fish is well established in the literature [15]. Antimicrobial action of chitosan can be related to disruption of the lipopolysaccharide layer of the outer membrane of gram-negative bacterial and its function as a barrier against oxygen transfer. Another mechanism of action is by formation of an impermeable layer around the cell preventing the transport of essential solutes and inhibition of the RNA and protein synthesis by permeation into the cell nucleus [16]. Increased concentration of chitosan further delayed the bacterial growth whereby the final increment was recorded as 2.03 log, 1.80 log and 1.52 log for 1%, 1.5% and 2% of chitosan respectively. Higher concentration of chitosan solution produced thicker film with higher barrier and mechanical properties [17] and hence offers more effective antimicrobial ability.

**Table 1.** Change in Aerobic Plate Count (APC) of tilapia fillet with different treatments during refrigerated storage at 4 ± 1°C.

| Treatment   | Day-0  | Day-3  | Day-6  | Day-9  | Day-12 | Day-15 |
|-------------|--------|--------|--------|--------|--------|--------|
| Control     | 5.52 ± 0.15 | 6.02 ± 0.15 | 6.43 ± 0.23 | 7.10 ± 0.30 | 7.77 ± 0.18 | 8.72 ± 0.42 |
| 1C          | 5.17 ± 0.22 | 5.82 ± 0.13 | 6.07 ± 0.08 | 6.30 ± 0.17 | 6.42 ± 0.13 | 7.20 ± 0.28 |
| 1.5C        | 5.10 ± 0.04 | 5.56 ± 0.21 | 5.89 ± 0.11 | 6.18 ± 0.23 | 6.38 ± 0.06 | 6.90 ± 0.64 |
| 2C          | 5.18 ± 0.05 | 5.48 ± 0.22 | 5.85 ± 0.19 | 6.16 ± 0.08 | 6.28 ± 0.10 | 6.70 ± 0.25 |
| 1C-2.5AA    | 5.17 ± 0.06 | 5.51 ± 0.09 | 5.83 ± 0.18 | 6.01 ± 0.28 | 6.43 ± 0.32 | 6.62 ± 0.21 |
| 1C-5.5AA    | 5.06 ± 0.08 | 5.43 ± 0.12 | 5.73 ± 0.08 | 6.02 ± 0.07 | 6.18 ± 0.05 | 6.45 ± 0.18 |
| 2C-2.5AA    | 5.00 ± 0.30 | 5.40 ± 0.12 | 5.67 ± 0.02 | 5.96 ± 0.06 | 6.13 ± 0.08 | 6.43 ± 0.06 |
| 1C-5AA      | 5.06 ± 0.07 | 5.29 ± 0.11 | 5.58 ± 0.10 | 5.57 ± 0.21 | 6.04 ± 0.24 | 6.24 ± 0.24 |
| 1.5C-5AA    | 4.83 ± 0.06 | 4.92 ± 0.13 | 5.41 ± 0.23 | 5.53 ± 0.16 | 5.74 ± 0.18 | 6.02 ± 0.15 |
| 2C-5AA      | 4.88 ± 0.04 | 4.99 ± 0.13 | 5.24 ± 0.16 | 5.11 ± 0.15 | 5.62 ± 0.21 | 5.80 ± 0.05 |

Abbreviation C denotes chitosan and AA denotes ascorbic acid. The figure attached to the abbreviation denotes the concentration used; e.g. 1C-5AA denotes coating with 1% (w/v) chitosan and 5% (w/v) ascorbic acid. Values represent means ± SE of three replicates.

Incorporation of ascorbic acid (AA) in the chitosan solution further enhanced the prevention of bacterial growth along with the increase of its concentration. The highest reduction of APC was observed for 2C-5AA, in which the bacteria count only raised 0.92 log in 15 days of refrigeration. The addition of various kinds of active compound in chitosan for preserving tilapia fillets was found effectively to retard the microbial growth during storage (Table 2). Similar to AA used in this study, these additives are additional hurdles incorporated to improve the protection effect of chitosan. In line with this, the shelf life of chilled tilapia fillets was significantly extended to be at least 15 days, 20 days or 30 days (Table 2). Apart from the active compounds involved, the difference in shelf life found may be attributed to the variances in the initial microbial load of the fish fillets, thickness of the coating used, dimension of fish fillets used and other non-identical experimental settings employed by different groups of researcher.

### 3.2. Lipid oxidation

The rancidity development of tilapia fillets was determined by quantifying the content of malonaldehyde (MDA), which is the main compound in lipid oxidation. Lipid oxidation in the fish product may lead to off-flavour, colour, and odour changes and eventually texture deterioration [21]. The initial TBA values were the same for all the fillets (p > 0.05). Continual increase in TBA values was observed along 15 days of storage (Table 3) due to partial dehydration and oxidation of unsaturated fatty acid in the fillet [22]. The TBA value for control elevated drastically after 6 days of storage. Based on the limit for acceptable level of oxidation for fish (5 mg/kg of MDA) [23], the shelf-
life for control and the other three chitosan coated samples (without AA) was the same (p > 0.05), only 3 days. As a good barrier for oxygen, chitosan film slowed down the diffusion of oxygen to the surface of tilapia fillets and hence retarded the lipid oxidation. The anti-oxidative activity of chitosan was further enhanced by its chelating ability with ferrous ions in fish proteins and binding capacity with lipid [24].

**Table 2.** Shelf life of chilled fresh fish fillets preserved by chitosan-based coating.

| Type of fish       | Type of coating                                                                 | Storage condition | Shelf life* (day) | Reference |
|--------------------|---------------------------------------------------------------------------------|-------------------|-------------------|-----------|
| Tilapia **(Oreochromis niloticus)** | Without coating                                                                 | Ice storage       | 12                | [7]       |
|                    | 0.5% chitosan                                                                   |                   | > 15              |           |
|                    | 0.5% chitosan + 0.5% or 1% Jicama starch                                         |                   |                   |           |
| Tilapia **(Oreochromis niloticus)** | 2% chitosan                                                                     | Ice storage       | 15                | [18]      |
|                    | 2% chitosan + 0.125 or 0.25% carvacrol essential oil                            |                   | > 20              |           |
| Tilapia **(Oreochromis niloticus)** | Without coating                                                                 | 4°C               | 15                | [19]      |
|                    | 2% (w/v) chitosan                                                               |                   | > 30              |           |
|                    | 2% (w/v) chitosan + 0.5% or 1.0% or 1.5% or 2.0% pomegranate peels extract     |                   |                   |           |
| Tilapia **(Oreochromis niloticus)** | 1% chitosan (fresh fillet)                                                      | 4°C               | > 30              | [20]      |
|                    | 1% chitosan (liquid smoked fillet)                                              |                   |                   |           |

* Based on Aerobic Plate Count

**Table 3.** Effects of chitosan and ascorbic acid coating on lipid oxidation (TBA value) of tilapia fillet during storage at 4 ± 1°C.

| Treatment                        | Day-0      | Day-3      | Day-6      | Day-9      | Day-12     | Day-15     |
|----------------------------------|------------|------------|------------|------------|------------|------------|
| Control                          | 3.52 ± 0.67a | 4.59 ± 1.86a | 6.84 ± 1.19a | 12.66 ± 1.62a | 15.60 ± 1.09c | 16.26 ± 1.01a |
| 1C                               | 3.06 ± 0.71a | 3.60 ± 0.71a | 6.46 ± 0.42bc | 7.22 ± 0.86b  | 10.16 ± 0.60d | 11.77 ± 1.55c |
| 1.5C                             | 2.50 ± 0.32a | 4.03 ± 1.26a | 5.41 ± 0.93bde| 7.67 ± 0.15b  | 9.70 ± 0.75d  | 10.42 ± 1.06c  |
| 2C                               | 3.26 ± 0.91a | 4.54 ± 1.24a | 5.59 ± 0.25cde| 7.34 ± 1.57b  | 9.14 ± 1.50d  | 10.23 ± 1.11de |
| 1C-2.5AA                         | 2.86 ± 0.83a | 3.91 ± 1.35a | 4.54 ± 0.37abcd| 5.95 ± 0.57bc | 7.21 ± 1.80bc | 9.25 ± 0.24de |
| 1.5C-2.5AA                       | 3.56 ± 0.45a | 3.79 ± 0.76a | 4.25 ± 1.11abc | 5.47 ± 0.32bc | 6.20 ± 1.07abc | 8.97 ± 0.26cd |
| 2C-2.5AA                         | 2.22 ± 0.05a | 3.20 ± 0.66a | 3.55 ± 1.01abc | 4.06 ± 0.45a  | 6.19 ± 0.94bc | 6.73 ± 0.80bc |
| 1C-5AA                           | 2.32 ± 0.18a | 2.92 ± 0.94a | 3.08 ± 0.33a  | 3.84 ± 1.00a  | 5.42 ± 0.74b  | 6.72 ± 1.36bc |
| 1.5C-5AA                         | 3.04 ± 0.77a | 3.15 ± 0.65a | 3.35 ± 0.50a  | 3.94 ± 1.48a  | 4.86 ± 0.55ab | 5.90 ± 0.91ab |
| 2C-5AA                           | 2.00 ± 0.27a | 2.51 ± 0.48a | 2.54 ± 0.45a  | 3.65 ± 0.42a  | 3.65 ± 1.41a  | 3.69 ± 0.39a |

Abbreviation C denotes chitosan and AA denotes ascorbic acid. The figure attached to the abbreviation denotes the concentration used; e.g. 1C-5AA denotes coating with 1% (w/v) chitosan and 5% (w/v) ascorbic acid.

Values represent means ± SE of three replicates.

Means with identical alphabet within the same column indicates insignificant difference (p < 0.05).

Meanwhile, addition of AA in chitosan coating enhanced the protection of fish fillets over lipid oxidation (p < 0.05); results obtained also exhibits decreased of lipid oxidation with increasing concentration of AA. AA is a well-known natural antioxidant in which it can scavenge singlet oxygen...
and reduce oxygen- and carbon-centred radicals [25]. The protection effect was further strengthened when higher concentration of chitosan was used in coating solution, e.g. the level of lipid oxidation for 1C-5AA, 1.5C-5AA and 2C-5AA was reported to be acceptable as 9 days, 12 days and 15 days respectively. Marginal level of lipid oxidation was reported for 2C-5AA throughout 15 days of chilling. Combination of chitosan and AA was also reported to be more effective in preventing the lipid oxidation of frozen Atlantic salmon fillet up to 5 months as compared to chitosan alone [26].

3.3. Water activity
Figure 1 displays water activity ($A_w$) of tilapia fillets during refrigerated storage. Increase of $A_w$ with storage can be related to the denaturation of myosin during rigor that resulted in the loss of water holding capacity and thus higher drip loss [14]. The use of edible coating was able to minimize the changes of $A_w$ with composite coatings (1.5C-5AA and 2C-5AA) being more effective than chitosan coating alone. Kester and Fennema [27] have reported that chitosan coatings may function as moisture-sacrificing agents instead of moisture barriers, thus, moisture loss from the product could be delayed until the moisture contained within the chitosan coating had evaporated. Chitosan-Jicama starch coating was also reported to retard the drip loss of fresh tilapia fillets during ice storage [7].

3.4. pH
In general, the pH values for all the samples rose with refrigerated duration (Figure 2). Since acidic chitosan solutions were used for coating the fish fillets, treated samples were found to have lower pH than control ($p<0.05$, data not shown). Addition of AA further reduced the pH values of the samples and hence contributing to more effective preservation effect. Increasing pH during storage may be correlated to the accumulation of basic compounds released by either endogenous or microbial enzymes actions [7] [28]. Thus, ability to maintain the pH of fish fillets at lower value indicates better preservation ability, which is again shown by coating consisted with higher concentration of chitosan and AA. Lower pH of the coated samples can enhance microbial inhibition and contribute to extend the preservation of fish samples by inhibiting the activity of the endogenous proteases [29]. Rainbow trout fillet coated with chitosan and sumac extract (with improved shelf life) was also reported to exhibit lower pH value than the control during refrigerated storage (4°C) [6].
4. Conclusions
The current study concluded that composite coating consists of chitosan and ascorbic acid is more effective to extend the shelf-life of chilled tilapia fish fillets when compared to chitosan alone. Addition of AA improved the efficiency of chitosan film in delaying the microbial growth and particularly in postponing the lipid oxidation. Profound preservation effects were seen in proportional to the amount of chitosan and ascorbic acid used in the coating solution. Based on the APC and TBA values, the uncoated chilled tilapia fillet has a shelf-life less than 6 days, with the shelf-life being extended to at least 15 days when coated with 2% chitosan and 5% AA.

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Acknowledgement
The authors wish to thank Malaysia Ministry of Higher Education for funding this project under Niche Research Grant Scheme (NRGS0006).