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CASSAVA STARCH-BASED EDIBLE COATINGS ENRICHED WITH *Alpina purpurata* AND *Kaempferia rotunda* ESSENTIAL OILS FOR PATIN FILLETS PRESERVATION

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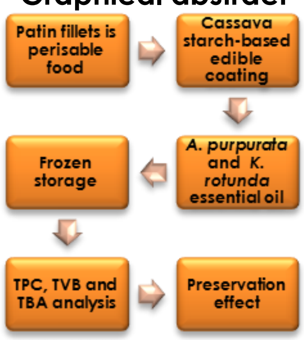
Graphical abstract



Abstract

Cassava starch–based edible coating enriched with essential oil of *Alpinia purpurata* and *Kaempferia rotunda* was applied to preserve patin fillets during frozen storage. The quality of fillets was analyzed based on microbiological and chemical properties. Different concentration of essential oil in distilled water, 0 % (control), 0.1 % and 1 % (v/v), was added in cassava starch–based edible coating. The addition of essential oil significantly inhibited the microbial growth by reducing protein deterioration and delaying lipid oxidation in fillets during 4 mo of frozen storage. The results indicates that essential oil of *Alpinia purpurata* and *Kaempferia rotunda* can be used as an alternative preservation as it could protect frozen fish fillets from deterioration and extend the shelf life.

Keywords: *Alpinia purpurata* (Vieill.) K. Schum, edible coating, essential oil, *Kaempferia rotunda* L., preservation

1.0 INTRODUCTION

Patin or catfish fish [*Pangasius sutchi* (Fowler, 1937)] is one of economically valuable cultivated fish in Indonesia. According to statistical data of Marine Affairs and Fisheries of Indonesia [1], the production of cultivated patin was the second highest in Indonesia at the period of 2008 to 2012 and is estimated to keep increasing as one of export commodities. In international markets, patin fish is available at various products including portions, steaks, fillets and others as it can improve the value of products [2]. Patin fillets have substituted the other white fleshed fish in the western markets [3]. However, fish is a perisable food due to the enzymatic, chemical oxidation and microbial activity [4].

Freezing is common preservation method to prolong fish shelf life with minor changes. However, microbial growth and chemical reactions were not completely inhibited, thus, promoted fish deterioration [5]. Combination of several preservation methods can be used as an alternative method to get longer shelf life [6].

Shelf life extension of frozen fish were reported by using edible coatings. Several studies have shown that edible coatings which was made from different coating materials could maintain fish quality and extend fish shelf life. Whey protein-based coating have been applied to preserve kilka fish [7–10] and Atlantic salmon [11] while chitosan-based coating to silver carp [5] and Atlantic salmon [12]. Beside, one of potential edible coating materials is cassava starch. Cassava starch-based edible coating is isotropic, odorless, tasteless, colorless, non-toxic, biologically degradable, have good flexibility and low water permeability [13].

Fortification of some active substances have been done to improve edible coatings characteristics and enhance the protective properties. Omega-3 fatty acids-fortified chitosan films application could be used to extend frozen lingcod shelf life and fortify omega-3 fatty acid [14]. Natural antioxidants from barley husks coated on low density polyethylene film could retard lipid damage of frozen Atlantic salmon [15], Atlantic halibut [16], and blue shark [17]. Addition of microbial transglutaminase on heated whey protein coatings delayed lipid oxidation of frozen Atlantic salmon [18].

Edible coatings incorporated with essential oils also have been used in food preservation [19]. Cinnamon oil incorporated on chitosan coating improved the storage quality of frozen rainbow trout [20]. Essential oils of *Alpinia purpurata* (Vieill.) K. Schum. and *Kaempferia rotunda* (Linnaeous) enrichment on cassava starch-based edible coating could maintain refrigerated patin fillets for several days [21, 22]. However, the addition of those essential oils on cassava starch-based edible coatings have not been further explored to extend frozen patin fillets shelf life. Preservation effect of essential oil is related to the antimicrobial and antioxidant activity of the chemical compounds. Cineole compound from *Alpinia purpurata* essential oil was related with the antimicrobial activity against *Bacillus cereus* (Frankland & Frankland 1887), *B. subtilis* (Ehrenberg 1835) Cohn, 1872*,* and *Pseudomonas aueruginosa* (Schröter, 1872) [23]. The free radical scavenging activity of *Alpinia purpurata* essential oil had been reported [24]. The main component of *Kaempferia rotunda* essential oil is benzyl benzoate [25] while tetradecane and benzyl benzoic also have been reported as the major compounds of *Kaempferia rotunda* essential oil [26]. Hence, this research aimed to investigate the preservative effects of *Alpinia purpurata* and *Kaempferia rotunda* essential oils enrichment on cassava starch-based edible coating to frozen patin fillets.

2.0 MATERIAL AND METHOD

2.1 Fish Fillets and Essential Oil Preparation

After quarantined for one day, fresh patin were prepared by removing the head, inner organs, removing the skin, making it a fillet, and cleaning on site. Skinless fillets were transported to the laboratory in a cooler box. Essential oils of *Alpinia purpurata* and *Kaempferia rotunda* were produced by water-vapor distillation. Prior to distillation, rhizomes were sliced (2 to 3) mm and air dried at room temperature for 4 h.

2.2 Coating Solution Preparation and Coating Aplication

The formulation and preparation of cassava starch-based edible coating solution was based on previous procedure [22]. Coating formula consisted of 5 g of cassava starch, 100 mL of distilled water and 2 mL of glycerol as plasticizer. Distilled water and cassava starch were mixed and heated at 60 °C for gelatinisation. Glycerol were added to the previous mixture and heated at 60 °C for 30 min. Each essential oil was mixed after the last heating of the solution. Different concentration of each essential oil enrichment were studied (0 %, 0.1 %, and 1 % (v/v of distilled water)).

The fillets were straightly dipped into solution and dried in drying box for 10 min at 70 °C. Coated samples were packed in sealed polyethylene frozen bag, placed at styrofoam box and stored at (-10 ± 2) °C for 4 mo. Samples were analyzed microbiologically and chemically at (0, 1, 2, 3, and 4) mo of storage. All samples were made in duplicates. Total plate count (TPC) value representing the microbial quality, were expressed as log CFU g^{-1} and were analyzed based on previous procedure [22]. Total volatile bases (TVB) value representing the protein deterioration, were expressed as mg N 100 g^{-1} and were analysed following Conway micro-diffusion method described in Indonesian National Standard SNI 2354.8:2009 [27]. Thiobarbituric acid (TBA) value representing the lipid oxidation, were expressed as mg malonaldehyde kg^{-1} and were determined following previous procedure [28].

2.3 Statistical Analysis

A completely randomized design was used. The data were subjected to one way analysis of variance (ANOVA) at the level of $p < 0.05$ in order to determine significant differences as a result of essential oil concentration and duration of frozen storage. Data were statistically evaluated using SPSS version 16 for Windows.

3.0 RESULTS AND DISCUSSION

3.1 Total Plate Count

The initial total plate count of samples ranged from 2.40 log CFU g^{-1} to 3.05 log CFU g^{-1} indicating good fillets quality (Figure 1). During frozen storage for 4 mo, TPC of all samples increased significantly ($P < 0.05$). Microorganisms grew faster in control samples, and reached 6.78 log CFU g^{-1} at the end of storage (4 mo). Essential oils enrichment on edible coating solution reduced the TPC in fillet samples ($P < 0.05$), from 1.44 log CFU g^{-1} to 1.9 log CFU g^{-1} (21 % to 28 %) by *Alpinia purpurata* and 0.77 log CFU g^{-1} to 0.84 log CFU g^{-1} (11 % to 12 %) by *Kaempferia rotunda*. After 4 mo frozen storage, the TPC values of 0.1 % and 1 % of *Alpinia* *purpurata* essential oil treatment samples still below the maximum acceptability limit (6 log CFU g^{-1})[29] which were 5.34 log CFU g^{-1} and 4.88 log CFU g^{-1}, respectively. This condition also occured in application of 1 % of *Kaempferia rotunda* essential oils treatment samples that showed 5.94 log CFU g^{-1} of TPC value. This indicated that essential oil of *Alpinia purpurata* and *Kaempferia rotunda* addition on coating solution could inhibit microbial proliferation *Alpinia purpurata* and *Kaempferia rotunda* essential oil enrichment on cassava starch-based edible coating also generated microbial inhibition of refrigerated patin fillets. Enrichment of 1 % of *Alpinia purpurata* essential oil maintained TPC value of patin fillets at 5.58 log CFU g^{-1} while control samples reached 6.06 log CFU g^{-1} after 8 d of storage [21]. In adequate concentration (1 %), addition of *Kaempferia rotunda* essential oil on edible coating solution also significantly inhibited microbial growth compared to that of the control treatment [22] of refrigerated patin fillets. Antimicrobial activity of *Alpinia purpurata* and *Kaempferia rotunda* essential oils contributed with this microbial growth inhibition.

Alpinia purpurata essential oil could inhibit the growth of pathogenic and food spoilage by bacteria with the inhibition zone between 7.25 mm to 11.17 mm [30]. Antimicrobial mechanism of cineole as the mayor compound of *Alpinia purpurata* essential oil related to its hydrophobicity. Cineole has prominent outer membrane disintegration and nucleus cytoplasm concentration or reduction [31]. Benzyl benzoate was reported as the main component of *Kaempferia rotunda* essential oil [25]. Benzyl benzoate arrested *C. albicans* cells at G2-M phase and induced apoptosis. Terpenoids including benzyl benzoate induced membrane fluidization, and modulating functions of membrane bound proteins involved in signaling and transport [32]. Essential oils enriched on edible films also could inhibit the growth of *Pseudomonas putida* and *Pseudomonas flourescens* as meat spoilage microorganisms with the inhibition zone 23.5 mm and 24.2 mm by *Alpinia purpurata* essential oils [24] and 29.44 mm to 29.99 mm by *Kaempferia rotunda* essential oils [33], respectively.

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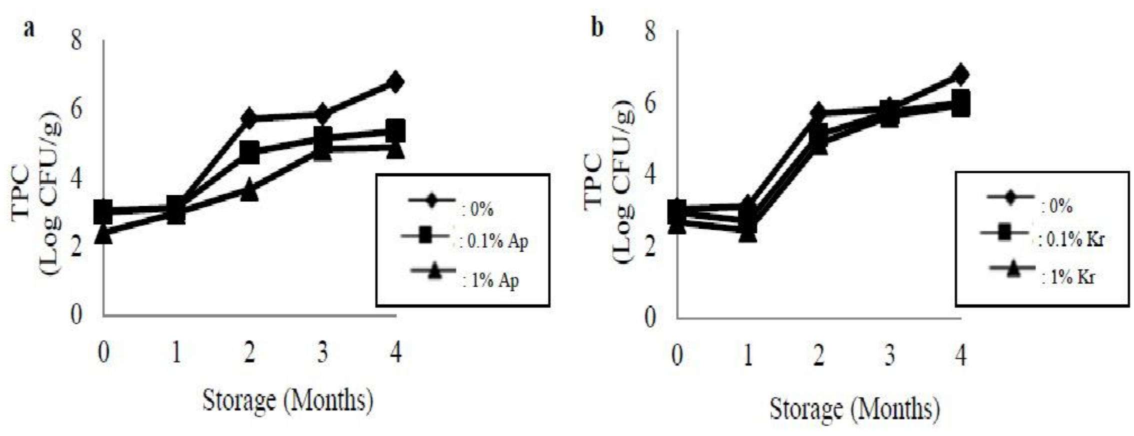

Figure 1 The effect of edible coating enriched with (a) *Alpinia purpurata* (Ap) (b) *Kaempferia rotunda* (Kr) essential oil on total plate count (TPC) values of patin fillets during storage at (-10 ± 2)°C

3.2 Total Volatile Bases

At the beginning of storage, TVB values ranged from (15.43 to 20.39) mg N·100 g^{-1} and significantly ($P < 0.05$) increased in all samples at various rate during frozen storage (Figure 2). According to limit of acceptability of fish regarding of TVB value (30 mg N 100 g^{-1}), the control sample exceeded the limit level and reached 32.98 mg N·100 g^{-1} after 2 mo of storage while all essential oil treated samples remained below maximum standard after 4 mo of storage (25.46 to 28.95) mg N 100 g^{-1}. TVB values represented the nitrogenous materials which resulted from proteins degradation of fish due to the action of proteolitic bacteria [6]. Antimicrobial activity of *Alpinia purpurata* and *Kaempferia rotunda* essential oil is related to the microbial growth inhibition. Microbial growth inhibition leads the reduction of the nitrogenous materials formation and lowering of the TVB values. In this study, the results of TVB values was compatible with the TPC values.

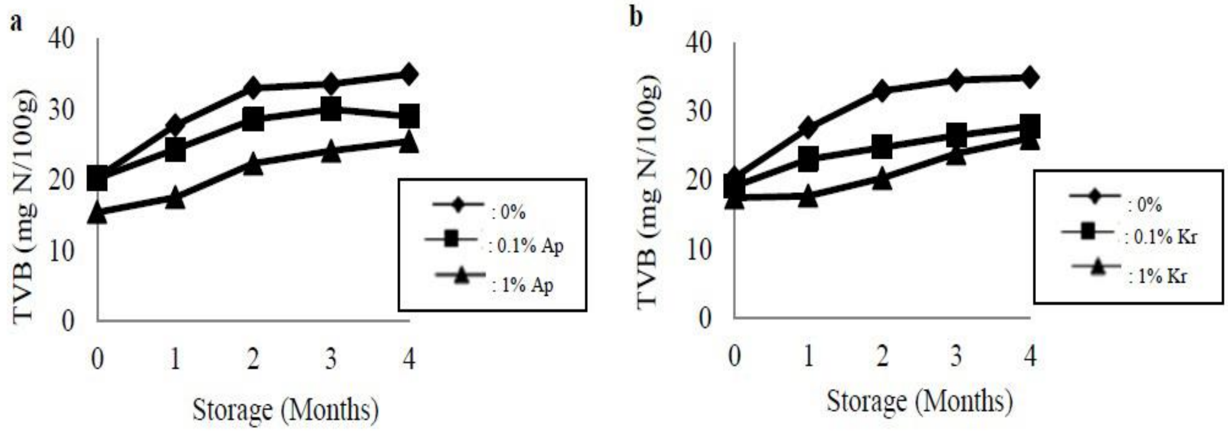

Figure 2 The effect of edible coating enriched with (a) *Alpinia purpurata* (Ap) (b) *Kaempferia rotunda* (Kr) essential oil on total volatile bases (TVB) values of patin fillets during storage at (-10 ± 2) °C

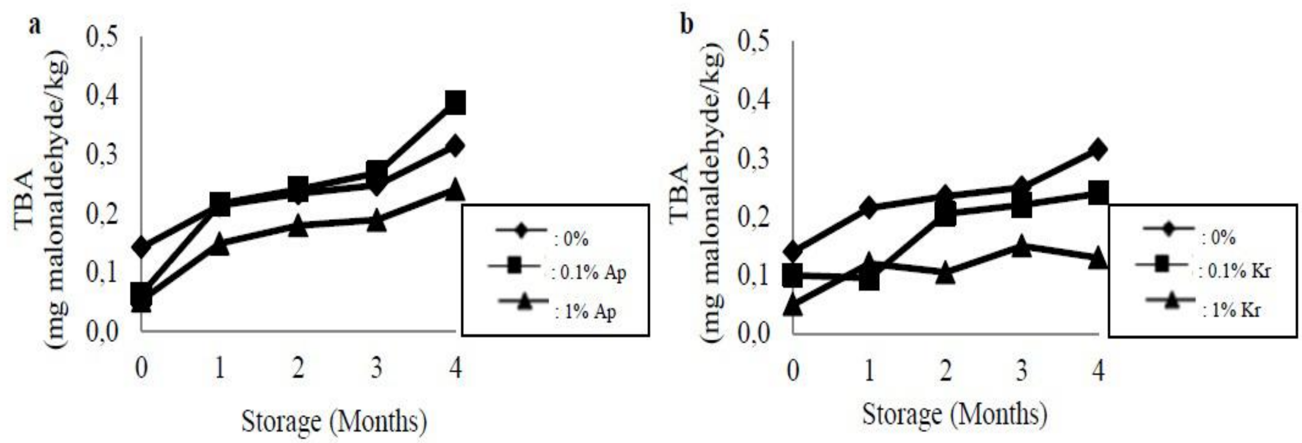

Figure 3 The effect of edible coating enriched with (a) *Alpinia purpurata* (Ap) (b) *Kaempferia rotunda* (Kr) essential oil on thiobarbituric acid (TBA) values of patin fillets during storage at (-10 ± 2)°C

3.3 Thiobarbituric Acid

Lipid oxidation of fish can be represented by the TBA value. A constant increased of TBA value was observed in all samples during storage. The addition of *Alpinia purpurata* and *Kaempferia rotunda* essential oils on cassava starch-based edible coating solution reduced the value of TBA in comparison to control samples. After 4 mo of frozen storage, TBA value of control sample significantly ($P < 0.05$) higher (0.32 mg malonaldehyde kg^{-1}) than those of 1 % essential oil treatment samples which were 0.24 mg malonaldehyde kg^{-1} for *Alpinia purpurata* and 0.13 mg malonaldehyde kg^{-1} for *Kaempferia rotunda* essential oil (Figure 3). No significant differences was obtained from TBA values in control samples and 0.1 % of each essential oil treatment samples which indicated that the delaying of lipid oxidation only occured at adequate essential oil concentration. Lipid oxidation protection of *Alpinia purpurata* and *Kaempferia rotunda* essential oils is most likely due to the antioxidant activity of its chemical compunds which were cineole [34] for *Alpinia purpurata* and benzyl benzoate [35] for *Kaempferia rotunda*. However, TBA values of all samples were lower than the maximum level of acceptability limit (1 to 2) mg malonaldehyde · kg^{-1} [36].

Several studies also reported the lowering of frozen fish lipid oxidation. Lipid oxidation was observed on frozen rainbow trout with chitosan coating enriched by cinnamon oil. The phenolic compounds of cinnamon oil that showed antioxidant and radical scavenging activity contributed to the mechanism of oxidative stability [20]. Antioxidant activity of natural substance from barley husks is related with the reduction of thiobarbituric acid concentration of frozen Atlantic salmon, Atlantic halibut, and blue shark which coated on low density polyethylene films. The higher concentration of natural antioxidant coated on films generated a greater lipid oxidation protection [15–17].

4.0 CONCLUSION

Enrichment of *Alpinia purpurata* and *Kaempferia rotunda* essential oil on cassava starch-based edible coating solution confirmed protection towards fish fillet samples against microbial growth, protein deterioration (the nitrogenous materials formation from proteins degradation) and lipid oxidation during frozen storage. Increasing essential oil concentration showed an increase in maintaining fish quality. In conclusion, the method of patin fillets coating treatment by cassava starch-based edible coating enriched with *Alpinia purpurata* and *Kaempferia rotunda* essential oils can be used as preservation method to extend shelf life.

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