

The effect of cassava starch-based edible coating enriched with *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oil on refrigerated patin fillets quality

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Abstract

The effects of cassava starch-based edible coating enriched with *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oil on patin fillets quality during 8 days refrigerated storage were investigated to determine their ability to extend fish shelf life. Fish quality was determined based on microbiological (Total Plate Count/TPC) and chemical (Total Volatile Bases/TVB, Thiobarbituric acid/TBA and pH) properties. Concentration of each *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oil was varied at 0.1% and 1% while without essential oil (0%) was named as control treatment. The results indicated that essential oil enrichment on edible coating were able to maintain the patin fillet's quality. Based on microbiological properties, patin fillet's quality only could be maintained by applying 1% essential oils during 8 day storage. Furthermore, 1% essential oils treatment also could retain patin fillet's quality longer than others treatments based on chemical properties. Therefore, *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils enriched on cassava starch-based edible coating could extend patin fillet's shelf life and use as an alternative fish preservation.

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Introduction

Patin is one of the potential types of fish in Indonesia. Based on Indonesian MMAF report, the production of Patin increased up to 54.41% from 2007 to 2011 (MMAF, 2011). Patin can be processed by filleting the meat or making it into surimi products (Suryaningrum, 2008). However fish has short shelf life due to enzymatic, chemical reaction and microbial proliferation (Ghaly *et al.*, 2010).

Previous methods have been developed to extend fish shelf life. Chilling is one of the effective methods to maintain fish quality (Opara *et al.*, 2007). Applying chilling temperatures for various fish fillets preservation such as rainbow trout (Chytiri *et al.*, 2004), wild turbot (Ozogul *et al.*, 2006), haddock (Olafsdottir *et al.*, 2006), *Pangasius sutchi* (Abbas *et al.*, 2006), sea bream (Senso *et al.*, 2007), hybrid catfish (Chomnawang *et al.*, 2007), cazon (Ocaño-Higuera *et al.*, 2009), meagre (Hernández *et al.*, 2009), grouper (Sharifian *et al.*, 2011) and ray fish (Ocaño-Higuera *et al.*, 2011) have been reported.

Furthermore, to extend refrigerated fish fillets shelf life, natural preservative treatments also have been evaluated. Shelf life extension of refrigerated fish fillets were reported by the using of liquid smoke (Siskos *et al.*, 2007), thyme powder (Attouchi and Sadok, 2010), extract of seaweed (Husni *et al.*, 2013) and rosemary or garlic (Guerrero *et al.*, 2011),

essential oil of thyme, rosemary (Albarracín *et al.*, 2012) and *Zataria multiflora boiss* (Rahimabadi *et al.*, 2013) and essential oil compounds such as thymol and carvacrol (Mahmoud *et al.*, 2004).

Kaempferia rotunda and *Curcuma xanthorrhiza* spices are the members of *Zingiberaceae* family which are traditionally used in Indonesia (Atun *et al.*, 2013; Husein *et al.*, 2009). The main component of *Kaempferia rotunda* essential oil is benzyl benzoate (Woerdenbag *et al.*, 2004). Sulianti and Chairul (2005) also reported that the major compounds of *Kaempferia rotunda* essential oil are tetradecane and benzyl benzoic. While, Helen *et al.* (2012) stated that essential oil of *Curcuma xanthorrhiza* contains xanthorrhizol, camphene, curcumin, pinene, α -thujene, β -pinene, myrcene, linalool and zingiberane. This essential oil has also antimicrobial activity which is against pathogenic bacteria. Due to those compounds, *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils can be used as a natural preservative.

However, the application of essential oils on foods have limitation because of their strong flavour which could have negative impact to consumers acceptability. The interesting method to minimize that problem is incorporation of essential oil into edible coating (Sa'nchez-Gonza'lez *et al.*, 2011; Hyldgaard *et al.*, 2012). Essential oil of clove (Gómez-Estaca *et al.*, 2010), cinnamon (Lu *et al.*, 2010; Ojagh *et al.*,

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2010), thyme (Chamanara *et al.*, 2012), lemongrass (Ahmad *et al.*, 2012) and *Zataria multiflora* Boiss (Rahimabadi *et al.*, 2012) have been enriched in edible coating and applied to extend fish shelf life at chilling storage.

Among sources of edible coating materials, starch have been considered one of the most promising materials due to its similar physical characteristics to synthetic polymers such as transparent, odorless, tasteless, semi-permeable to CO₂ and resistant to O₂ passage (Vásconez *et al.*, 2009), low cost, renewability and biodegradability (Veiga-Santos *et al.*, 2007). Cassava, the second largest agricultural commodity of Indonesia (FAO stat, 2011), is one of the main sources of industrial starch. Cassava starch indicated as potential starch material for edible coating because cassava starch-based edible coatings are isotropic, odorless, tasteless, colorless, non-toxic, biologically degradable, have good flexibility and low water permeability (Maran *et al.*, 2013).

The use of cassava starch-based edible coating which incorporated with *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils could be a method to extend refrigerated patin fish fillets shelf life. Therefore, the objective of this study is to investigate the effect of enrichment of *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils on cassava starch-based edible coating to Patin's quality during storage at 4 ± 1°C. This investigation was shown by the measurement of microbiological and chemical properties of fish during storage. Finally, the ability of *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils to extend fish shelf life will be determined.

Materials and Methods

Fish

Fresh Patin (approximately 200 - 300 g) were obtained from Lembah Hijau Multifarm (Sukoharjo, Indonesia). Being quarantined for one day, fish were beheaded, gutted, filleted, skinned and cleaned at the farm. Skinless fillets were immediately transported to the laboratory in a cooler box within 10 minutes.

Preparation of coating solution and treatments of fish fillets

Coating solution was obtained from cassava starch edible coating. Edible coating formula were 5 g cassava starch, 100 ml distilled water, and 2 ml glycerol. Edible coating solution were prepared by dissolving cassava starch on distilled water at 60°C heating temperature on a hotplate and then stirred until the mixtures became clear. Glycerol then added to the mixtures and followed by heating at 60°C during 30

minutes. Each essential oil was mixed after the last heating of solution. The applied of each *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oil concentrations were 0.1% and 1%. No essential oil addition edible coating was used as control treatment. Patin fish were dipped in each coating solution and dried at drying box. Coated fillets were placed at styrofoam plates, wrapped by wrapping plastic, and stored at refrigerator (4 ± 1°C) for 8 days. Samples were analyzed microbiologically and chemically at 0, 2, 4, 6 and 8 days of storage.

Microbiological analysis

Total plate count (TPC) was determined by analyzing 10 g samples of patin fillets. Aseptically minced fillets were homogenized with 90 ml of sterilized 0.85% NaCl saline. After serial dilution in the same saline solution (9 ml), 1 ml diluted samples were plated in duplicate plate count agar (PCA) (Merck). The inoculated plates were incubated at 37°C for 2 days. TPC was expressed as log₁₀CFU/g.

Chemical analysis

Chemical analysis of fish fillets were determined to evaluate the changes of fish quality according to chemical spoilage properties such as total volatile base (TVB), thiobarbituric acid (TBA) and pH values. TVB value was analyzed by Conway micro-diffusion method which was described in Indonesian National Standard SNI 2354.8:2009 (SNI, 2009). TVB value performed in mg N/100 g of fish. TBA value was determined according to the method which was described by Apriyantono *et al.* (1989) and expressed as mg malonaldehyde/kg of fish. pH was measured using pH meter after sample had been homogenized in distilled water in same ratio (AOAC, 1995).

Statistical analysis

All experiments were used completely randomized design and were replicated twice. Data were subjected to one way analysis of variance (ANOVA) at 0.05 significance level and differences in the mean values were determined with Duncan's test (p < 0.05) by SPSS Statistics 16 program.

Result and Discussion

Microbial analysis

The initial TPC in patin fillets ranged from 3.11 - 5.22 log₁₀CFU/g (Figure 1). During storage at 4 ± 1°C for 8 days, TPC of all samples increased significantly (p < 0.05). At the end of storage (day 8), TPC of 1% essential oil of *Kaempferia rotunda* and *Curcuma xanthorrhiza* samples were 5.91 log₁₀CFU/g (Figure 1a) and 5.50 log₁₀CFU/g (Figure 1b), respectively.

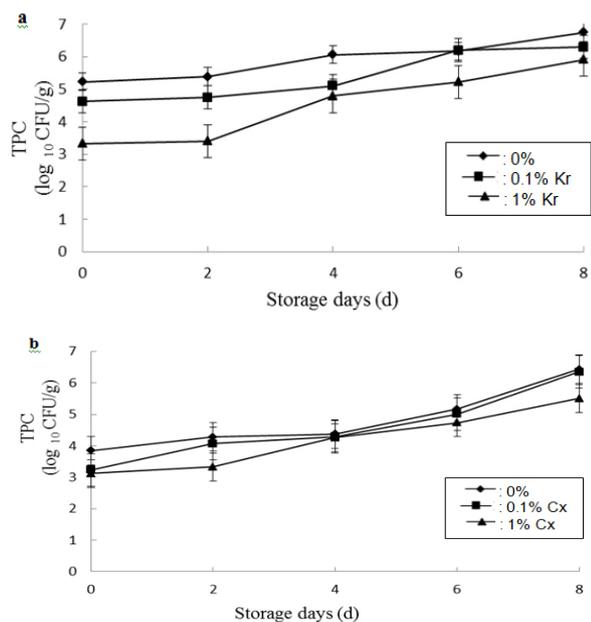


Figure 1. The effect of edible coating enriched with (a) *Kaempferia rotunda* (Kr) (b) *Curcuma xanthorrhiza* (Cx) essential oil on total plate count (TPC) values of patin fillets during storage at $4 \pm 1^\circ\text{C}$. Mean \pm standard deviation of the values (two replicates) are presented.

Those TPC values still below the maximum acceptable limit $6 \log_{10}\text{CFU/g}$ (Mahmoud *et al.*, 2006). While, TPC of the control and 0.1% samples ranged from 6.29 - $6.87 \log_{10}\text{CFU/g}$ and exceeded the standard level. This indicated that *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oil enrichment on edible coating solution in adequate concentration could inhibit microbial growth.

Similar result found that cinnamon oil enriched on alginate-calcium coating solution could inhibit the increasing of bacterial growth on fresh northern snakehead fish fillets during refrigeration storage. Total bacterial of treated sample was $5.27 \log_{10}\text{CFU/g}$ while that of control sample reached $8.10 \log_{10}\text{CFU/g}$ (Lu *et al.*, 2010). Incorporation of clove oil on gelatin-chitosan film also significantly ($p < 0.05$) inhibit microbial growth on cod fillets (Gómez-Estaca *et al.*, 2010). Furthermore, in refrigerated rainbow trout fillets, total viable count remained below $6 \log_{10}\text{CFU/g}$ because of clove oil enrichment on chitosan coatings after 16 days chilling storage (Ojagh *et al.*, 2010). Ahmad *et al.* (2012) also reported that gelatin film incorporated with lemongrass essential oil could inhibit 2 log microbial counts of sea bass slices.

Chemical analysis

Total volatile base (TVB)

The TVB values for all treatments during storage at $4 \pm 1^\circ\text{C}$ are presented in Figure 2. During storage, TVB values of patin fillets coated by edible coating enriched with *Kaempferia rotunda* and *Curcuma*

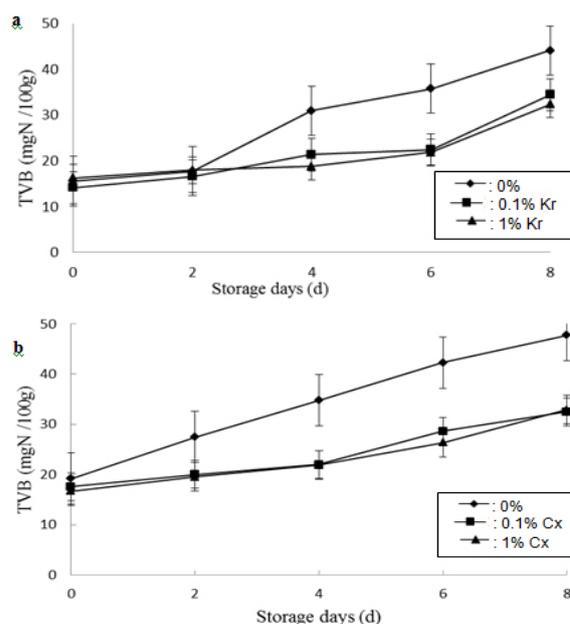


Figure 2. The effect of edible coating enriched with (a) *Kaempferia rotunda* (Kr) (b) *Curcuma xanthorrhiza* (Cx) essential oil on total volatile base (TVB) values of patin fillets during storage at $4 \pm 1^\circ\text{C}$. Mean \pm standard deviation of the values (two replicates) are presented.

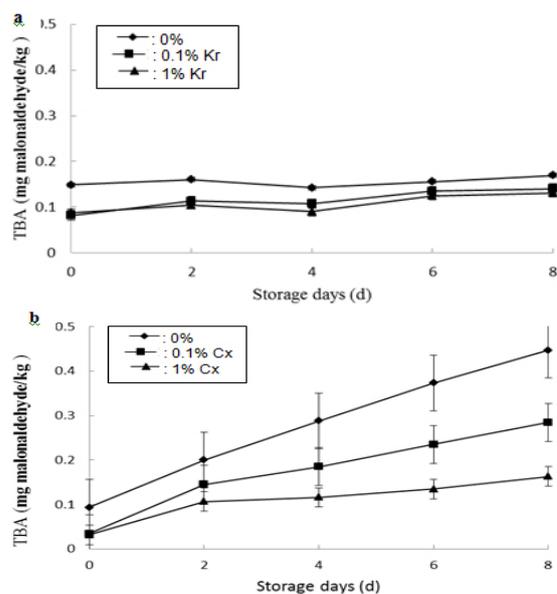


Figure 3. The effect of edible coating enriched with (a) *Kaempferia rotunda* (Kr) (b) *Curcuma xanthorrhiza* (Cx) essential oil on thiobarbituric acid (TBA) values of patin fillets during storage at $4 \pm 1^\circ\text{C}$. Mean \pm standard deviation of the values (two replicates) are presented.

xanthorrhiza essential oil significantly increased ($p < 0.05$). Patin fillets coated by 0.1% and 1% *Kaempferia rotunda* essential oil enriched edible coating showed TVB value significantly lower ($p < 0.05$) than the value of control samples during 4 - 8 days storage (Figure 2a). The initial TVB values of each 0%, 0.1% and 1% *Kaempferia rotunda* essential oil enriched samples were 15.60, 14.16, and 16.27 mg N/100 g, respectively. TVB values at day 8 were 44.12 mg N/100 g for control samples, 34.44 mg

N/100 g for 0.1% samples and 32.39 mg N/100 g for 1% samples (Figure 2a). This result suggested that 0.1% and 1% *Kaempferia rotunda* essential oil enrichment on edible coating could inhibit TVB values below the maximum limit of acceptability (35 mg N/100 g) (Ozyurt *et al.*, 2009).

Similar result was also shown by *Curcuma xanthorrhiza* treatments samples. Began at the initial level ranged from 16.72 to 19.18 mg N/100 g, the TVB values significantly increased ($p < 0.05$) at varied rate during storage. The highest increasing of TVB values was shown by control samples (47.74 mg N/100 g) whereas 0.1% and 1% *Curcuma xanthorrhiza* samples remained below the maximum limit of acceptability (35 mg N/100 g) (Ozyurt *et al.*, 2009) which were 32.49 mg N/100 g and 32.88 mg N/100 g, respectively at day 8 (Figure 2.b).

Clove oil enriched on gelatin-chitosan film also could inhibit the increasing of TVB values of cod fillets significantly ($p < 0.05$) (Gómez-Estaca *et al.*, 2010). Ojagh *et al.* (2010) reported that although TVB values increased during storage, cinnamon oil effected on lowering the TVB values of samples coated with chitosan + cinnamon essential oils significantly ($p < 0.05$) in comparison with the samples coated with chitosan or controls at 16 days refrigeration storage. Furthermore, TVB values of sea bass slices wrapped with gelatin film incorporated with lemongrass essential oil were reported at lower value (15.84 mg N/100 g) than TVB values of sea bass slices wrapped with gelatin film (18.34 mg N/100 g) (Ahmad *et al.*, 2012).

Thiobarbituric acid (TBA)

The changes of patin fillet TBA values which were related to lipid oxidation are shown in Figure 3. During storage, an increasing trend of TBA values was presented by all *Kaempferia rotunda* essential oil concentration treatments samples though at an insignificant level ($p > 0.05$). At the day 8, from an initial 0.148 mg malonaldehyde/kg of fillet, TBA values of control samples increased to 0.169 mg malonaldehyde/kg. Whereas the values of 0.1% samples from 0.081 mg malonaldehyde/kg increased to 0.139 mg malonaldehyde/kg and the values of 1% samples from 0.087 mg malonaldehyde/kg to 0.130 mg malonaldehyde/kg. Furthermore, the TBA values for control samples were higher than those for 0.1% and 1% samples at all day analysis (Figure 3a). An increasing trend of TBA values during storage was also presented by *Curcuma xanthorrhiza* essential oil treatments samples, significantly ($p < 0.05$). The initial TBA values ranged from 0.032 to 0.094 mg malonaldehyde/kg. At day 8, the TBA values were

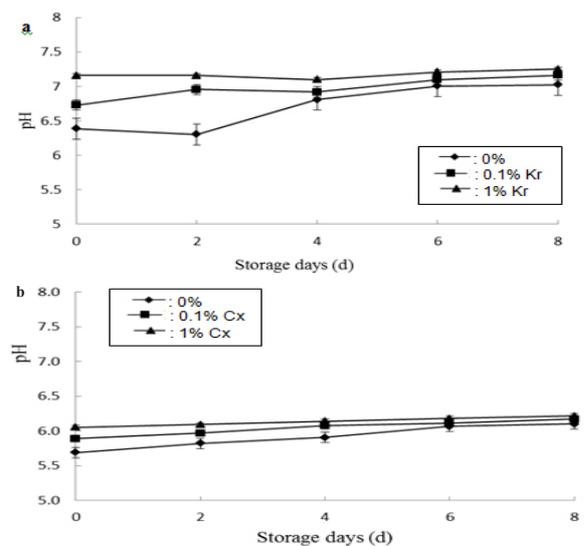


Figure 4. The effect of edible coating enriched with (a) *Kaempferia rotunda* (Kr) (b) *Curcuma xanthorrhiza* (Cx) essential oil on pH of patin fillets during storage at $4 \pm 1^\circ\text{C}$. Mean \pm standard deviation of the values (two replicates) are presented.

0.447 mg malonaldehyde/kg for control samples, 0.284 mg malonaldehyde/kg for 0.1% treatment samples and 0.163 mg malonaldehyde/kg for 1% treatment samples (Figure 3b). All resulted TBA values were remaining below the upper limit of acceptability (1 - 2 mg malonaldehyde/kg) based on Gill (1990) report.

Previous research also reported the same pattern of fish TBA values during refrigeration storage. Lu *et al.* (2010) stated that snakehead fish fillet treated by clove oil showed lower TBA values (1.58 mg malonaldehyde/kg) than control fillet (3.10 mg malonaldehyde/kg) at day 6 cold storage. Inhibition of the increasing TBA values were also affected by cinnamon oil adding on chitosan coating solution of refrigerated rainbow trout (Ojagh *et al.*, 2010). Ahmad *et al.* (2012) mention that TBA values of sea bass slices wrapped with gelatin film enriched by lemongrass essential oil were lower than those of the control.

pH

Due to accumulation of alkaline compounds indicating fish spoilage, pH of patin fillets increased gradually during storage (Figure 4). The initial pH of fillets with various *Kaempferia rotunda* essential oil treatments were varied at 6.39, 6.73 and 7.16 for control, 0.1% and 1% treatments samples, respectively. The increasing of pH were significant ($p < 0.05$) for control and 0.1% treatments samples whereas insignificant ($p > 0.05$) for 1% treatment samples. The highest increase of pH was shown by control samples, followed by 0.1% treatment

samples and 1% treatment samples (Figure 4a). Due to affection of *Curcuma xanthorrhiza* essential oil treatments, pH of 1% essential oil enriched coated fillets showed the lowest increase of pH among other treatments. pH of 1% treated samples increased from 6.05 to 6.21 whereas 0.1% treated samples increased from 5.89 to 6.17 and control samples increased from 5.68 to 6.10 (Figure 4b).

Related on pH, Lu *et al.* (2010) reported that cinamon oil affected on maintaining quality of fresh snakehead fish fillets coated with calcium coating materials. Clove oil application could also maintain pH of cod fillets below 7 (Gómez-Estaca *et al.*, 2010). The increase of rainbow trout pH was inhibited by thyme essential oil enrichment on chitosan coating solution (Chamarana *et al.*, 2012). Similar result also reported by Ahmad *et al.* (2012) that lemongrass essential oil incorporation on gelatin film wrapped to sea bass slices effected on the lowering of increasing pH.

Conclusion

This research suggested that enrichment of *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils on edible coating affected patin fillets quality during refrigeration storage. Due to microbiological and chemical analysis results, patin fillets quality could be maintained by the enrichment of essential oil on edible coating treatments than that by control treatment. Both 1% of *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oil enrichment treatments more effectively inhibited microbial growth and reduced chemical spoilage degree such as TVB, TBA and pH than 0.1% and control treatments. In conclusion, coating treatment by edible coating enriched with *Kaempferia rotunda* and *Curcuma xanthorrhiza* essential oils can be used as preservation method to extend shelf life of patin fillets.

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