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Preservative effects of kaffir lime (*Citrus hystrix* DC) leaves oleoresin incorporation on cassava starch-based edible coatings for refrigerated fresh beef

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Abstract

The chemical compounds of kaffir lime leaves oleoresin and the preservative effects of kaffir lime leaves oleoresin incorporation on cassava starch-based edible coatings for fresh beef during 14 days refrigerated storage were investigated to determine their ability to extend beef shelf life. Beef characteristic was determined based on microbiological (Total Plate Count/ TPC), chemical (Total Volatile Bases/TVB, Thiobarbituricacid/TBA) properties, pH and color). Concentration of kaffir lime leaves oleoresin incorporated on cassava starch-based edible coatings was varied at 0.01% and 0.075% while without kaffir lime leaves oleoresin (0%) was named as control. The chemical compounds of kaffir lime leaves oleoresin were nerolidol (58.27%), citronellal (15.5%), citronellol (2.78%), linalool (2.28%), isopulegol (1.61%), citronellyl acetate (1.28%), trans-caryophyllene (1.17%), geranyl acetate (0.69%), alpha-copaene (0.34%) and alpha-farnesene (0.27%). Kaffir lime leaves oleoresin incorporation on cassava starch-based edible coatings affected the microbiological, chemical properties, pH and color of beef. Microbiologically, 0.075% kaffir lime leaves oleoresin incorporated cassava starch edible coating resulted in 1.48 log reduction of TPC on beef at the end of storage than control samples. Kaffir lime leaves oleoresin treatment more maintained beef quality based on physico-chemical characteristics than control. Therefore, the enrichment of kaffir lime leaves oleoresin on cassava starch-based edible coatings could extend the shelf life of fresh beef and use as an alternative preservation methods.

Keywords

Beef
Citrus hystrix DC
Kaffir lime
Oleoresin
Preservation

Introduction

Beef meat and products are widely produced and consumed all over the world. Based on value, meat indigenous of cattle being the fourth world commodity ranking (Food and Agriculture Organization, 2013). Beef meat also valuable because of the nutritional composition such as high biological value protein and important micro nutrient for human life (Williams, 2007). However, as perishable food, meat provides favorable condition for microbial growth and susceptible to spoilage due to chemical and enzymatic activities (Dave and Ghaly, 2011). Hurdle technology, combination preservation methods, could be applied to inhibit meat spoilage. Combinations of existing and new preservation techniques is a challenging hurdle technology to extend meat shelf life (Zhou *et al*. 2010).

Refrigeration is the most widely used traditional food preservation method. Subjecting meat at sub-optimal temperatures by chilling or freezing could inhibit the microbial growth. By refrigeration, the appearance, texture and flavour of meat also could be retained (Wilson, 2005). However, the psychrotrophic microbes especially Pseudomonas are present at spoiled refrigerated meat (James and James, 2002).

The promising types of new preservation techniques is antimicrobial packaging. Microbial contamination of meat which usually present at the surface could be more efficient to inhibited by antimicrobial packaging due to slow migration of antimicrobial agents to the meat surface (Coma, 2008). Among the categories of antimicrobial packaging, bioactive edible coating has received attention on recent years because consumable, biodegradable and eco-friendly (Bourtoom, 2008). Coating material that had been used to extend meat shelf life such as wheat gluten (Wu *et al*. 2000), gelatin (Antoniewski *et al*. 2007), chitosan (Beverlya *et al*. 2008), sour whey protein (Haque *et al*. 2009), and soy protein isolate (Shon *et al*. 2010). Besides, one of potential edible coating materials is cassava starch. Cassava starch-based edible coating is isotropic, odorless, tasteless, colorless, non-toxic, biologically degradable, have

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good flexibility and low water permeability (Maran *et al*. 2013). Recently, bioactive compounds enrichment on cassava starch-based edible coatings had been reported extend food shelf life. Cinnamon bark or fennel essential oils enrichment could maintain apple slices quality (Oriani *et al*. 2014). Essential oil of Kaempferia rotunda and Curcuma xanthorrhiza which incorporated on cassava starch-based edible coatings also revealed the preservative effect to patin fish (Utami *et al*. 2014). Essential oil containing bioactive edible coating also could prevent beef deterioration. Thyme and oregano essential oil which incorporated on soy edible films shows antimicrobial activity on fresh ground beef patties (Emiroglu *et al*. 2010). Others antimicrobial and antioxidant effects also have been performed by milk protein-based film containing pimento and oregano essential oil on whole beef muscle (Oussalah *et al*. 2004). Zinoviadou *et al*. (2009) mention that oregano oil that enriched on whey protein isolates films also show antimicrobial action against fresh beef spoilage flora. Antimicrobial and antioxidant activity of essential oils associated with their bioactive compounds (Sanchez-Gonzalez *et al*. 2011).

Kaffir lime (*Citrus hystrix* DC), an Indonesian and other Southeast Asian origin herb, contains large of bioactive compounds and shows antimicrobial and antioxidant activity. Citronellal and the other minor volatile compounds such as α-pinene, camphene, β-pinene, sabinene, myrcene, limonene, trans-ocimene, β-terpinene, ρ-cymene, terpinolene, copaene, linalool, β-cubebene, isopulegol, caryophyllene, citronellyl acetate, citronellol, geranyl acetate, δ-cadinene have been found in the essential oil of kaffir lime leaves (Lawrence *et al*. 1971). Nanasombat and Lohasupthawee (2005) reported that both essential oils and ethanolic extracts of kaffir lime leaves exhibited their antibacterial activity against several serotypes of *Salmonella* and *Enterobacteria*. Previous studies also reported that essential oils of kaffir lime leaves have inhibitory activity against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus* (Chaisawadi *et al*. 2005), *Aspergillus flavus* (Thanaboripat *et al*. 2006), and *Propionibacterium acnes* (Luangnarumitchai *et al*. 2007). Several studies also have been reported the antioxidant activity of fresh, boiled, deep-fat fried (Butryee *et al*. 2009), volatile oil, methanolic extract (Wungsintaweekul *et al*. 2010), and ethanolic extract (Tachakittirungrod *et al*. 2007; Ayusuk *et al*. 2009; Jamilah *et al*. 2011) kaffir lime leaves.

Due to of those activities, kaffir lime potentially used as bioactive compounds sources which enriched in bioactive edible packaging. Essential oils of kaffir lime leaves have been incorporated into fish skin gelatin film to be developed as bioactive edible packaging (Tongnuanchan *et al*. 2012, Tongnuanchan *et al*. 2013). However, the enrichment of kaffir lime leaves oleoresin on cassava starch-based edible coatings has not been found to extend fresh beef shelf life. Hence, this research aimed to investigate the chemical compounds of kaffir lime leaves oleoresin and the preservative effects of kaffir lime leaves oleoresin incorporation on cassava starch-based edible coatings to fresh beef.

Materials and Methods

Production of kaffir lime leaves oleoresin

Kaffir lime leaves oleoresin that used in this research is a mixture solution of kaffir lime leaves essential oil and ethanolic extract of kaffir lime leaves distillation waste. The essential oil produced by water-steam distillation of clean crused (1.5 cm) kaffir lime leaves. One portion of dried (14% moisture content) kaffir lime leaves distillation waste macerated on five portions 96% ethanol at 73.6°C for 5 hours 14 minutes (Khasanah *et al*. 2013). Evaporated kaffir lime leaves distillation waste extract mixed with previous essential oil to obtain kaffir lime leaves oleoresin. The chemical compounds of kaffir lime leaves oleoresin analyzed by GCMS (GC-2010 and GC-MS-QP2010 by Shimadzu, Suzhou, Cina). GCMS equipped with capillary coloum AGILENT J&W DB-1 (30 m × 0.25 mm id, Shimadzu, Japan) and operated with Helium as carrier gas.

Coating solution preparation and coating applications

The formulation and preparation of coating solution followed Utami *et al*. (2014) procedure. Edible coating formula were 5 g cassava starch, 100 ml distilled water, and 2 ml glycerol. Kaffir lime leaves oleoresin was mixed after the last heating of the solution. The minimum concentration that could inhibit both *Pseudomonas putida* FNCC 0070 and *Pseudomonas fluorescens* FNCC 0071 (meat spoilage indicator bacteria) growth being the minimum level of oleoresin enrichment (0.01%). While the maximum level of that was based on the high concentration of organoleptic assay result (0.075%).

Tenderloin part of beef meat which obtained from local market (Surakarta, Indonesia) devided into approximately 35-40 g (2.5 cm thickness). Samples of beef were double dipped in coating solutions and dried at drying box. No oleoresin addition edible coating was used as control treatment. Coated beef samples were placed at styrofoam plates, wrapped by wrapping plastic, and stored at refrigerator (4±1ºC) for 14 days. Samples were analyzed microbiologically, chemically, pH and color at 0, 4, 7, 10 and 14 days of storage.

Microbiological analysis

Aseptically minced beef (10 g) in duplicated 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 37ºC for 2 days. Total Plate Count (TPC) was expressed as the logarithm of the colony forming units per gram (log CFU/g).

Chemical analysis

Chemical analysis of beef were determined to evaluate the changes of beef quality according to chemical spoilage properties such as total volatile base (TVB), thiobarbituric acid (TBA). TVB value was analyzed by Conway micro-diffusion method which was described by Min *et al*. (2007). The results of TVB value reported in mgN/100g meat. TBA value was determined according to the distillation method which was described by Tokur *et al*. (2006) and expressed as mg malonaldehyde/kg meat.

pH analysis

Beef pH was measured using pH meter Eutech Instrument Handheld Series after samples (10 g) have been homogenized in distilled water (10 ml).

Color analysis

Color intensity was determined triplicate by Chromameter Konica Minolta CR-400/410 (Minolta Co., Osaka, Japan). The chromaticity coordinates recorded were L^* (Lightness), a^* (redness) and b^* (yellowness).

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 levels and differences in the mean values were determined with Duncan's test ($p < 0.05$) by SPSS Statistics 16 program.

Result and Discussion

Chemical compounds of kaffir lime leaves oleoresin

Kaffir lime leaves oleoresin contained nerolidol (58.27%) and citronellal (15.5%) as mayor compounds. The minor compounds were citronellol (2.78%), linalool (2.28%), isopulegol (1.61%), citronellyl acetate (1.28%), trans-caryophyllene (1.17%), geranyl acetate (0.69%), alpha-copaene (0.34%) and alpha-farnesene (0.27%). Citronellal also as mayor compound of kaffir lime leaves oleoresin which no distillation proces while the others compunds (citronellyl acetate (8.89%), citronellol (8.75%), trans-caryophyllene (9.13%), germacrene B (13.41%), linalool (5.56%)) as minor compounds (Kawiji *et al*. 2015). Citronellal, linalool and citronellol shown as volatile compounds of kaffir lime leaves extracted using Pressurized Liquid Extraction or soxhlet extraction (Haiyee *et al*. 2012). Norkaew *et al*. (2013) reported that nerolidol was one of the major terpenoids constituens in kaffir lime leaf oils extracted by supercritical CO_2. Besides, Nor (1999) found that nerolidol was contained in *Citrus hystrix* oil as trace elements.

Antimicrobial activity of each kaffir lime leaves oleoresin compounds widely investigated. Orhan *et al*. (2012) menthion that citronellol, citronellal, isopulegol and linolool showed antimicrobial activity against *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecalis*, *B. subtilis*, *C. albicans*, and *C. parapsilosis*. Linalool also inhibited the growth of *Klebsiella spp*, *Pseudomonas spp* and *Staphylococcus aureus* (Jaroenkit *et al*. 2011), while citronellol and citronellyl acetate showed antimicrobial activity against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Staphylococcus mutans* (Singh *et al*. 2012), and nerolidol inhibited the growth of *Staphylococcus aureus* (Tao *et al*. 2013). Antimicrobial effect of those compounds related to the membrane permeability of bacteria. Terpenes affected cells swelling and partial dissipation of pH gradient (Siklema *et al*. 1994). Nerolidol, a sesquiterpenoid compound could disrupt the barrier function of bacterial cell membranes (Brehm-Stecher *et al*. 2003). Citronellal affected the membrane structure and disrupted membrane integrity (Singh *et al*. 2006).

The free radical scavenging activities of Citronellal have been determined. Three different methods including FRAP assay (Ferric reducing antioxidant potential), DPPH radical scavenging assay (1,1-diphenyl-2-picryl hydrazyl radical reducing power methods), and β-carotene bleaching assay confirmed the antioxidants activity and capasity of citronellal (Lu *et al*. 2014). The antioxidant activity of nerolidol had been investigated. The mechanism of antioxidative action of nerolidol might be because of the ability of nerolidol to scavenge free radicals (Vinholes *et al*. 2014). The radical-scavenging effects of citrus essential oil such citronellol, citronellal, linalol, and geranyl acetate also have been reported

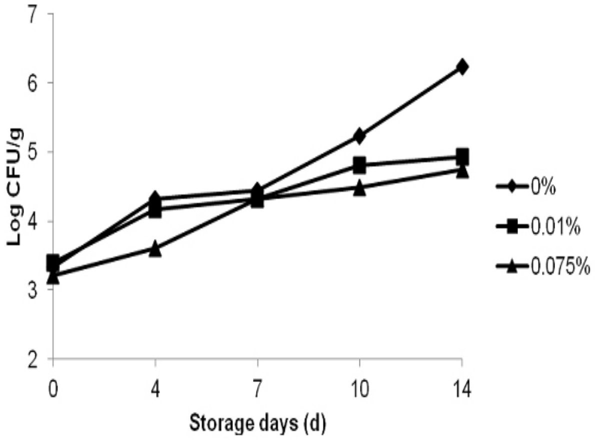

Figure 1. The effect of edible coating enriched with kaffir lime leaves oleoresin on total plate count (TPC) values of beef during storage at 4±1°C

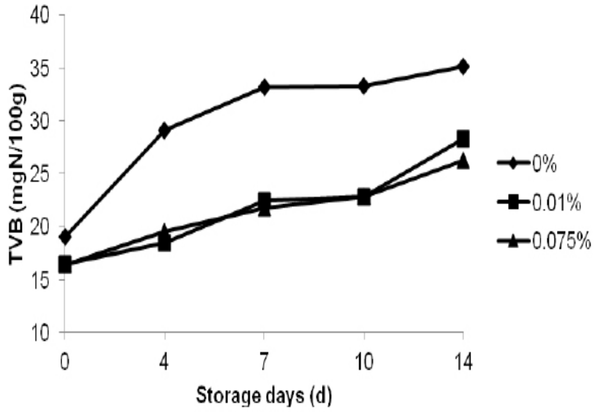

Figure 2. The effect of edible coating enriched with kaffir lime leaves oleoresin on total volatile base (TVB) values of beef during storage at 4±1°C

(Choi *et al*. 2000). Yaudim *et al*. (2002) stated that linalool that also contained on thyme oil showed antioxidant activity.

Microbiological analysis

Beef can be categorized as good quality meat if the TPC does not exceed $1x10^6$ CFU/g or 6 log CFU/g (Indonesian National Standards 3932:2008). The initial TPC in all beef samples ranged from 3.21-3.4 log CFU/g (Figure 1) that indicated the freshness of meat. During storage at 4±1ºC, TPC of control samples increased significantly ($p<0.05$) and reached 6.23 log CFU/g at the end of storage (day 14). Enrichment of cassava based edible coating with kaffir lime leaves oleoresin could inhibit microbial growth. At day 14, TPC of enrichment samples were 4.93 log CFU/g (0.01%) and 4.75 log CFU/g (0.075%). Compared with the control, 0.075% kaffir lime leaves oleoresin incorporated cassava starch edible coating resulted in 1.48 log reduction of TPC on beef at the end of storage. Similar result reported that enrichment of 1.5% oregano essential oil on whey protein isolates edible film affected to the reduction of microbial growth of fresh beef cuts as compared with the control (Zinoviadou *et al*. 2009).

Microbial growth inhibition also performed by several essential oil applications on films. Microbial growth inhibition correlated with the increasing concentration of Chinese cinnamon essential oil in alginate-based edible coating (Oussalah *et al*. 2006). During refrigeration storage, the counts of *Pseudomonas spp*. and coliform bacteria were reducted by oregano and thyme essential oil incorporated films on ground beef patties (Emiroglu *et al*. 2010). Cinnamon essential oil which coated on polypropyle films significantly inhibited the growth of bacteria ($p<0.05$) (Han *et al*. 2014). Those antimicrobial effects due to the chemical compounds of herbs.

Total volatile bases

The initial TVB values of all samples were unsignificantly different ($p>0.05$) which ranged from 16.39 to 19.08 mgN/100g (Figure 2). TVB values of all treatments increased significantly ($p<0.05$) during the storage at 4±1ºC. The highest increasing of TVB value was revealed by the control sample. At the end of storage, TVB values of control samples significantly higher ($p<0.05$) than the values of kaffir lime leaves oleoresin enrichment samples. Based on the meat freshness indicator (Xiao *et al*. 2014), 7th days control samples have been exceeded the standard level (> 30 mgN/100g) while the TVB values of enrichment samples remained below rotting categories at the end of storage (14 days). This indicated that kaffir lime leaves oleoresin enrichment on edible coating solutions could inhibit meat deterioration.

Maintaining of TVB values also was performed by antimicrobial film application for fresh beef steaks packaging. At day 8, TVB values of control samples exceeded the acceptable level while at day 12, the values of antimicrobial film treatment samples within acceptable limits (Han *et al*. 2014). Coating solution contained kaffir lime leaves oleoresin also retained the TVB values of frozen beef sausages. After four months storage, the values of enrichment samples significantly lower ($p<0.05$) than the values of control samples (Utami *et al*. 2014) indicating the antimicrobial activity of kaffir lime leaves oleoresin to prevent microbial protein degradation.

Thiobarbituric acid

Lipid oxidation is also an important factor limiting

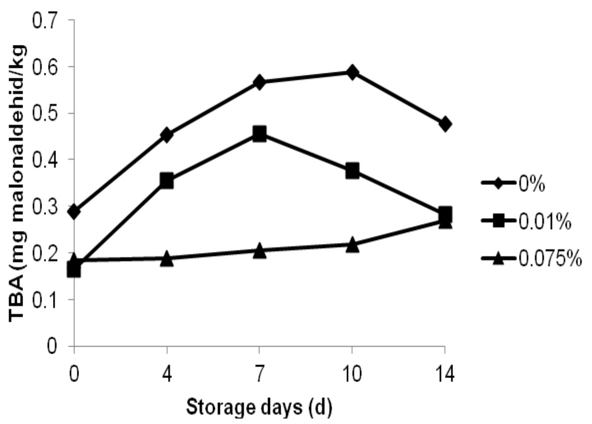

Figure 3. The effect of edible coating enriched with kaffir lime leaves oleoresin on thiobarbituric acid (TBA) values of beef during storage at 4±1°C

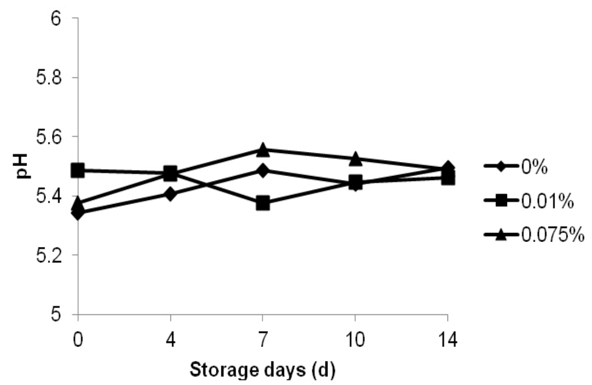

Figure 4. The effect of edible coating enriched with kaffir lime leaves oleoresin on pH values of beef during storage at 4±1°C

the shelf life of beef. No differences were observed among samples at the beginning of storage (Figure 3). During storage, beef samples would expose with the oxygen and this caused the increasing of TBA values. TBA values increased more rapidly in control samples as compared to kaffir lime leaves oleoresin treatment samples. The addition of kaffir lime leaves oleoresin on edible coating solutions suppressed the accumulation of lipid oxidation by-products indicating the antioxidant activity of kaffir lime leaves oleoresin chemical compounds. According to the threshold values for the rancidity perception of consumers at 0.5 mg malonaldehyde/kg (Shon *et al*. 2010), all treatment samples did not exceed that values until the end of storage (day 14) while control samples perceived as rancid after day 7 of storage.

Several natural antioxidant compounds have been applied to improve the lipid oxidative stability. Mixing kaffir lime peel essential oil on Chinese sausage significantly reduced the increasing of TBA reactive substance values compared to the control sample after five days storage (Kingchaiyapum *et al*. 2012). Active film coated with oregano extracts (Camo *et al*. 2011) and rosemary extracts (Barbosa-Pereira *et al*. 2014) enhanced oxidative stability of beef samples. The oxidative effects of rosemary oleoresin also noted on lipid oxidation inhibition of ground beef patties (Parks *et al*. 2012).

Beef pH

The pH values of fresh beef samples did not show

Table 1. The effect of edible coating enriched with kaffir lime leaves oleoresin on color intensity of beef during storage at 4±1°C

	Oleoresin Concentration	Storage days (d)				
		0	4	7	10	14
	0%	$39.80\pm2.11^{a}_{A}$	$38.54\pm3.88^{a}_{A}$	$39.95\pm1.11^{b}_{A}$	$43.92\pm1.16^{a}_{A}$	$39.28\pm4.49^{a}_{A}$
L*	0.01%	$36.97\pm0.11^{a}_{A}$	$36.07\pm2.50^{a}_{A}$	$35.89\pm0.20^{a}_{A}$	$44.01\pm6.00^{a}_{A}$	$40.76\pm0.07^{a}_{A}$
	0.075%	$38.35\pm2.08^{a}_{A}$	$37.61\pm4.94^{a}_{A}$	$37.75\pm0.78^{ab}_{A}$	$42.28\pm1.74^{a}_{A}$	$40.58\pm0.82^{a}_{A}$
	0%	$14.26\pm0.28^{a}_{C}$	$11.83\pm0.39^{a}_{AB}$	$12.63\pm0.00^{a}_{B}$	$11.54\pm0.01^{a}_{AB}$	$10.71\pm1.15^{a}_{A}$
a*	0.01%	$15.77\pm0.79^{a}_{B}$	$13.97\pm1.49^{a}_{AB}$	$14.15\pm0.65^{a}_{AB}$	$11.70\pm0.66^{a}_{A}$	$11.093\pm2.48^{a}_{A}$
	0.075%	$15.61\pm1.07^{a}_{A}$	$13.67\pm3.51^{a}_{A}$	$13.47\pm2.73^{a}_{A}$	$13.04\pm1.59^{a}_{A}$	$13.56\pm3.04^{a}_{A}$
	0%	$9.50\pm0.06^{a}_{A}$	$8.31\pm1.32^{a}_{A}$	$11.79\pm3.32^{a}_{A}$	$9.37\pm1.01^{a}_{A}$	$8.15\pm0.27^{a}_{A}$
b*	0.01%	$10.30\pm0.41^{a}_{A}$	$9.21\pm1.36^{a}_{A}$	$11.20\pm0.77^{a}_{A}$	$8.57\pm0.64^{a}_{A}$	$8.43\pm0.73^{a}_{A}$
	0.075%	$10.45\pm2.05^{a}_{A}$	$10.34\pm1.70^{a}_{A}$	$9.93\pm0.32^{a}_{A}$	$9.05\pm0.22^{a}_{A}$	$9.29\pm0.35^{a}_{A}$

Mean±SD with different superscripts column wise and subscript row wise differ significantly (P<0.05) (n = 2) significant differences (p>0.05) among treatments and during storage (Figure 4). After refrigeration storage at 4±1ºC, the pH values of all samples generally unsignificantly increased (p>0.05). Control samples had higher increased pH values than kaffir lime leaves oleoresin treatment samples. Kaffir lime leaves oleoresin treatment appeared to be effective mainained beef pH. The increasing of pH values indicating the meat spoilage due to protein degradation for the formation of free amino acids leading to the production of alkaline compounds such as NH_3 and amines (Karabagias *et al*. 2011). Similarly, coating of grapefruit seed extracts on film more maintained the pH value of beef than plain film (Ha *et al*. 2001).

Color intensity

No treatment differences were observed for Hunter L* (lightness) or b* (yellowness) values (Table 1) among treatments variation and storage time of all beef samples. Besides, color intensity of Hunter a* (redness) reduced after refrigeration storage. The reduction degree of control samples higher than 0.075% treatment samples. This demonstrated that the incorporation of kaffir lime leaves oleoresin on edible coating at certain concentration could protect meat color. The protective effect against discoloration of beef related to antioxidant activity of kaffir lime leaves oleoresin that inhibited the conversion of oxymyoglobin to metmyoglobin.

Discoloration inhibition of beef also depended on oregano extracts addition on active film formulation. Concentration over 1% oregano extracts maintained Hunter a* (redness) values above 10, while nine values for 0.5% oregano extracts after storage (Camo *et al*. 2011). Ground beef samples with rosemary oleoresin addition were redder than no rosemary oleoresin addition samples after abusive temperature storage (Parks *et al*. 2012). High concentration of antimicrobial agents by rhubarb ethanolic extracts (REE) and cinnamon essential oil (CEO) mixture didn't always maintained a* (redness) values. Loss color protection of middle concentration treatment (1% REE and 0.08% CEO) was strongest than low concentration treatment (0.5% REE and 0.04% CEO) and high concentration treatment (2% REE and 0.16% CEO or 4% REE and 0.32% CEO) due to the influence of the REE and CEO color (Han *et al*. 2014).

Conclusion

The chemical compounds of kaffir lime leaves oleoresin were nerolidol (58.27%), citronellal (15.5%), citronellol (2.78%), linalool (2.28%), isopulegol (1.61%), citronellyl acetate (1.28%), trans-caryophyllene (1.17%), geranyl acetate (0.69%), alpha-copaene (0.34%) and alpha-farnesene (0.27%). Kaffir lime leaves oleoresin incorporation on cassava starch-based edible coatings affected the microbiological, chemical properties, pH and color of beef. Microbiologically, 0.075% kaffir lime leaves oleoresin incorporated cassava starch edible coating resulted in 1.48 log reduction of TPC on beef at the end of storage than control samples. Kaffir lime leaves oleoresin treatment maintained beef quality did not exceeded TVB value standard level until the end of storage (14 days). Beef pH of all samples stable at 5.34-5.56. The addition of kaffir lime leaves oleoresin on edible coating solutions suppressed the accumulation of lipid oxidation by-products related to the lower TBA values increasing. The 0.075% treatment sample more maintained the redness off the fresh beef samples than others. In conclusions, the enrichment of kaffir lime leaves oleoresin on cassava starch-based edible coatings had been found extended the fresh beef shelf life.

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