

Research Article

Antimicrobial and antioxidative activities of essential oils in Chinese sausage (Kun-Chiang)

Wisaruta Kingchaiyaphum and Chitsiri Rachtanapun*

Department of Food Science and Technology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900 Thailand.

*Email: chitsiri.t@ku.ac.th

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Abstract

The efficiency of antioxidative and antimicrobial activities of kaffir lime peel (*Citrus hystrix* DC.) essential oil (EO) and fingerroot (*Boesenbergia pandurata* Roxb.) EO in Chinese sausage (Kun-Chiang) was investigated. A mixture of Chinese sausage ingredients was prepared; EO in a proportion of 5, 10 and 20% (w/w) was added, then the mixture was stuffed into collagen casing and oven dried. The samples were stored at room temperature (30°C) for 25 days. The antioxidative activity of essential oil in Chinese sausages was determined by Thiobarbituric Acid Reactive Substance (TBARS) method. Meanwhile, the antimicrobial activity was examined by enumerating total viable counts on plate count agar and total yeast and mold counts on potato dextrose agar adjusted to pH 3.5. The results showed that kaffir lime peel EO and fingerroot EO could significantly retard the lipid oxidation of Chinese sausages. The kaffir lime peel EO showed stronger antioxidative effect than fingerroot EO. However, there was no significant effect between EO concentration and its antioxidative activity. For antimicrobial activity, 10% kaffir lime peel and fingerroot EO could extend shelf-life of Chinese sausages by 5 and 10 days, respectively, when compared to the control (without EO addition). Moreover, fingerroot EO had stronger antimicrobial activity than kaffir lime peel EO and the antimicrobial effect increased as its concentration increased. Both kaffir lime peel and fingerroot EO have potential to use as a natural preservative against lipid oxidation and microorganisms in food products, in particular high fat and low a_w meat products such as Chinese sausages.

Keywords: food additives, kaffir lime (*Citrus hystrix* DC.), fingerroot (*Boesenbergia pandurata* Roxb.), TBARS, meat, Thailand

Introduction

Dry/semi-dry sausage is one of the popular foodstuffs among meat products. However, during storage, quality attributes of the product deteriorate due to lipid oxidation and microbial growth. Lipids oxidation is responsible for reduction in nutritional quality as well as changes in flavour [1], while microbial contamination can cause major public health hazards and economic loss in

terms of food poisoning and meat spoilage. Thus, application of suitable agents possessing both antioxidant and antimicrobial activities may be useful for maintaining meat quality, extending shelf-life and preventing economic loss [2]. Many researchers have indicated that lipid oxidation and microbial growth in meat products can be controlled or minimized by using either synthetic or natural food additives [3, 4]. Various synthetic antioxidants, such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT), are commonly used to delay the development of rancidity in food products [5, 6]. However, consumers are concerned about the safety of synthetic food additives. This concern has led to arouse a greater interest in natural additives [7]. Natural agents possessing antioxidant and antimicrobial properties have the advantage of being readily accepted by consumers, as they are considered natural.

Recently, research in essential (volatile) oils has shown strong antioxidative properties and antimicrobial activity *in vitro*; however, their applications in food are limited. The potential herbs and spices are kaffir lime peel and fingerroot. EO of kaffir lime (*Citrus hystrix* DC) peel exhibited antimicrobial activity against Gram positive bacteria namely *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, Gram negative bacteria namely *Escherichai coli*, *Salmonella* Typhimurium, lactic acid bacteria and mold [8, 9, 10]. Essential oil of fingerroot (*Boesenbergia pandurata*) could inhibit the activity of various foodborne pathogenic microorganisms including *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichai coli*, *Salmonellae*, lactic acid bacteria and mold [9, 10, 11, 12, 13]. Furthermore, both kaffir lime peel and fingerroot EO also exhibited antioxidant effects [11, 14, 15]. Therefore, the aim of this study was to investigate the antioxidative and antimicrobial activity of kaffir lime peel essential oil (EO) and fingerroot EO in high fat, low a_w meat product by using Chinese sausage (Kun-Chiang) as a model.

Materials and Methods

Essential oils

Essential oils of kaffir lime peel and fingerroot were provided by Thai-Chinese Flavour and Fragrance (TCFF), Ayutthaya, Thailand.

Preparation of Chinese sausage

Chinese sausage was prepared as follows. Hip pork and lard were ground and mixed with salt, phosphate powder and essential oil (kaffir lime peel or fingerroot EO at concentration of 5%, 10% and 20%, w/w), then chopped with a silent cutter (Scharfen GmbH & Co., Postfach, Germany) and stuffed in collagen casing with a sausage stuffer (Dadaux, Bersaillin, France). The sausages were dried in a tray oven (Reliance Tech-Service co., Ltd., Samutsakhon, Thailand) at 50°C for 1 h, and then the temperature adjusted to 60°C for 2 days. Sausage samples were packed in sterile polyethylene (PE) bags and kept at room temperature for 25 days. Samples were taken to determine lipid oxidation and number of microorganisms at 5 day intervals.

Measurement of water activity (a_w)

Ten grams of sample was analyzed by water activity instrument (Testo 650; Tesco, Inc., Lenzkirch, Germany).

Determination of antioxidant activity of essential oils in sausage

The antioxidative activity was determined by Thiobarbituric Acid-Reactive Substances (TBARS) as modified from Fernandez-Lopez *et al* [16]. A 10 g sample was blended with 50 ml distilled water for 2 min and then transferred to a distillation tube. The cup used for blending was washed with an additional 47.5 ml distilled water, which was added to the same distillation tube with 2.5 ml 4N HCl. The mixture was distilled and 50 ml distillate collected. Five ml of

0.02 M 2-thiobarbituric acid in 90% acetic acid (TBA reagent) was added to a vial containing 5 ml of the distillate and mixed well. The vials were capped and heated in a boiling water bath for 30 min to develop the chromogen and cooled to room temperature. The absorbance was measured at 538 nm, against a blank prepared with 5 ml distilled water and 5 ml TBA-reagent, using a spectrophotometer (LaboMed, Inc., CA, USA). TBARS were calculated from a standard curve (8–50 nmol) of malondialdehyde, freshly prepared by acidification of TEP (1,1,3,3-tetraethoxy propane). TBARS were reported as milligrams of substance equivalent to malonaldehyde/kg dry weight.

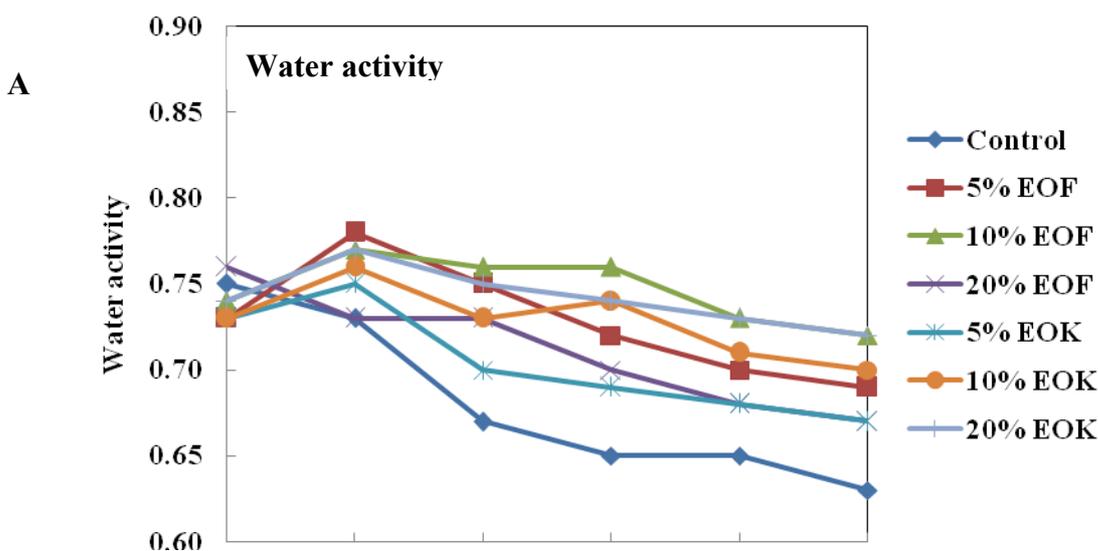
Microorganisms evaluation

Total viable counts and yeast and mold counts were evaluated on trypticase soy agar (TSA; Merck, Darmstadt, Germany) and potato dextrose agar pH 3.5 (PDA; Merck), respectively, by using spread plate technique as described; 25 g of fermented sausage was added with 225 ml 0.1% peptone water, pummeled in a stomacher (Stomacher 400; Steward, London, UK) for 2 min, then serial dilution was spread on solidified suitable agar. TSA plates were incubated at 37°C for 24-48 h and PDA plates were incubated at 30°C for 3-5 days. All experiments were repeated three times with two replications per experiment.

Results and Discussion

Changes in water activity (a_w)

The water activity value of Chinese sausage was gradually reduced from 0.75 to 0.63 during storage for 25 days. All values obtained for the various groups tested were between 0.76 and 0.67 for 1 and 25 days. Addition of essential oil increased the water activity of Chinese sausage (Fig. 1A), the addition of 10% EO exhibited the highest a_w in the sausage. However, at high concentration of 20% there was some oil droplet leakage during storage and the product had a pungent odour.



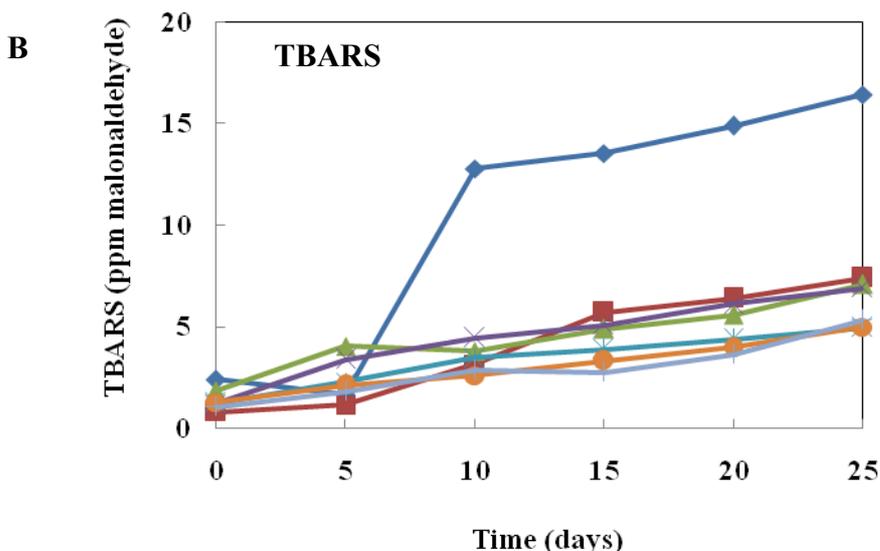


Figure 1. Water activity (a_w) and TBARS in Chinese sausage with fingerroot EO (EOF) or kaffir lime peel EO (EOK) at 5%, 10% and 20% (w/w), stored at room temperature (30°C) for 25 days.

Points represent average values (n=3).

Lipid oxidation of Chinese sausage containing kaffir lime peel EO and fingerroot EO

The presence of kaffir lime peel EO and fingerroot EO significantly decreased lipid oxidation after day 5 of storage compared to the controls (Fig. 1B). Kaffir lime EO showed stronger lipid stability than fingerroot EO. Increasing concentration of EOs did not affect to the antioxidation activity. The lower lipid oxidation values obtained with the kaffir lime peel EO and fingerroot EO treatments would be due to the protective effect of oxidation during processing and storage. The agents responsible for the antioxidant activity in both kaffir lime peel EO and fingerroot EO oil are the bioactive compounds they contain and, mainly, polyphenols. Many studies have pointed to the effect of the polyphenols on lipid oxidation in meat products [17, 18, 19]. Also, the antioxidant effect of the essential oils on meat products is generally accepted [4, 19, 20, 21]. This antioxidant activity is related to the capacity of polyphenols to act as metal-chelators, free radical scavengers, hydrogen donors and inhibitors of the enzymatic systems responsible for initiating oxidation reaction. Furthermore, they can act as substrate for free radicals like superoxide or hydroxyl or intervene in propagation reactions [22, 23].

Antimicrobial activity of kaffir lime peel EO and fingerroot EO addition to Chinese sausage

Initial total viable counts for the seven treatments were less than 3 log CFU/g and met the local official general hygienic requirement (5.0 log CFU/g) [24]. The initial counts in sausage with added kaffir lime peel EO was lower than fingerroot EO and control. Changes in aerobic counts during storage are shown in Table 1. In the control samples, the counts increased after 10 days storage and in the 5% EOs they increased progressively during 15 days storage. A comparison between fingerroot EO and kaffir lime peel EO, fingerroot EO showed stronger antimicrobial effect. In the 10% fingerroot EO samples, the aerobic counts slightly increased during storage and were less than 5.0 log CFU/g after 25 days, indicating that addition 10% fingerroot EO could extend shelf-life of Chinese sausage by over 10 days. In addition, there was no yeast and mold detected in the experiment.

Table 1. Total viable counts in the Chinese sausage with added fingerroot EO or kaffir lime peel EO and stored at room temperature for 25 days.

Sample	Total viable counts (log CFU/g)					
	0	5	10	15	20	25
Control	2.15±0.21	2.86±0.45	2.22±0.00	6.35±0.00	6.60±0.00	6.60±0.00
5% Fingerroot	2.28±0.71	2.76±0.40	2.68±0.38	6.09±0.00	6.60±0.00	6.60±0.00
10% Fingerroot	2.42±1.58	2.66±1.24	2.45±0.52	3.72±1.70	4.00±1.79	4.63±2.79
20% Fingerroot	1.50±0.71	2.09±0.55	2.28±0.53	3.58±1.65	3.84±1.79	4.81±2.54
5% Kaffir lime peel	1.50±0.71	2.33±0.21	3.81±0.00	5.33±0.00	6.60±0.00	6.60±0.00
10% Kaffir lime peel	1.50±0.71	2.19±0.41	3.38±0.47	4.28±1.35	5.70±1.27	5.70±1.27
20% Kaffir lime peel	1.50±0.71	1.80±0.71	3.32±0.42	4.14±1.11	5.70±1.27	5.70±1.27

Numbers represent average values (n=3) ± standard deviation

Jantan *et al.* [25] reported that the major constituents of fingerroot EO obtained from Malaysia, Indonesia and Thailand are camphor, geraniol, 1,8 cineol, β -ocimene, camphene and methyl cinnamate. These phytochemicals have been reported for their antimicrobial activity. Geraniol and 1,8 cineol had strong antibacterial activity against Gram positive and Gram negative bacteria [9]. Furthermore, the mechanism on antimicrobial effect of the fingerroot EOs has been demonstrated by causing degradation of cell walls and detachment of cell membranes, leading to proteinous leakage [12, 13].

Conclusion

Essential oils of kaffir lime peel and fingerroot were found to exhibit potent lipid oxidation activity and antimicrobial activity in Chinese sausage. The addition of 10% fingerroot EO in sausage could retard the growth of total viable counts and extend shelf-life by 10 days.

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