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Effect of capsicum oleores and garlic oil on sensorial quality and microbial growth of mixed salads for Thai green papaya salad

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Abstract

The purpose were to investigate the effect of capsicum oleores (CS), garlic oil (GL) and CS incorporated with GL (CS+GL) on sensorial attributes and microorganisms growth of mixed salads, the mixture of shredded green papaya, shredded carrot and cherry tomato fruit, during storage. The mixed salads were kept in a semi-rigid polypropylene plastic box containing the CS, GL or CS+GL pad. Gases in package (O_2 and CO_2 concentration), sensorial attributes (colour, odour and overall appearance scores) and microbial growth (total microbial count and the growth of inoculated *E. coli* and *S. aureus*) were determined. In the package of the mixed salads treated with GL and CS+GL, CO_2 was lower and O_2 was higher than control and CS treated mixed salads. At the end of storage, colour and overall appearance scores of the mixed salads treated with GL and CS+GL were above the acceptable level and higher than those of the mixed salads treated with CS and control which the both scores were under the acceptable score, whilst odour score of all treatments was above the acceptable level. All treatments delayed the increase in total microbial count, inoculated *E. coli* and *S. aureus* when compared to the control. GL had potentially delayed the growth of *E. coli* and *S. aureus* in the mixed salads during storage.

Introduction

Regarding the development of the world, the life-style of people has been changed including food consumption. Recently, fresh-cut or minimally processed products are popular and the requirement of the products has been increasing as convenience to eat and cook and the health benefit concern of consumers (Cantwell and Suslow, 2002). Fresh-cut products have been currently taken a large market share for food product around the world. In Thailand, fresh-cut products including fresh-cut fruit, mixed salads and ready to cook fresh vegetables are widely sold in both local markets and supermarkets. One of the most popular mixed salads in Thailand is spicy green shredded papaya salad so called 'Som Tam'. It generally consists of shredded green papaya as a main ingredient, cut tomato, and shredded carrot. However, it is recognized that fresh-cut product is highly perishable with a short shelf-life after processing. The loss of quality in green shredded papaya include the shriveling of shreds, losses of weight, softening and colour change (Chareekhot *et al*., 2014; Chareekhot *et al.*, 2016). The undesirable effects could be reduced by dipping in organic acid (Lichanporn and Kanlayanarat, 2006), heat treatment (Kakaew *et al.*, 2007), and controlled atmospheric conditions (Techavuthiporn *et al*., 2010). However, this product is also easily contaminated from microorganisms known as cross contamination during processing, especially foodborne pathogens (Berger *et al*., 2010; Francis *et al*., 2012). Although most researches have focused on maintaining physiological and physic-chemical qualities, few reports document the microbiological control of green shredded papaya salad.

In recent years, interest in natural antimicrobial compounds has increased and numerous studies have been reported on the antimicrobial activity of a wide range of natural compounds. Many pathogenic microorganisms, which can be the cause of foodborne diseases or fresh food decay, can be inhibited using natural compounds. Among these, several essential oils, alcohols, organic acids and aromatic compounds have been shown to be biologically active (Ayala-Zavala and González-Aguilar, 2010). Garlic and chili are important economic produces of Thailand. It is widely used as an ingredient in exotic foods, pharmaceutical, cosmetic and other industries. They contain a unique smell, taste and flavour derived from both non-volatile and volatile oils. One of those interested properties is antimicrobial agent affecting

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for both foodborne pathogenic and food spoilage microorganisms. Pranoto *et al.* (2005) showed that the application of garlic oil in biodegradable film could inhibit a range of foodborne microorganisms such as *Escherichia coli, Staphylococcus aureus, Salmonella* Typhimurium, *Listeria monocytogenes* and *Bacillus cereus.* In addition, capsaicin (8-methyl-N-vanillyl-6-nonenamide), the main compound in chili, showed antimicrobial activity, antibiotic synergism and bacterial virulence removal (Dorantes *et al.*, 2000; Omolo *et al.*, 2014). Nascimento *et al.* (2014) demonstrated that active compound in chili against the microbial growth in both Gram-positive bacteria *(Enterococcus faecalis, Bacillus subtillis, Staphylococcus aureus)* and Gram-negative bacteria *(Pseudomonas aeruginosa, Klebsiella pheumoniae, Escherichia coli)* and yeast *(Candida albicans).* Antimicrobial agents can be incorporated into a packaging system through simple blending with packaging materials, immobilization or coating differently depending on the characteristics of packaging system, antimicrobial agent and food products. It can migrate from packaging materials to foods, while the immobilized agent cannot migrate (Appendini and Hotchkiss, 1997). This contributes to the improvement of food safety and extension of shelf life of the packaged food (Cooksey, 2005).

Therefore, this study is aimed to study the application of garlic oil and capsicum oil onto package for 'sustainable antimicrobial packaging' by using 'Som Tam' as the mixed salads model. This supposes to be a model for reducing chemical application to fresh cut products and implementing trade in which has been sold on shelf in convenience stores and supermarket to reduce the risk of contamination.

Materials and Methods

Bacterial cultures

Two food pathogenic bacteria, which are represented gram positive and gram negative were used in this study. *S. aureus* TISTR 1466 HCUMIB 050 and *E. coli* ATCC 8739 HCUMIB 022 were obtained from the culture collection at Microbiological Industry Laboratory, Huachiew Chalermprakiet University, Thailand. The bacterial cultures were grown on the nutrient agar slant and kept at 4°C. In order to prepare the seeding culture, a loopful of bacteria from agar slant was taken and inoculated into 100 ml of nutrient broth in 250 ml flask. The flask was transferred to incubate at 37°C for 18 hr. Serial dilution was conducted to obtain required concentration which both strains had an initial concentration of bacteria inoculation approximately 10^5-10^6 CFU/ml.

Antimicrobial pad preparation

Capsicum oleores (CS) and garlic (GL) oil were purchased from Four Foods Company Limited, Thailand. For incorporating plant oil to control matrix, 5% (w/w) of CS, GL and mixed CS + GL oils was dissolved in 2% (w/v) carboxyl methyl cellulose (CMC; Fluka, Finland) and 2% (w/v) glycerol. Then, the solution was homogenised using a mechanical stirrer (Model: LMS-HTS-1003, Bunkyo-Ku, Tokyo, Japan) for 30 min. Two ml of the antimicrobial solution was applied to a sterilised cotton pad (2 × 4 cm) and adhered to the inner side of the lid of package to create the antimicrobial packaging with the releasing system of headspace system with control matrix. All antimicrobial pad were left to dry at 40°C overnight.

Sample preparations

Unripe papaya fruit, cherry tomato fruit and carrot were purchased from a supermarket. No physical damaged and without any defect samples were selected and cleaned with tap water to reduce the contamination. Each kind of plant material was separately processed excepting cherry tomato which was directly dipped in 150 µg/ml sodium hypochlorite for 2 min and left to dry at room temperature (approximately 25°C). The papaya fruit and carrot were peeled using a 2-edges stainless-steel knife and rinsed again with tap water. Then both peeled papaya and carrot were dipped in 50 µg/ml sodium hypochlorite for 1 min and left to dry at room temperature. They were then shredded into approximately 10-12 cm length using a shredding stainless-steel knife. The shreds were moved to dip in 150 µg/ml sodium hypochlorite for 2 min. They were dried using a salad spinner for 2 min to remove excess water. Mixed green papaya salad were prepared by mixing 80 g of shredded papaya, 40 g of shredded carrot and 20 g of cherry tomato fruit (per box).The mixed green papaya salad were randomly divided into 3 sets for different bacteria inoculations namely (a) without inoculation, (b) *Staphylococcus aureus* and (c) *Escherichia coli*. For bacteria inoculation, 0.1 ml of strain with concentration of 10^5-10^6 CFU/ml was transferred to obtain bacteria concentration of approximately 10^4 CFU/ml. Each set of bacterial inoculated mixed salads was divided into 4 lots for different antimicrobial treatments; firstly without treated with herb oil (as control), secondly treated with 5% Capsicum oleores oil (CS), thirdly treated with 5% Garlic oil (GL) and fourthly treated with 5% CS incorporated with 5% GL. The samples were mixed in plastic bag (low density polyethylene, 15×22 cm dimension) for 30 sec. They were then placed in polypropylene semi-rigid box (15 cm × 15 cm × 9 cm) and covered with plastic lid (polypropylene, 15 x 15 cm dimension with 90 µm thickness) containing different antimicrobial pad. The salad boxes were held at 4°C and 90-95% RH for 6 days. All analyses were performed at 0, 2, 4, and 6 days of storage. Each treatment was applied to four replicates in randomly of four boxes.

Gas headspace analyses

Both O_2 and CO_2 inside the package were determined using a handheld Gas Analyser (model: Oxybaby M+X, Germany). Gas concentrations were expressed in terms of partial pressure (kPa).

Sensorial quality attributes

Sensory evaluations were performed and defined as colour, odour and overall appearance. The subjective measurements were done by 30 semi-trained panelists. The samples were evaluated using 5 points hedonic scoring test which scale 5 is like extremely, 4 is like moderately, 3 is neither like nor dislike, 2 is dislike moderately and 1 is dislike extremely, respectively.

Measurement of microbial count

Twenty-five grams of mixed green papaya salads were removed from each package and homogenised with 225 ml of 0.85% (w/v) NaCl solution (normal saline) in sterile plastic bag using a stomacher for 2 min. Tenfold dilution series were made using normal salinity. The appropriate sample dilution (0.1 ml) were spread plated on plate count agar (PCA, HiMedia), MacConkey agar (HiMedia) and Mannitol Salt agar (MSA, HiMedia) and then were incubated at 37°C for 24-48 hr for Total Bacterial Count, *E. coli* and *S. aureus* counts, respectively. Microbial counts were expressed as $\log_{10}$ CFU/g.

Statistical analysis

All data points represent the mean ± standard mean error of a least 4 replications. The significance of all data was determined using a 2-way ANOVA (analysis of variance) followed by Duncan's Multiple Range Test (DMRT) at the 5% level.

Results and Discussion

Gases concentration in package

The changes of gases concentration in the package of mixed salad during storage were shown in Figure 1. The increase in CO_2 (Figure 1A) and decrease in O_2 (Figure 1B) were found throughout the storage. CO_2 concentration in the package of mixed salads treated with 5% CS and control markedly increased and was significantly higher than that in the package of the mixed salads treated with 5% GL and 5% CS+GL ($P < 0.05$). On day 6, the CO_2 in the package of mixed salads treated with 5% CS was significantly higher than other treatments ($P < 0.05$) which reached to 3.5 kPa; whilst that of the control was about 3 kPa and the mixed salads treated with 5% GL and 5% CS+GL was about 1.4 kPa. In contrast, O_2 in the package of the mixed salads treated with 5% GL and 5% CS+GL were higher than that in the package of the mixed salads treated with 5% CS and the control. Especially on day 6, the O_2 in the package of mixed salads treated with 5% CS was significantly lower than that of other treatments ($P < 0.05$) which it was about 18 kPa whilst that in the package of mixed salads treated with GL and CS+GL was approximately 20 kPa. Among all the treatments, storage period and interaction of treatments and storage period, the change in CO_2 and O_2 were statistically significant with p-value < 0.01 (Table 1). Interestingly, we found that GL and CS+GL retarded respiratory rate of the mixed salads due to the lower CO_2 level and

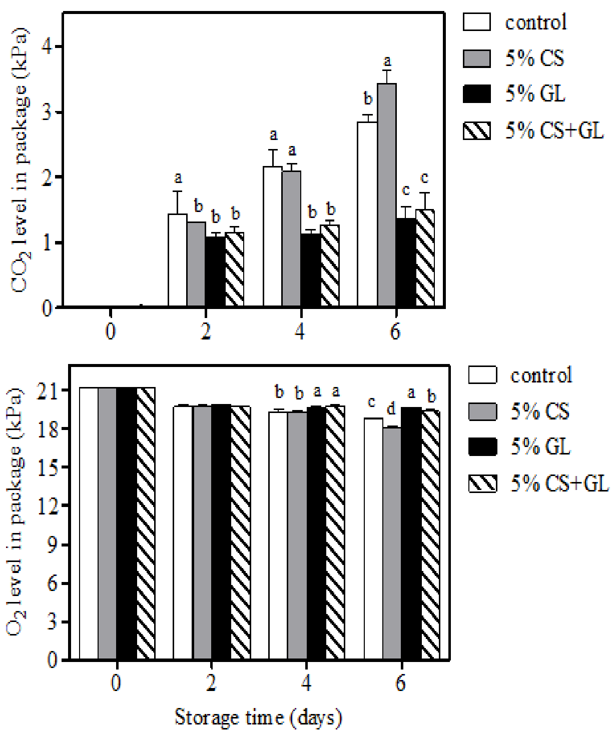

Figure 1. Carbon dioxide (CO_2) (A) and oxygen (O_2) (B) levels in package of mixed salads for Thai green papaya salad treated with capsicum oleores (CS), garlic oil (GL) and CS incorporated with GL (CS+GL) during refrigerated storage at 4±1°C. Data are the average of at least four replicates ± standard deviation. The different letters denote significance among treatments.

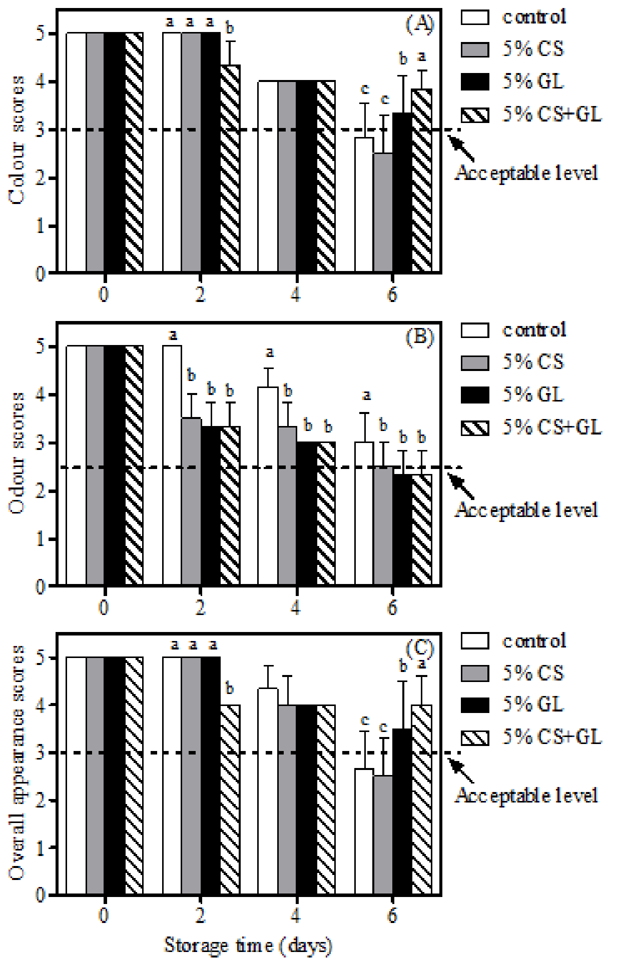

Figure 2. Colour (A), odour (B) and overall appearance (C) scores of mixed salads for Thai green papaya salad treated with capsicum oleores (CS), garlic oil (GL) and CS incorporated with GL (CS+GL) during refrigerated storage at 4±1°C. Data are the average of at least four replicates ± standard deviation. The different letters denote significance among treatments.

higher O_2 level in the package when compared to the control and CS. No previous work has reported the respiratory inhibition by GL. It is widely accepted that high respiratory rate indicates high metabolism in plants. The increase in metabolism might be induced the short-shelf life of fresh commodities, especially in fresh-cut form. Thus, the high colour and overall appearance scores of the mixed salads treated with 5%GL and 5%CS+GL (Figure 2) might be related to the CO_2 and O_2 levels in package.

Sensorial quality attributes

Sensorial quality attributes involving colour, odour and overall appearance of the mixed salads were presented in Figure 2. A five hedonic scoring test was used to estimate those sensorial quality attributes. Colour score of all treatments decreased over storage and the score was higher than the acceptable level (score 3) during storage for 4 days (Figure 2A).

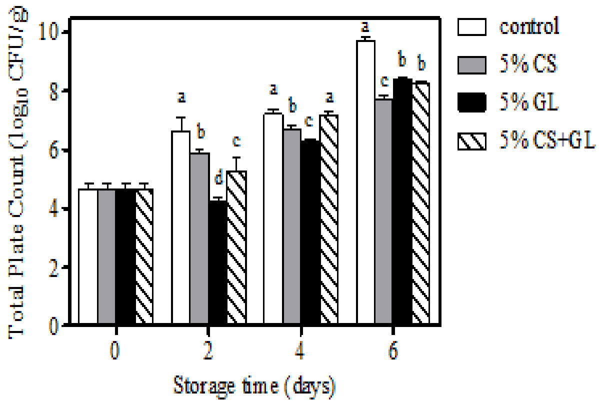

Figure 3. Total microbial count of mixed salads for Thai green papaya salad treated with capsicum oleores (CS), garlic oil (GL) and CS incorporated with GL (CS+GL) during refrigerated storage at 4±1°C. Data are the average of at least four replicates ± standard deviation. The different letters denote significance among treatments.

On day 6, the colour score of the control and 5% CS treated mixed salads was below the acceptable level whilst that of both 5% GL and 5% CS+GL treated mixed salads was above the acceptable level. The score of 5% CS+GL treated mixed salad was higher than that of 5% GL treated mixed salads but a significance of colour score of the both treatments was not found. The odour score of all treatments was also decreased throughout storage (Figure 2B). All treated mixed salads had odour score lower than the control; however, that of all treatments was above the acceptable level (score 2.2) over storage as well as no significant difference between treatments was found. The changes in overall appearance score as shown in Figure 2C was similar to the changes in the colour score during storage. This shows that the change in overall appearance score of the mixed salads was obviously depended upon the change in its colour score.

Ayala-Zavala and González-Aguilar (2010) studied the impact of garlic oil-containing sachets on the odour acceptance of fresh-cut tomatoes by using a 5-point hedonic scale. None of the tested concentrations of garlic oil encapsulated in sachets affected the general acceptance, which remained between the terms like moderately and neither like nor dislike, thus above the acceptance threshold.

Total microbial count

As shown in Figure 3, total microbial count of the mixed salads in all treatment on initial day was about 4.5 log_{10} CFU/g and increased during storage. In the control, the total microbial count increased higher than other treatments over storage and reached to 9.5 log_{10} CFU/g on day 6. Total microbial count of 5% GL treated the mixed salads was significantly lower

Table 1. ANOVA indicating the effect of natural oil and storage period on gas concentration inside package, sensory attributes and microbial growth

Parameter	Treatment (a)			Time (b)			a × b (Interaction)		
	DF	MS	S/NS	DF	MS	S/NS	DF	MS	S/NS
CO	3	2.73	***	3	14.03	***	9	0.82	***
O	3	0.80	***	3	13.45	***	9	0.39	***
CS	3	0.41	*	3	35.69	***	9	1.67	***
OS	3	8.35	***	3	50.30	***	9	1.45	***
OAS	3	2.49	NS	3	160.50	***	9	26.50	***
TPC	3	3.79	***	3	44.98	***	9	1.31	***
EC	3	7.44	***	3	16.31	***	9	2.05	***
SA	3	11.78	***	3	5.23	***	9	1.41	***

DF degree of freedom, MS mean sums of squares, S significance, NS non-significance, CD critical difference ($P \leq 0.01$), CO; gas carbon dioxide inside package, O; gas oxygen inside package, CS; colour scores, OS; odour scores, OAS; overall appearance scores, TPC; total plate count, EC; *E. coli*, SA; *S. aureus.*

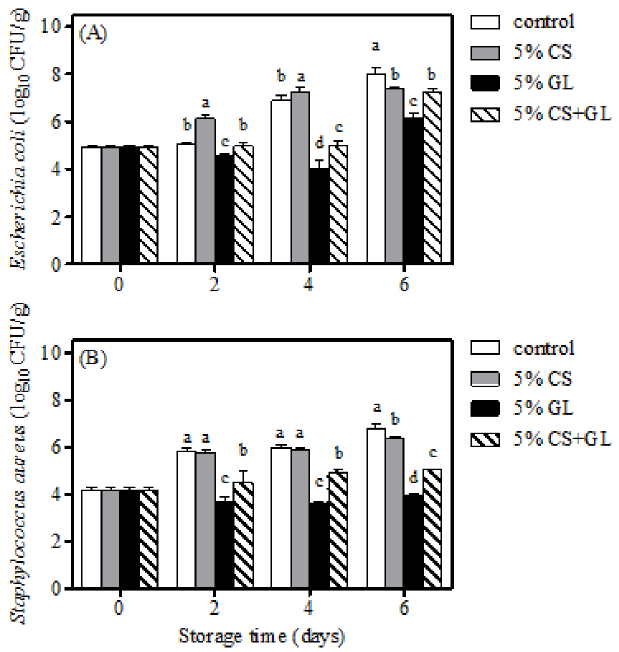

Figure 4. The growth of E. coli (A) and S. aureus (B) in mixed salads for Thai green papaya salad treated with capsicum oleores (CS), garlic oil (GL) and CS incorporated with GL (CS+GL) during refrigerated storage at 4±1°C. Data are the average of at least four replicates ± standard deviation. The different letters denote significance among treatments.

than that of others during storage for 4 days. On day 6, total microbial count of 5% GL and 5% CS+GL treated mixed salads were approximately 8 $\log_{10}$ CFU/g whilst that of 5% CS treated mixed salads was approximately 7.5 $\log_{10}$ CFU/g. These show that CS, GL and CS+GL could delay the increase in total microbial growth in the mixed salads during storage which 5% GL showed the best result when compared to others.

The growth of inoculated E. coli *and* S. aureus

E. coli and *S. aureus* at the concentration of 4 $\log_{10}$ CFU/g were inoculated in the mixed salads and the effects of CS, GL and CS+GL on inhibiting the growth of *E. coli* and *S. aureus* were investigated, as the result shown in Figure 4. During storage for 4 days, *E. coli* count of 5% GL treated mixed salads decreased whilst that of 5% CS+GL remained constant and that of both control and 5% CS treated mixed salads was increased markedly (Figure 4A). On day 6, *E. coli* count of all treatments obviously increased; however the lowest *E. coli* count was found in 5% GL treated mixed salads and the highest was found in the control. The growth of *S. aureus* was obviously inhibited by 5% GL which a slight decrease in *S. aureus* count was also detected during storage (Figure 4B). 5% CS+GL delayed the increase in *S. aureus* count in the mixed salads over storage whilst 5% CS could not inhibit the growth of *S. aureus* in the mixed salads when compared to that in the control. These show that GL potentially inhibit the growth of contaminated *E. coli* and *S. aureus* in salad. Among all the treatments, storage period and interaction of treatments and storage period, the change in microbial populations were statistically significant with *p*-value < 0.01, indicating that the growth rate varied depending upon the treatments applied (Table 1). Iorizzi *et al.* (2002) and Curtis *et al.* (2004) also suggested that among the different plant extracts screened, those from Allium showed high levels of antimicrobial activity towards plant pathogens. The major constituent of GL has been widely reported as allyl disulfide, and several reports attribute the antimicrobial activity of GL's sulfur constituents to the inactivation of key metabolic enzymes of microorganisms (Harris *et al.*, 2001;

Benkeblia, 2004).

Antimicrobial packaging slowed down the rate of the microorganism growth by 1 to 2 logarithmic cycle in *E. coli* and by 1 to 3 logarithmic cycle in *S. aureus* as compared with control. This result agrees with several other studies that have shown the inhibitory effect of phenolic compounds in plant essential oils. This compound is more effective on Gram-positive than on Gram-negative bacteria (Beuchat and Golden, 1989). Resistance of Gram-negative bacteria is usually associated with the presence of a lipopolysaccharide layer, which might be involved in reducing the sensitivity against those compounds (Sivaroonban *et al*., 2008).

The ANOVA table (given in Table 1) explained that all the main effects of treatment (natural oil; a), time (storage period; b) and a x b two factor interactions were strongly significant. Different treatment and time had a significant effect ($p < 0.00$) on gases concentration in package, sensorial attributes and microbial growth.

Conclusion

The GL and CS+GL could prevent the increase in CO_2 and decrease in O_2 in package and maintained sensorial quality attributed involving colour and visual appearance of the mixed salads better that CS during storage. For microbiological quality, CS, GL and CS+GL delayed the increased in total microbial count in the mixed salads. The GL treatment effectively inhibited the growth of *E. coli* and *S. aureus* in the mixed salads. We suggest that the use of GL is a suitable treatment controlling the changes in sensorial quality attributes and inhibiting the growth of certain foodborne pathogens such as *E. coli* and *S. aureus* contaminated in the mixed salads for Thai green papaya salad.

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