Use of *Rhodomyrtus tomentosa* ethanolic leaf extract for the bio-control of *Listeria monocytogenes* post-cooking contamination in cooked chicken meat

Grace Fiyinfoluwa Odedina^{1,2} · Kitiya Vongkamjan³ ·
Supayang Piyawan Voravuthikunchai^{1,2}

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Abstract Controlling foodborne pathogen in ready-to-eat food is important in food safety. The present study accessed the potential use of *Rhodomyrtus tomentosa* ethanolic leaf extract as a bio-control agent against *Listeria monocytogenes* in cooked chicken meat model system. The antilisterial activity of the plant extract was better under microwave condition and enhanced as storage temperature increased from 4 to 37 °C. The extract could reduce *L. monocytogenes* numbers at low (10^4 CFU/g) and high (10^6 CFU/g) inoculum levels in cooked chicken by both rinse and injection application methods. A 5 min rinse in 8% w/v *R. tomentosa* extract reduced the bacterial number by ≥ 2 -log before storage and ≥ 3 -log after storage at 4 °C for 5 days. Injection with 0.4% w/w *R. tomentosa* extract resulted in approximately 2-log reduction in the cell numbers both before and after storage at 4 °C for 5 days. Five minutes rinse in the extract bath demonstrated better sensory preferences which were not significantly different from the control. Addition of black pepper powder to the

extract rinsed samples improved odour but not appearance, colour, and texture preferences. *Rhodomyrtus tomentosa* extract was significantly effective for the bio-control of *L. monocytogenes* contaminations in cooked chicken meat model. The extract was observed as a potent bio-additive agent to control contaminations from *L. monocytogenes* and ensure safety in ready-to-eat meat.

Keywords *Listeria monocytogenes* · *Rhodomyrtus tomentosa* · Bio-control · Thermal stability · Ready-to-eat

Practical application

Rhodomyrtus tomentosa ethanolic leaf extract could be applied to raw meats intended for cooking under a microwave without losing stability. The extract could be used as a potent food bio-additive to ensure safety in ready-to-eat meat using either the rinse or injection delivery methods. Flavourful agents could improve preference of *R. tomentosa* extract treated meat.

Introduction

Consumption of foods contaminated with *L. monocytogenes* is majorly implicated in human listeriosis cases (WHO 2015). Listeriosis ranks as the leading cause of death attributed to foodborne illnesses in European Union member states with the highest hospitalization rate (99%) and highest case fatality rate (15.6%) (EFSA 2015) while it is estimated to be the third highest cause of foodborne disease related deaths in the United States (Doyle 2015). Incidence of listeriosis increased by 8.6% in European Union trends from 2012 to 2013 indicated by 1763

✉ Supayang Piyawan Voravuthikunchai
supayang.v@psu.ac.th

Grace Fiyinfoluwa Odedina
odedinagrace@gmail.com

Kitiya Vongkamjan
kitiya.v@psu.ac.th

¹ Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

² Excellence Research Laboratory on Natural Products, Faculty of Science and Natural Product Research Center of Excellence, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

³ Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand

confirmed cases and 191 deaths (EFSA 2015). A variety of ready-to-eat food products including fish, meat, poultry, and dairy have been associated with outbreaks (Knabel 2015; EFSA 2015). *Listeria monocytogenes* is capable of forming matured biofilm on food within 24 h (Srey et al. 2014), persisting on food processing equipments and/or slicing materials from which post-cooking contamination of ready-to-eat meat may occur. Moreover, this Gram-positive pathogen is capable of growing at refrigeration temperatures which is a major concern in refrigerated foods.

Furthermore, increasing resistance of *L. monocytogenes* to chemical food sanitizers including silver dihydrogen citrate (Masuku et al. 2014), fulvic acid-based and sodium silicate-based sanitizers (Zhu et al. 2014) as well as consumer awareness to the hazardous nature of chemical sanitizers have shifted research focus to biological sources of food additives.

Antimicrobial food additives from natural sources can serve as alternatives to increase safety of ready-to-eat meat and poultry products. These include essential oils, aqueous extracts and bioactive compounds from medicinal plants that have been used for thousands of years, especially in food preservation. Bio-preservatives from plant origin against *L. monocytogenes* have been widely studied. For example *Syzygium aromaticum*, *Cinnamomum cassia*, *Origanum vulgare*, and *Brassica nigra* extracts were evaluated as antimicrobials and antioxidants in raw chicken meat against *L. monocytogenes* (Babuskin et al. 2014). Essential oil from *Thymus capitata* was studied as a preservative in minced meat (El Abed et al. 2014), and *Vaccinium* spp. powder was used as a bio-control agent in cured cooked meat (Xi et al. 2011) against *L. monocytogenes*. However, sensory acceptability of food products is a major setback in certain plant antimicrobial applications. For instance, aqueous extract of *Brassica juncea* inhibited contaminating foodborne pathogens in situ but consistently affected the food odour in sniffing assay (Miceli et al. 2014). Sourcing for plant antimicrobials as food preservatives is therefore still ongoing.

Rhodomyrtus tomentosa (Aiton) Hassk is a medicinal flowering plant in the Myrtaceae family, native to South-east Asia. Ethanol extract from *R. tomentosa* leaves has been demonstrated to exhibit excellent potency against a wide range of Gram-positive bacteria (Limsuwan et al. 2009a, b), including food related pathogens such as *Bacillus cereus* (Voravuthikunchai et al. 2010), *Staphylococcus aureus* (Saising et al. 2011), and *L. monocytogenes* (Odedina et al. 2015) at low minimum inhibitory concentrations. The extract also showed the ability to reduce *B. cereus* viable cells and endospores significantly when applied in precooked rice and tuna steak model (Voravuthikunchai et al. 2010).

The study was therefore aimed to investigate the effects of *R. tomentosa* ethanol extract applied on *L. monocytogenes* in vitro and in ready-to-eat chicken meat model system. The extract was rinse and injection methods and sensory evaluation was conducted.

Materials and methods

Plant extract preparation

Rhodomyrtus tomentosa leaves (NPRC0057) deposited in the Faculty of Traditional Thai Medicine, Prince of Songkla University, Thailand, were extracted following a previous method (Limsuwan et al. 2009a, b). Dried leaves were grounded followed by extraction with 95% ethanol, and subsequent evaporation of the ethanol using a rotary evaporator. Stock solution was prepared by dissolving the extract in 90% ethanol (Ensure, Damstadt, Hesse, Germany) and stored at -20°C until use.

Bacterial strains, culture conditions and cell suspension

Three strains of *L. monocytogenes* were used in the study including PSU-KV-133 (isolated from food), FSL R2-574 (CDC isolate associated with outbreak in 1985, isolated from cheese) obtained from the Food Safety Lab, Cornell University, Ithaca, New York, and Scott A (clinical isolate from 1983 foodborne listeriosis outbreak) kindly provided by Prof. Dr. Catherine Nettles Cutter, Department of Food Science, Pennsylvania State University. Each strain kept at -80°C in Tryptic Soy Broth (TSB; Difco, Le Port de claix, France) supplemented with 30% glycerol was revived on Tryptic Soy Agar (TSA; Difco Le Port de claix, France) at 37°C for 24 h prior to use. The bacterial cells were separately cultivated in TSB containing 0.6% yeast extract (LabM, Lancashire, UK) (TSB-YE) and incubated at 37°C for 16 h. Broth culture of each strain was pelleted by centrifugation at 5000 rpm for 5 min and washed three times in equal volumes of sterile 0.85% normal saline solution (NSS; Emsure, Billerica, MA, USA). Pelleted cells were resuspended in NSS, adjusted to final concentrations of approximately 10^4 and 10^6 CFU/mL followed by mixing of each strain in ratios of 1:1:1 to produce *L. monocytogenes* cocktails used in the study.

Effects of cooking and storage temperatures on extract stability

The experiment was set up to study the effects of *R. tomentosa* ethanolic leaf extract against *L. monocytogenes* when subjected to various cooking and storage

temperatures following a slightly modified methodology (Temilade 2009). The concentration of the extract which significantly inhibited *L. monocytogenes* cells in 1 g of chicken meat as compared with the control ($P < 0.05$) after storage at 4 °C, for 5 days (0.1% w/v) was determined from a preliminary trial and used in the study. The extract was subjected to heat treatments by sterilization at 121 °C for 15 min, microwaving (Sharp, Chachoengsao, Thailand) at medium power for 3 min to achieve an inner temperature of 80 °C or heating at 65 °C for 30 min in a water bath (Julabo, Seelbach, Germany). The various treatments were cooled at normal room temperature, subsequently inoculated with *L. monocytogenes* at approximately 10^6 CFU/mL in 96-well microtitre plates (Thermo Fisher Scientific, Suzhou, Jiangsu, China) and incubated at 37, 28, and 4 °C, for 48 h. One hundred microliter aliquots of sampled at time 0, 24, and 48 h were serially diluted with NSS and counted on TSA plates following incubation at 37 °C for 24 h. Control cells incorporated with 1% ethanol were counted under the same conditions. The assay was set up in duplicates for three independent studies.

Preparation and inoculation of cooked chicken meat pieces

Fresh, lean, boneless chicken breast was purchased from grocery stores in Hat Yai, Thailand and transported to the laboratory immediately. The meat was cut manually into cubes of $1 \times 1 \times 1$ cm and weighed between 6 and 7 g. Chicken meat pieces were arranged into petri dishes, sprayed with distilled water to allow for easy removal after sterilization and sterilized by autoclaving at 121 °C for 15 min. Sterilized chicken meat samples weighed 5 g, were cooled at room temperature and aseptically spiked with *L. monocytogenes* cocktails at either 10^4 or 10^6 CFU/g of chicken meat. Cells were allowed to attach for 2 h in a flow cabinet. Negative control consisted of uninoculated chicken meat cube.

Chicken meat rinsing with *Rhodomyrtus tomentosa* ethanolic leaf extract

A slightly modified rinse method previously outlined by Higginbotham et al. (2014) was used. Inoculated cooked meat cubes were immersed in a bath incorporated with or without *R. tomentosa* ethanolic leaf extract for 5, 10, 15, and 20 min to achieve a final concentration of 0.4% w/w extract per gram of chicken meat. Based on preliminary trials, 5 g of chicken meat cubes could absorb approximately 250 µL of 8% w/v solution of *R. tomentosa* ethanolic leaf extract when immersed for 20 min, resulting in about 0.4% w/w of the extract per gram of chicken meat. Samples were allowed to air dry in a flow cabinet for 1 h,

packed individually into sterile bags, sealed, and stored at 4 °C for 5 days. Surviving *L. monocytogenes* cells were enumerated on day 0, 3, and 5 using serial dilution and plate count method.

Chicken meat injection with *Rhodomyrtus tomentosa* ethanolic leaf extract

Injection of chicken meat pieces was performed following a modified method (Yusop et al. 2012). Two hundred and fifty microliters of the extract and the control solutions were injected into inoculated chicken meat piece using a syringe and a needle. Chicken meat pieces were injected in random locations in order to mimic the multi-injector used in the modified method. The samples were allowed to air dry in a flow cabinet for 1 h, individually packaged into stomacher bags, sealed and stored at 4 °C for 5 days. Surviving cells of *L. monocytogenes* were enumerated on day 0, 3, and 5 using serial dilution and plate count method.

Microbiological analysis

Counts of *L. monocytogenes* from the meat cubes were analyzed by adding 5 mL of NSS to each sample in a stomacher bag, followed by homogenizing in a stomacher machine (Seward, England) at 230 rpm for 60 s. Aliquots were serially diluted appropriately and plated on both TSA and modified oxford agar (MOX; Oxoid, Basingstoke, England) to determine both *L. monocytogenes* counts and the presence of any sublethally injured cells. Tryptic soy agar plates and MOX agar plates were incubated at 37 °C, overnight and 30 °C for 48 h, respectively. The experiment was performed in duplicates for three independent studies.

Colour determination

Colour evaluation of control and treatments by both application methods was set up following a previous method (Mitumoto et al. 2005) with slight modifications. A colour meter (HunterLab ColorFlex, Reston, Virginia, USA) was used to determine chicken meat colour using the coordinates L^* (lightness), a^* (redness), and b^* (yellowness) after storage at 4 °C on day 0, 3, and 5. Colour readings from 3 replicates were taken from chicken meat samples. The average was determined and statistically analyzed.

Sensory evaluation

Sensory evaluation was performed using uninoculated chicken meat samples following a previous method (Lazaridou et al. 2007) with a slight modification. Chicken meat cubes were prepared as described earlier in the Food

Laboratory, Microbiology Department, Faculty of Science, Prince of Songkla University, Thailand. Five gram chicken meat cubes were rinsed without or with *R. tomentosa* ethanolic leaf extract at $\leq 0.4\%$ w/w for 5, 10, 15, and 20 min. Two set of samples were prepared. The first set consisted of treatments and control. Another set of samples included treatments and control meat pieces covered with black pepper powder. Black pepper powder helped in masking the appearance of control and treated samples. Prepared samples were coded with random numbers and stored in separately sealed containers at 4 °C for 5 days.

Stored samples warmed under medium power of a microwave for 3 min were served individually in small disposable cups. Twenty-five untrained panelists (males and females, aged 25–40 years) recruited from Prince of Songkla University, were asked to assess sensory evaluation on using a 9-point hedonic scale. Panelists were also asked to give comments on sample taste. A general sensory score was determined from the average of all the scores and meat samples were considered acceptable if their mean scores for overall acceptability were above 5 (neither like nor dislike).

Fig. 1 Effects of *Rhodommyrtus tomentosa* ethanolic leaf extract at 0.1% w/v, subjected to pasteurization (65 °C, 30 min) or microwave (inner temperature of 80 °C) conditions on growth of *Listeria monocytogenes* Scott A cells at different incubation temperatures, 37 °C (a); 28 °C (b); and 4 °C (c). The detection limit was 10^2 CFU/mL

Statistical analysis

Microsoft Excel 2007 (Microsoft, Redmond, WA, USA) was used to calculate means, standard deviations and construct graphs. Differences between more than two groups were evaluated using One-Way ANOVA (Analysis

of Variance) with Sidak post hoc test for data normally distributed and Dunnett's Cs post hoc test for data not normally distributed (IBM SPSS Statistics 20, NY, USA). A probability level of $P < 0.01$ for microbiological analysis and $P < 0.05$ for colour determination and sensory analysis were used in testing statistical significance.

Fig. 2 Effects 0.4% w/w of *Rhodomyrtus tomentosa* ethanolic leaf extract by rinse method on *Listeria monocytogenes* cocktail in cooked chicken meat at 10^4 CFU/g. Samples were stored at 4 °C for 5 days. Surviving cell numbers are represented as means with standard

deviation. Different lowercase and uppercase letters are significantly different ($P < 0.01$) between treatments within a storage period and within a treatment between storage periods, respectively. The detection limit was 10^2 CFU/mL.

Fig. 3 Effects of 0.4% w/w *Rhodomyrtus tomentosa* ethanolic leaf extract by rinse method on *Listeria monocytogenes* cocktail in cooked chicken meat at 10^6 CFU/g. Samples were stored at 4 °C for 5 days. Surviving cell numbers are represented as means with standard

deviation. Different lowercase and uppercase letters are significantly different ($P < 0.01$) between treatments within a storage period and within a treatment between storage periods, respectively. The detection limit was 10^2 CFU/mL.

Results and discussion

Effects of post-thermal treatments of *Rhodomyrtus tomentosa* ethanolic leaf extract on growth of *Listeria monocytogenes* at different incubation temperatures

The extract was subjected to various heat treatments that tend to initiate post-processing in the food industry. The extract could not withstand high temperature and pressure during sterilization at 121 °C for 15 min as indicated by precipitate formation. However, the extract was stable after subjecting to pasteurization (65 °C, 30 min) and microwave (inner temperature of 80 °C). Plant extract antilisterial activity was better under microwaving condition. Post-thermal treated extract by pasteurization and microwave could inactivate *L. monocytogenes* from 24 to 48 h (Fig. 1). Incubation temperatures showed significant ($P < 0.05$) effects on the reduction of *L. monocytogenes*. Antilisterial activity was enhanced as incubation temperature increased from 4 to 37 °C. A study, (Veldhuizen et al. 2007) found a great reduction in the antilisterial activity of carvacrol, a major component of thyme and oregano essential oil when incubation temperature reduced from 30 to 10 °C. Similarly, Solomakos et al. (2008) reported a stronger activity of thyme essential oil and nisin against *L. monocytogenes* contaminated on minced beef when incubation temperature was increased from 4 to 10 °C.

Effects of *Rhodomyrtus tomentosa* ethanolic leaf extract on *Listeria monocytogenes* in cooked chicken meat by rinse or injection application method

Rhodomyrtus tomentosa ethanolic leaf extract could reduce *L. monocytogenes* cell numbers at low (10^4 CFU/g) and high (10^6 CFU/g) inoculum levels by both application methods in cooked chicken meat over a storage period of 5 days at 4 °C (Figs. 2, 3, 4). Rinsing cooked meat in *R. tomentosa* ethanolic leaf extract bath for 5 min resulted in reduction of the bacterial cell numbers by at least 2-log unit at time 0, before storage for both low and high levels of inoculum (Figs. 2, 3). After storage at 4 °C for 5 days, a 3-log reduction was observed for high *L. monocytogenes* contamination (Fig. 3), while inhibition was maintained beyond the detection limit for low inoculum contamination (Fig. 2). Comparing the rinse and injection methods for extract application in cooked chicken meat model, rinse method was more effective against *L. monocytogenes* at high inoculum level. Rinsing cooked meat in the extract for 20 min which was equivalent to injection of extract into chicken meat at 0.4% w/w could reduce bacterial cell numbers by greater than 2-log unit before storage and beyond the detection limit (>4-log unit reduction) after

storage at 4 °C for 3 days (Fig. 3). Injection of cooked meat pieces with the extract at the same concentration yielded approximately 2-log reduction in cell numbers before storage and after storage at 4 °C for 3 days (Fig. 4). The rinse application method was more effective especially for higher inoculum contamination of cooked chicken meat. Since post cooking contamination is likely to introduce *L. monocytogenes* on chicken meat surface, it was expected that the rinse method would be more effective because of the homogeneous distribution of plant extract on sample surface as opposed to introduction into cooked chicken meat cubes in the injection method. Interestingly, cells of *L. monocytogenes* were not sublethally injured but killed by the extract. Surviving bacterial cells recovered on TSA were not significantly different ($P > 0.05$) from cell numbers recovered on MOX agar. The results demonstrated that *R. tomentosa* ethanolic leaf extract possess

Fig. 4 Effects of 0.4% w/w *Rhodomyrtus tomentosa* ethanolic leaf extract by injection method on *Listeria monocytogenes* cocktail in cooked chicken meat at 10^4 CFU/g (a) and 10^6 CFU/g (b). Samples were stored at 4 °C for 5 days. Surviving cell numbers are represented as means with standard deviation. Different lowercase and uppercase letters are significantly different ($P < 0.01$) between treatments within a storage period and within a treatment between storage periods, respectively. The detection limit was 10^2 CFU/mL

excellent antilisterial properties. The extract is rich in bioactive components including rhodomyrtone, displaying antibacterial potency against a wide range of Gram-positive organisms (Limsuwan et al. 2009a, b) and flavonoid compounds which has earned recognition as free radical scavenging and antioxidant agents (Lavanya et al. 2012). Previous in situ model system which rinsed hot dog contaminated with *L. monocytogenes* in a bath of 12% w/v *Hibiscus sabdariffa* calyces extract found 1.8-log and 2-log reduction in the bacterial cell numbers after rinsing for 60 min, before storage and after storage at 4 °C for 24 h, respectively (Higginbotham et al. 2014). In the present study, rinsing cooked chicken meat in 8% w/v *R. tomentosa* ethanolic leaf extract resulted in 3-log and >4-log unit (beyond the detection limit) reduction in *L. monocytogenes* cell numbers after rinsing for 15 min, before storage, and after storage at 4 °C for 5 days, respectively (Fig. 3). Furthermore, Solomakos et al. (2008) reported a 2-log unit reduction in *L. monocytogenes* cells when thyme essential oil at 0.6% v/w was applied in minced beef and stored for 2 days at 4 °C. In our study, using the injection method of application (Fig. 4), *R. tomentosa* ethanol extract at 0.4% w/w inactivated *L. monocytogenes* cells on chicken meat by approximately 2-log unit at the initial time before storage.

Colour determination

The L^* value which represents lightness value was higher in samples injected with *R. tomentosa* ethanolic leaf extract than samples rinsed with the extract (Table 1). Injection

introduced plant extract into chicken meat pieces, while rinsing allowed a uniform absorption of extract on chicken meat surfaces. However, there was no significant difference ($P > 0.05$) in L^* values of meat samples as the rinse time increased. Lower lightness value of treatments as compared with the control may be an impact from chlorophyll pigmentation on the green leaves of *R. tomentosa*. Furthermore, average redness (a^*) values and yellowness (b^*) values of treatments were significantly higher than the control ($P < 0.05$). Higher a^* and b^* values observed with the treatments may be attributed to the carotenoid and flavonoid components of *R. tomentosa* leaves detected by the colour meter. The extract has been reported with abundant flavonoid components and tagged as a potent antioxidant (Lavanya et al. 2012). Carotenoid component can also act against lipid oxidation of meat stored under aerobic condition (Muthukumar et al. 2014). There was no significant difference ($P > 0.05$) in the colour of treated meat samples over storage time.

Sensory evaluation

All samples rinsed with the extract attained acceptance limit for overall acceptability (Table 2) set to be above an average score of 5 (neither like nor dislike) (Lazaridou et al. 2007). However, control samples were scored higher ($P < 0.05$) than the treatments. The panelists could be more familiar with chicken meat without the extract since rinse method impacted a light golden green colour on samples, contrasting the whitish colour of untreated meat pieces. However, there was no significant difference ($P > 0.05$) in

Table 1 Objective colour L^* , a^* , b^* values of cooked chicken meat treated with *Rhodomyrtus tomentosa* ethanolic leaf extract at 0.4% and stored at 4 °C

Colour parameters	Day of storage	Treatments						Overall mean (days)
		Control	Injection	Rinse 5 min	Rinse 10 min	Rinse 15 min	Rinse 20 min	
L^*	0	83.48 ± 0.68	73.51 ± 1.05	56.59 ± 3.44	56.48 ± 5.50	52.93 ± 4.06	54.88 ± 1.42	58.88 [#]
	3	85.91 ± 0.68	62.40 ± 0.47	57.00 ± 2.60	59.57 ± 4.80	51.35 ± 4.70	53.36 ± 1.04	61.60 [#]
	5	83.04 ± 0.85	66.37 ± 5.47	61.29 ± 2.31	53.79 ± 3.23	56.67 ± 1.30	52.50 ± 1.76	62.28 [#]
	Overall mean (treatments)	84.47 ^a	67.43 ^b	58.30 ^c	56.61 ^c	53.65 ^c	53.58 ^c	
a^*	0	0.56 ± 0.41	1.74 ± 0.40	3.72 ± 0.07	3.70 ± 1.02	3.42 ± 1.61	4.27 ± 0.33	2.90 [#]
	3	1.17 ± 0.22	3.28 ± 1.16	3.28 ± 0.39	4.17 ± 0.03	4.15 ± 1.09	3.87 ± 0.67	3.32 [#]
	5	1.05 ± 0.53	3.01 ± 0.53	2.53 ± 0.17	3.48 ± 0.31	4.00 ± 0.46	4.20 ± 0.19	3.04 [#]
	Overall mean (treatments)	0.93 ^a	2.68 ^b	3.18 ^b	3.78 ^b	3.86 ^b	4.11 ^b	
b^*	0	15.27 ± 1.77	26.97 ± 3.67	35.49 ± 3.59	35.81 ± 4.56	33.85 ± 3.46	36.30 ± 0.99	30.61 [#]
	3	14.93 ± 0.24	27.03 ± 3.26	32.60 ± 1.10	33.06 ± 6.76	34.27 ± 5.21	34.89 ± 2.37	29.46 [#]
	5	14.59 ± 0.45	23.85 ± 2.92	34.02 ± 1.62	32.74 ± 2.16	37.77 ± 4.93	36.15 ± 3.87	29.85 [#]
	Overall mean (treatments)	14.93 ^a	25.95 ^b	34.04 ^c	33.87 ^c	35.30 ^c	35.78 ^c	

L^* = lightness; a^* = redness; b^* = yellowness

^{a-c} Mean ± standard deviation. Different letters within the same row are significantly different ($P < 0.05$)

[#] Mean ± standard deviation. Different letters within the same column are significantly different ($P < 0.05$)

Table 2 Sensory scores of cooked chicken meat rinsed with *Rhodomomyrtus tomentosa* ethanolic leaf extract at various time intervals, covered with or without black pepper powder and stored at 4 °C for 5 days ($n = 25$)

Sensory attributes	Rinse time (min)		<i>Rhodomomyrtus tomentosa</i> + black pepper							
	<i>Rhodomomyrtus tomentosa</i>									
	0	5	10	15	20	0	5	10	15	20
Appearance	6.72 ± 0.35 ^a	6.38 ± 0.27 ^a	5.79 ± 0.36 ^{ab}	5.90 ± 0.37 ^{ac}	5.86 ± 0.34 ^{ad}	4.33 ± 0.41 ^{bcd}	4.07 ± 0.37 ^b	4.13 ± 0.39 ^{bc}	4.12 ± 0.38 ^b	4.20 ± 0.47 ^{bcd}
Colour	6.67 ± 0.29 ^a	5.85 ± 0.31 ^a	5.56 ± 0.40 ^a	6.00 ± 0.33 ^a	5.85 ± 0.37 ^a	3.59 ± 0.44 ^b	3.42 ± 0.28 ^b	3.35 ± 0.31 ^b	3.60 ± 0.38 ^b	3.41 ± 0.30 ^b
Odour	6.32 ± 0.41 ^a	4.69 ± 0.41 ^{ab}	4.00 ± 0.53 ^b	4.39 ± 0.50 ^{ab}	4.56 ± 0.47 ^{ab}	4.94 ± 0.52 ^{ab}	3.93 ± 0.42 ^b	4.77 ± 0.55 ^{ab}	5.00 ± 0.63 ^{ab}	4.24 ± 0.45 ^{ab}
Texture	6.25 ± 0.35 ^a	4.81 ± 0.44 ^{ab}	4.67 ± 0.44 ^{ab}	5.07 ± 0.43 ^{ab}	5.00 ± 0.49 ^{ab}	4.18 ± 0.41 ^b	3.47 ± 0.34 ^b	3.95 ± 0.39 ^b	4.56 ± 0.43 ^{ab}	3.88 ± 0.42 ^b
Overall acceptability	6.60 ± 0.28 ^a	5.36 ± 0.17 ^b	5.03 ± 0.24 ^{bde}	5.09 ± 0.24 ^{bde}	5.15 ± 0.30 ^{bd}	4.22 ± 0.31 ^{bcde}	3.65 ± 0.28 ^c	3.91 ± 0.27 ^{cd}	3.96 ± 0.33 ^{cd}	3.83 ± 0.29 ^{ce}

Scores of sensory evaluation were based on a nine-point hedonic scale (1 = extremely dislike; 5 = neither like nor dislike; 9 = extremely like)

Acceptable limit = score above 5 (neither like nor dislike)

^{a–e} Mean ± standard error. Different letters within the same row are significantly different ($P < 0.05$) from the control

the average preference scores for appearance and colour. Treatments were scored an approximate value of 6, while controls had an approximate value of 7. Overall, preference scores reduced for odour and texture in both control and treatments when compared with other sensory attributes. While preference scores of control samples reduced from odour to texture, average preference scores attributed to texture were generally higher than odour for treatments. Treated samples predominantly attained the acceptance limit or an average preference score of 4.5 for all sensory attributes, except for preference score of odour. This exception was found in samples rinsed with the extract for 10 and 15 min. However, both samples received increased scores beyond the average score when black pepper powder was incorporated on the surface. Although black pepper powder could improve odour preference of samples rinsed with the extract for 10 and 15 min, it caused a significant decrease ($P < 0.05$) in preference scores for appearance and colour in both control and treatments. Nevertheless, no significant effect ($P > 0.05$) on preference scores for texture of treated samples was observed. Reduced preference scores for appearance and colour of black pepper incorporated chicken meat may be due to unfamiliarity of panelists with the resulting black colour. The aroma from black pepper powder may have improved the odour preferences of samples. Overall, the results suggest that preference of chicken meat rinsed with the extract may be positively or negatively influenced by incorporation of flavourful components such as black pepper. A similar observation was reported by Du et al. (2012); Carvacrol at 0.5% incorporated in edible apple film reduced the preference of wrapped cooked chicken meat, while the same concentration of carvacrol in edible tomato film showed no effect on preference of wrapped cooked chicken meat. Comments from untrained panelists indicated that the extract had a slightly bitter taste on chicken piece surface. However, there was no significant difference ($P > 0.05$) in taste preference of black pepper incorporated control and extract rinsed meat piece (data not shown). Information from sensory evaluation in the present study was in agreement with Sun-Waterhouse and Wadhwa (2013). This review proposed that minimizing bitter taste from bio-active phytochemicals in food application could be achieved through industry relevant approaches, including incorporation of taste enhancing flavouring agents amidst others.

Conclusion

The results from the present study indicated that *R. tomentosa* ethanolic leaf extract exhibited potent antibacterial activity against *L. monocytogenes* in situ using cooked chicken meat model system. Both the rinse and

injection methods were effective for application in chicken meat model. However, the rinse method was the more effective, especially for higher *L. monocytogenes* inoculum contamination in chicken meat. Since post-processing contamination is likely to introduce *L. monocytogenes* on chicken meat surface, it was expected that the rinse method would allow for more effective delivery of plant extract to meat piece surfaces. Sensory attributes showed higher preferences at 5 min rinse time and was not significantly different ($P > 0.05$) from the control. This observation indicates that 5 min rinse time in *R. tomentosa* extract could be appropriate application in chicken meat. Overall, *R. tomentosa* ethanolic leaf extract could serve as a potent food bio-additive to ensure safety in ready-to-eat meat. In addition, the eating quality of extract rinsed cooked chicken meat could be improved by the incorporation of appropriate flavourful agent.

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Author contributions All the authors designed the study and interpreted the results. GFO completed the experimental work and drafted the manuscript. SPV and KV supervised and provided expertise throughout the process. All the authors reviewed and contributed to the scientific contents of the paper.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest with any organization regarding the materials and details discussed in the manuscript.

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