Quality Assurance and Safety of Crops & Foods

ICC MoniQA

Quality Assurance and Safety of Crops & Foods 2012, **4**, 33–40

ORIGINAL ARTICLE

Bactericidal effects of roselle (*Hibiscus sabdariffa*) against foodborne pathogens *in vitro* and on romaine lettuce and alfalfa sprouts

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Key words
bactericidal effects; *Escherichia coli* O157:H7; fresh produce; *Listeria monocytogenes*; roselle; *Salmonella enterica*.

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Author's Roles
Divya Jaroni designed and performed research study, collected and analyzed data, provided roselle calyces and leaves, and wrote the paper.
Sadhana Ravishankar designed and performed research study and collected data.

Received 20 December 2010; Revised 4 August 2011; Accepted 4 August 2011

doi: 10.1111/j.1757-837X.2011.00117.x

Abstract

Introduction An increased fresh-produce consumption trend accompanied by increased associated outbreaks suggests a need for better intervention strategies. Limitations of chemical sanitizers have necessitated alternative strategies. Research on antimicrobial properties of roselle against food pathogens is limited. *Objectives* The study aims to investigate *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes* survival in roselle calyx aqueous (RCA) or roselle leaf aqueous (RLA) extracts over 72 h at 4, 8 and 25 °C; bactericidal effects of roselle calyx concentrate (RCC) and roselle tea (RT) against *E. coli* O157:H7 on lettuce and of RCC against *Salmonella* on alfalfa sprouts. *Methods* Microbiological analyses and preparation of RCA, RLA, RCC and RT were done according to standard and established methods. *Results* No *E. coli* O157:H7 and *Salmonella* survivors were detected in RCA or RLA at 24 h and all temperatures. *L. monocytogenes* population was reduced by 5 and 3 logs in RCA and RLA, respectively, at 24 h and all temperatures; by 4–6 logs at 4 °C and 8 °C and to undetectable levels at 25 °C, at 48 h. At 24 h, *E. coli* O157:H7 and *Salmonella* were not detected on RCC- or RT-treated lettuce or sprouts. *Conclusion* These observations suggest the application of roselle extracts as potential antimicrobials in foods.

Jaroni D, Ravishankar S (2012). Bactericidal effects of roselle (*Hibiscus sabdariffa*) against foodborne pathogens *in vitro* and on romaine lettuce and alfalfa sprouts. *Quality Assurance and Safety of Crops & Foods*, **4**, 33–40.

Introduction

According to the new estimates from Centers for Disease Control and Prevention (CDC), foodborne illnesses affect 48 million people, resulting in 128,000 hospitalizations, and 3000 deaths annually in the United States (Scallan *et al.*, 2011). Some important pathogens attributed to foodborne outbreaks include *Salmonella enterica*, *Escherichia coli* O157:H7, *Campylobacter* spp. and *Listeria monocytogenes* (Castillo & Rodriguez-Garcia, 2004). The CDC estimates that 3.6 million (39%) foodborne illnesses are caused by bacteria, with nontyphoidal *Salmonella* spp. (1.0 million, 11%) causing the most illnesses. Out of the 128 000 hospitalizations, 64% are caused by bacteria, with the leading causes of hospitalization being nontyphoidal *Salmonella* spp. (35%), *Campylobacter* spp. (15%) and *E. coli* O157:H7 (4%) (Scallan *et al.*, 2011). An estimated 1632 foodborne cases of listeriosis occur in the United States annually,

resulting in 266 deaths (16%), and about $200 million in monetary losses (CDC, 2002).

Consumption of ready-to-eat, fresh, and/or minimally processed fruits and vegetables has increased over the last several years due to a more health-conscious consumer. However, this trend has also been accompanied with an increase in the reported outbreaks of foodborne illnesses associated with fresh produce. *E. coli* O157:H7 and *S. enterica* alone are responsible for about 61% of all produce-related illnesses (Olsen *et al.*, 2000). Although control measures are in place in the fresh produce industry, recent foodborne outbreaks related to fresh produce suggest a critical need for better intervention strategies.

Interventions currently used by the fresh produce industry include chemical sanitizers, the most common being 50–200 ppm chlorine. However, studies show that chlorine is not very effective in reducing bacterial populations on fresh produce (Brackett, 1992; Beuchat, 1999; Cherry, 1999; Taormina & Beuchat, 1999). Additionally, chlorine is rapidly inactivated by organic matter and can even react with it to form carcinogenic compounds (chloramines, trihalomethanes) (Dychdala, 1991; Gallard & Gunten, 2002; Komulainen, 2004).

Limitations and drawbacks of traditional sanitizers, combined with the fact that consumers today prefer a 'natural' product, have led to an increased research interest in assessing natural intervention approaches as an alternative for the fresh produce industry. Research in this area has revealed a strong possibility that natural antimicrobials can replace traditional sanitizing agents, in addition to providing other health benefits (Graham, 1997; Cherry, 1999). Plant antimicrobials, including extracts and essential oils of herbs and spices have been shown to exhibit antibacterial effects in numerous studies (Gupta & Ravishankar, 2005; Uhart *et al.*, 2006; Ravishankar *et al.*, 2008, 2009, 2010; Friedman *et al.*, 2009). These herbs and spices, which are considered to be GRAS (Generally Regarded As Safe) substances, are becoming widely popular in the food industry.

Roselle (*Hibiscus sabdariffa*) is an edible plant with its seeds, leaves, fruits and roots used in various applications including foods. Among them, the most popular are the fleshy red calyces used for making wine, juice, jam, syrup, pudding, cakes, ice cream or tea. Roselle is also known for its antiseptic, diuretic, antioxidant and antimutagenic properties (Chewonarin *et al.*, 1999; Onyenekwe *et al.*, 1999; Salleh *et al.*, 2002). The dried flowers of this plant contain gossipetine and hibiscin (anthocyanins); the petals yield glucoside hibiscritin (flavanol); and the calyces are rich in riboflavin, ascorbic acid, niacin, carotene, calcium and iron (Duke, 1983).

Although much is known about its medicinal and nutritional properties, limited information is available on the antimicrobial properties of roselle, especially against foodborne pathogens. Liu *et al.* (2005) reported the *in vitro* inhibitory effects of roselle calyx on nosocomial pathogens responsible for bloodstream infections in Taiwan. Olaleye (2007) also determined the minimum inhibitory concentrations of the methanolic extract of roselle against nosocomial pathogens. Chao & Yin (2009) examined the antibacterial effects of roselle calyx aqueous (RCA) extracts against foodborne pathogens in ground beef and apple juice at 25 °C (room temperature). The objectives of the present study were to investigate: (a) the fate of *E. coli* O157:H7, *S. enterica* serotype Newport and *L. monocytogenes* in aqueous extracts of roselle calyx and leaf; (b) bactericidal effects of roselle calyx concentrate and tea against *E. coli* O157:H7 on romaine lettuce; and (c) bactericidal effects of roselle calyx concentrate against *S.* Newport in alfalfa sprouts.

Materials and methods

Bacterial cultures and media

The test bacteria included in the study were *E. coli* O157:H7 (ATCC® 43888 for *in vitro* studies and a cocktail of 3 strains-F4546, 960218, SEA13B88 for studies with lettuce), *S. enterica* serotype Newport (for both *in vitro* and sprouts studies) and *L. monocytogenes* (strain LM101M for *in vitro* studies only). Frozen cultures (−70 °C; in 20% glycerol) of test pathogens were sub-cultured into tryptic soy broth (TSB; Difco/Becton Dickinson, Sparks, MD, USA) and incubated for 18–24 h at 37 °C. Three transfers were done before a working overnight culture was prepared from subcultures. Overnight cultures were prepared by inoculating 0.1 mL of the subculture into 10 mL fresh TSB and incubating for 18–24 h at 37 °C. Buffered peptone water (BPW; Difco/Becton Dickinson) was used as the diluent in all experiments. Following each treatment, viable organisms were enumerated by plating on sorbitol MacConkey (SMAC; Difco/Becton Dickinson) agar for *E. coli* O157:H7, xylose lysine desoxycholate (XLD; Difco/Becton Dickinson) agar for *S. enterica*, and modified Oxford formulation (MOX; Difco/Becton Dickinson) agar for *L. monocytogenes.*

Preparation of aqueous extracts of roselle calyces and leaves for *in vitro* studies

Fresh roselle calyces and leaves were obtained from the hibiscus farm at Southern University Agricultural Research and

Extension Center (SUAREC), Baton Rouge, LA. Aqueous extracts of calyces (RCA) and leaves (RLA) were prepared as described by Liu *et al.* (2005) with slight modifications. A 50-g edible portion of roselle calyx or leaf was thoroughly washed in de-ionized water three times and kept on clean dry paper towels for 5 min to drain the excess water and facilitate drying of the samples. The calyces or leaves were then kept under UV light for 30 min to reduce background microflora, and blended with equal amounts of sterile de-ionized water (50 g in 50 mL) using a Waring blender. The aqueous extract thus prepared was used for *in vitro* studies.

Preparation of roselle calyx concentrate

Fresh roselle calyces obtained from the SUAREC farm were washed thoroughly in running tap water with a final rinse in de-ionized water. To prepare the concentrate, aqueous extract was prepared as described earlier with the exception that the resulting homogenate was boiled for 5 min. A 50-g edible portion of the calyces was added to 50 mL of sterile de-ionized water and homogenized in a Waring blender, followed by boiling the homogenate for 5 min. The homogenate was then cooled and filtered through four layers of sterile cheesecloth to obtain the concentrate. The concentrate was stored at 4 °C until further use. The concentrate thus prepared was used at 100% concentration (v/v) for the studies in fresh produce.

Preparation of roselle tea from dried calyces

Twenty grams of dried roselle calyx was ground to a fine powder and 300 mL of de-ionized water was added. The mixture was then boiled for 5 min to prepare a strong concoction and filtered through four layers of sterile cheesecloth to obtain the tea. The tea was used at 100% (v/v) concentration in experiments with lettuce.

Determination of *in vitro* antimicrobial activity of roselle calyx and leaf aqueous extracts

Fifteen grams each of the prepared aqueous extract of roselle calyx (RCA) or leaf (RLA) was dispensed into stomacher bags (Whirl Pak, Nasco, Fort Atkinson, WI, USA). Approximately 150 μL of the overnight culture [10^9 colony forming units (CFU) mL^{-1}] of one of the test pathogens (*E. coli* O157:H7, *S.* Newport or *L. monocytogenes*) was inoculated into the RCA or RLA extract and mixed well. Samples for each test pathogen were taken immediately after inoculation into the aqueous extracts to obtain data for 0 h. Controls were prepared by inoculating 150 μL of the test pathogen into 15 mL TSB and sampled immediately for 0 h. The treated and control samples for each test pathogen were then stored either at 4, 8 or 25 °C (room temperature). Samples for enumeration of bacterial survivors were taken at 24, 48 and 72 h. Serial dilutions were carried out in BPW and aliquots plated on selective agars, as described previously.

Determination of antimicrobial activity of roselle calyx concentrate and tea against *E. coli* O157:H7 on romaine lettuce

Romaine lettuce was obtained from the local grocery stores in Tucson, AZ, and outer leaves were discarded. Individual lettuce leaves were washed thoroughly under running tap water to remove any soil or other organic matter present on the leaves, followed by a final rinse in sterile de-ionized water. Approximately 10-g sample of the lettuce was inoculated with 0.1 mL of *E. coli* O157:H7 cocktail culture (to obtain 10^6 CFU g^{-1}; diluted from overnight culture) on the outer surface of the leaves and dried under a bio-safety hood for 20 min to allow for bacterial attachment to the surface. The inoculated lettuce samples (10 g each) were treated with 2 mL sterile de-ionized water as control and 2 mL roselle calyx concentrate or tea (100%; v/v). The leaf samples were placed in a stomacher bag and the entire leaf surface was covered by one of the treatments, agitated gently for 2 min and then drained. To enumerate the survivors, one 10-g sample of lettuce for each treatment was taken immediately (0 h) and the other after storage at 4 °C for 24 h. Samples were stomached (Seward, London, UK) in 90 mL BPW at normal speed for 1 min. Dilutions were done in BPW as needed. Plating was done on SMAC agar and survivors were enumerated after incubation at 37 °C for 18–24 h.

Determination of antimicrobial activity of roselle calyx concentrate against *S.* Newport in alfalfa sprouts

Alfalfa sprouts were purchased from the local grocery stores in Tucson, AZ, washed three times in de-ionized water and kept under UV light in a bio-safety hood for 30 min to reduce background microflora. Thirty grams of the sprout samples were inoculated by immersing them for 2 min in 200 mL of *S.* Newport culture (10^6 CFU g^{-1}, diluted from overnight culture), drained and dried under the bio-safety hood for 30 min for bacterial attachment. The sprouts were

sampled immediately after inoculation to obtain the initial counts on sprouts (positive control) before treatment. Inoculated sprout samples (15 g each) were then immersed either in 30 mL of 100% (v/v) roselle calyx concentrate, prepared as described earlier, or in 30 mL of de-ionized water for control. These were then dispensed in 5 g quantities into stomacher bags and one batch of treated and control samples was taken at 0 h to obtain the bacterial counts soon after treatment. The remaining samples were then stored at 4 °C and survival of *S.* Newport was determined at 24 h and 48 h. For enumeration, 5 g alfalfa sprouts were mixed with 95 mL BPW in a stomacher bag and stomached at normal speed for 1 min. Further dilutions were made in BPW and samples were plated on XLD agar. Plates were incubated at 37 °C for 24 h and CFUs were counted.

Statistical analysis

All the studies (*in vitro* and fresh produce) were repeated three times. Within each study/experiment, one replicate per sample was performed. The data, obtained as bacterial counts (CFU mL^{-1} or CFU/g), were log transformed prior to analysis and mean and standard deviations (±SD) were calculated for each treatment at various temperatures and time points. As *E. coli* O157:H7 and *S.* Newport populations were below the detection limits (less than 1.0 log CFU mL^{-1}), CFU counts could not be obtained for the two test pathogens at 24 h and 48 h. The experimental design for *in vitro* study was a $3 \times 3 \times 3$ factorial with three treatments (Control, RCA, RLA), three temperatures (4, 8 and 25 °C) and three times (0, 24, 48 h). Data for *in vitro* studies were analyzed using PROC GLM (SAS 9.3, SAS Institute Inc., Cary, NC, USA) and treatments, temperatures and times were included as main effects in the model. All two-way and three-way interactions were also included in the analysis. Significant differences were calculated at $P < 0.0001$. For studies in fresh produce, mean ± SD for the surviving bacterial populations is reported in Figures 1 and 2. Error bars are included in the figures.

Results and discussion

Over the past two decades, foodborne outbreaks associated with fresh produce including lettuce and alfalfa sprouts have been on the rise in the United States. Although commercial antimicrobial/sanitizing treatments are in place, the efficacy and safety of these treatments are often questioned. To explore the possibility of an alternative treatment that could

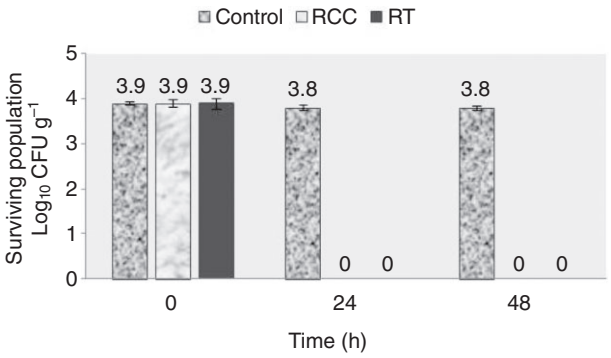

Figure 1 Bactericidal effects of roselle calyx concentrate (RCC) and roselle tea (RT) against *Escherichia coli* O157:H7 (Log_{10} CFU g^{-1}) on romaine lettuce at 4 °C.

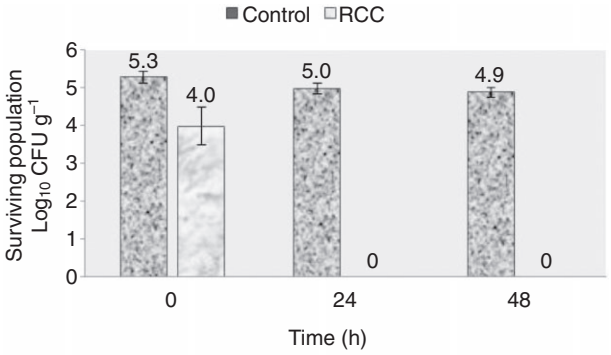

Figure 2 Bactericidal effects of roselle calyx concentrate (RCC) against *Salmonella enterica* Newport (Log_{10} CFU g^{-1}) on alfalfa sprouts at 4 °C.

be safe and effective, we examined the *in vitro* bactericidal effects of RCA and RLA extracts on three major foodborne pathogens, *E. coli* O157:H7, *S.* Newport and *L. monocytogenes*, over 72 h in fresh RCA and RLA extracts at refrigeration (4 °C), abuse (8 °C) and room (25 °C) temperatures. We also investigated the antibacterial effects of roselle calyx concentrate and tea against *E. coli* O157:H7 and *S.* Newport on lettuce and alfalfa sprouts. Surviving populations of the test bacteria were monitored at 0, 24 and 48 h to measure the bactericidal effects of roselle calyx concentrate and tea over time.

In vitro antimicrobial activity of RCA and RLA extracts against *E. coli* O157:H7, *S.* Newport and *L. monocytogenes*

Results for the *in vitro* antibacterial effects of RCA and RLA extracts against *L. monocytogenes*, *E. coli* O157:H7 and *S.* Newport are presented in Tables 1–3, respectively. Table 1

Table 1 Bactericidal activity of roselle calyx and leaf aqueous extracts against *Listeria monocytogenes* (Log_{10} CFU mL^{-1}) at 4, 8 and 25 °C

		L. monocytogenes population (Log_{10} CFU mL^{-1}) at time (h)			
Treatment	Temperature (°C)	0	24	48	72
Control	4	7.2 ± 0.1[a]	7.1 ± 0.1[a]	7.2 ± 0.0[a]	7.2 ± 0.0[a]
	8	7.2 ± 0.1[a]	7.2 ± 0.1[a]	7.4 ± 0.2[a]	8.0 ± 0.4[ac]
	25	7.2 ± 0.1[a]	9.1 ± 0.1[c]	9.3 ± 0.1[c]	9.2 ± 0.0[c]
RCA	4	7.0 ± 0.3[a]	1.4 ± 1.2[b]	1.5 ± 1.5[b]	ND[b]
	8	7.0 ± 0.3[a]	1.9 ± 0.4[b]	1.0 ± 0.3[b]	ND[b]
	25	7.0 ± 0.3[a]	0.5 ± 0.9[b]	ND[b]	ND[b]
RLA	4	6.9 ± 0.4[a]	2.7 ± 1.1[b]	1.3 ± 2.3[b]	ND[b]
	8	6.9 ± 0.4[a]	3.6 ± 0.5[b]	1.4 ± 1.2[b]	ND[b]
	25	6.9 ± 0.4[a]	1.9 ± 1.8[b]	ND[b]	ND[b]

ND, no detectable survivors (<1.0 log_{10} CFU mL^{-1}); RCA, roselle calyx aqueous extract; RLA, roselle leaf aqueous extract.
Data expressed as mean ± SD for three separate trials. Values with different superscript letters (a–c) are significantly different ($P < 0.0001$).

Table 2 Bactericidal activity of roselle calyx and leaf aqueous extracts against *Escherichia coli* O157:H7 (Log_{10} CFU mL^{-1}) at 4, 8 and 25 °C

		E. coli O157:H7 population (Log_{10} CFU mL^{-1}) at time (h)		
Treatment	Temperature (°C)	0	24	48
Control	4	6.9 ± 0.1	6.9 ± 0.1	6.7 ± 0.3
	8	6.8 ± 0.1	6.6 ± 0.3	7.0 ± 0.3
	25	7.0 ± 0.2	7.1 ± 0.5	7.1 ± 0.1
RCA	4	6.9 ± 0.2	ND	ND
	8	6.9 ± 0.2	ND	ND
	25	6.8 ± 0.1	ND	ND
RLA	4	6.6 ± 0.7	ND	ND
	8	6.7 ± 0.6	ND	ND
	25	6.7 ± 0.4	ND	ND

ND, no detectable survivors (<1.0 log_{10} CFU mL^{-1}); RCA, roselle calyx aqueous extract; RLA, roselle leaf aqueous extract.
Data expressed as mean ± SD.

Table 3 Bactericidal activity of roselle calyx and leaf aqueous extracts against *Salmonella enterica* Newport (Log_{10} CFU mL^{-1}) at 4, 8 and 25 °C

		S. Newport population (Log_{10} CFU mL^{-1}) at time (h)		
Treatment	Temperature (°C)	0	24	48
Control	4	7.0 ± 0.0	7.0 ± 0.1	7.0 ± 0.1
	8	7.0 ± 0.0	7.2 ± 0.1	8.1 ± 0.0
	25	7.0 ± 0.0	9.0 ± 0.0	9.2 ± 0.1
RCA	4	ND	ND	ND
	8	ND	ND	ND
	25	ND	ND	ND
RLA	4	2.1 ± 0.3	ND	ND
	8	2.1 ± 0.3	ND	ND
	25	2.1 ± 0.3	ND	ND

ND, no detectable survivors (<1.0 log_{10} CFU mL^{-1}); RCA, roselle calyx aqueous extract; RLA, roselle leaf aqueous extract.
Data expressed as mean ± SD.

shows results of *in vitro* studies with *L. monocytogenes* with mean ± SD and *P* values. Tables 2 and 3 show results of the *in vitro* studies with *E. coli* O157:H7 and *S.* Newport with mean ± SD for the surviving bacterial populations. As shown in Table 1, for control treatment, *L. monocytogenes* population remained the same at 4 °C and 8 °C but increased by 2 logs at 25 °C, over 72 h ($P < 0.001$). The cultures exposed to RCA extract showed a 4–5 log reduction and those exposed to RLA extract showed a 3–5 log reduction in *L. monocytogenes* population at all temperatures over a period of 24 h. At 48 h, no survivors for *L. monocytogenes* were detected in either the RCA or the RLA extract stored at 25 °C. At 72 h, no survivors for *L. monocytogenes* were detected in both aqueous extracts stored at 4, 8 or 25 °C. The differences observed between control and antimicrobial treatments (RCA and RLA extracts), in the surviving *L. monocytogenes* population, were highly significant ($P < 0.0001$). Both aqueous extracts were equally effective in reducing the populations of *L. monocytogenes* compared with the control at all three temperatures; however, the reduction was significantly higher ($P < 0.0001$) at 25 °C compared with 4 °C and 8 °C.

As shown in Table 2, for the control samples, population of *E. coli* O157:H7 remained the same at all three temperatures (4, 8 and 25 °C) over a period of 48 h. However, at these three temperatures, none of the *E. coli* O157:H7 cultures exposed to RCA or RLA extracts had any survivors detected at 24 h or 48 h. Both RCA and RLA extracts exhibited strong bactericidal activity at 24 h against *E. coli* O157:H7.

As shown in Table 3, for the control samples, population of *S.* Newport increased by 2 logs at 25 °C, and at 24 h and 48 h and also increased by 1 log at 8 °C over 48 h. When exposed to RLA extract, the reduction in *S.* Newport population was approximately 5 logs at 0 h, with no survivors detected at 24 h. However, upon exposure to RCA extract, no survivors were detected at 0 h and at all three test tempera-

tures. These results indicate that *Salmonella* populations were extremely susceptible to the two extracts with no detectable survivors in RCA extract and significant reductions upon exposure to RLA extract at 0 h.

Results from our *in vitro* studies showed that both the RCA and RLA extracts exhibited strong antibacterial activity against the three pathogens tested. No significant differences were observed between the RCA and RLA extracts and both were equally effective in reducing the populations of all three pathogens. Results obtained with RCA extracts were similar to those obtained in previous studies (Liu *et al*., 2005; Yin & Chao, 2008; Chao & Yin, 2009). However, to our knowledge, this is the first study conducted to test the *in vitro* effects of RLA extract against *E. coli* O157:H7, *S.* Newport and *L. monocytogenes*. Additionally, the *in vitro* studies carried out in our laboratories examined the survival of the three pathogens, from 0 h to 72 h, in RCA and RLA extracts at three different temperatures (4, 8 and 25 °C) to understand the effects over time with storage.

Antimicrobial activity of roselle calyx concentrate and tea against *E. coli* O157:H7 on romaine lettuce

Results for the antimicrobial activity of roselle calyx concentrate and tea against *E. coli* O157:H7 on lettuce are shown in Figure 1. The initial counts (0 h) of *E. coli* O157:H7 in the control and the treated samples were approximately 4 $\log_{10}$ CFU g^{-1}. However, similar to the *in vitro* studies, the *E. coli* O157:H7 population in the control samples remained the same over a period of 24 h, whereas the roselle calyx concentrate and the tea inactivated *E. coli* O157:H7 population to undetectable levels on lettuce within 24 h. Thus, both the concentrate and the tea were equally effective in reducing *E. coli* O157:H7 population on lettuce.

Antimicrobial activity of roselle calyx concentrate against *S.* Newport on alfalfa sprouts

The alfalfa sprouts were treated with only roselle calyx concentrate (100%; v/v). Results of the antimicrobial activity of roselle calyx concentrate against *S.* Newport in alfalfa sprouts are presented in Figure 2. The initial count (0 h) of *S.* Newport in control sprout samples was 5 $\log_{10}$ CFU g^{-1} and that in the sprouts treated with calyx concentrate was 4 $\log_{10}$ CFU g^{-1}, indicating a 1 log reduction in *S.* Newport population immediately upon exposure. Similar to the results observed in lettuce, while the population of *S.* Newport in alfalfa sprouts remained the same for the control, roselle calyx concentrate reduced the population to undetectable levels in alfalfa sprouts within 24 h, indicating its strong bactericidal activity against *S.* Newport.

Our studies with lettuce and alfalfa sprouts were conducted at refrigeration temperatures to simulate a typical fresh produce storage scenario at processing, retail or consumer levels. We achieved inactivation of *E. coli* O157:H7 and *S.* Newport to undetectable levels at 24 h in romaine lettuce and alfalfa sprouts, respectively, treated with roselle calyx concentrate or tea. Roselle calyx concentrate used in the fresh produce studies was prepared from fresh calyces that were homogenized in de-ionized water, filtered through sterile cheesecloth and boiled for 5 min. Our preliminary investigations of four different preparation methods of roselle calyx concentrate revealed that the concentrate prepared in this manner exhibited the strongest antibacterial activity (data not shown). On the other hand, roselle calyx tea used in the study was prepared from dry calyces. However, it was observed that both the concentrate and the tea reduced the bacterial populations to undetectable levels within 24 h, indicating that a concoction prepared from dry calyces was equally effective in reducing the foodborne pathogens. In the current study, both the concentrate and the tea were used at 100% (v/v) concentration. However, in keeping with the fresh produce industry practices for washing fresh produce, where such higher concentrations may not be practical, future studies will be aimed at looking at the effects of different concentrations of the concentrate or tea on the survival of foodborne pathogens in fresh produce.

Yin & Chao (2008) have studied the antimicrobial effects of RCA extract against *Campylobacter* species in ground beef stored for 6 days at 15 °C. Chao & Yin (2009) also investigated the effects of RCA extracts against foodborne pathogens (*Salmonella typhimurium*, *E. coli* O157:H7, *L. monocytogenes*, *Staphylococcus aureus* and *Bacillus cereus*) in ground beef and apple juice after 3 days of storage at 25 °C. Both studies showed a reduction (approximately 2–4 $\log_{10}$ CFU mL^{-1} or g) in the growth of the pathogens in ground beef and apple juice. The results obtained in the current study were similar to these studies; however, we observed an enhanced inactivation (to undetectable levels) of *E. coli* O157:H7 and *S.* Newport in lettuce and alfalfa sprouts, respectively, within 24 h. In addition, surviving populations of the test bacteria were monitored at 0, 24 and 48 h to measure the bactericidal effects of roselle calyx concentrate and tea over time. Differences observed in the results between our study in fresh produce and those

obtained in other studies with ground beef and apple juice (Yin & Chao, 2008; Chao & Yin, 2009) could be due to the utilization of a completely different food matrix. Thus, application of the extracts from roselle might provide enhanced antibacterial protection for fresh produce.

Conclusions

Observations from the current study suggest that RCA and RLA extracts/concentrate (prepared using fresh calyces and leaves), and the roselle calyx tea (prepared using dried calyx) can be used as potential antimicrobial treatments in the postharvest processing of fresh produce. Owing to their strong bactericidal effects, RCA extracts/concentrate/teas can also find potential applications in consumer households for reduction of foodborne pathogens. Future studies will therefore be directed toward testing the application of roselle extracts, concentrates and tea (at various concentrations) as antimicrobials in the fresh produce. Although the current study is reporting *in vitro* survival potential of *E. coli* O157:H7, *S.* Newport and *L. monocytogenes* in roselle aqueous extracts at 0, 24 and 48 h, future studies will be conducted to study bacterial survival at shorter intervals to further elucidate the effectiveness of roselle extracts. Mechanisms of action responsible for the antimicrobial effects of RCA and RLA extracts will also be examined in future studies. Antimicrobial effects of roselle extracts in other commodities such as fruits, other fresh-cut vegetables, tree nuts, as well as meat and poultry, also merit further investigation. Results from the study can be used toward optimizing various applications of roselle calyx and leaf extracts as natural plant antimicrobials for use as interventions at pre- and postharvest production of various food commodities. The results will immensely benefit the fresh produce industry and will improve microbiological safety of fresh produce.

Acknowledgements

Research in Dr. Jaroni's laboratory was supported by the Agricultural Research Department at SUAREC. Research in Dr. Ravishankar's laboratory was supported by the College of Agriculture and Life Sciences, University of Arizona. Research in both laboratories was also supported by the USDA-NIFA OREI grant 2010–51300-21760. The authors thank Libin Zhu for technical support and Dr. Kit Chin for providing roselle calyces and leaves for the study.

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