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RESEARCH ARTICLE

Improving the safety and shelf-life of orange and mango juices using *Panax ginseng*, malic acid and potassium sorbate

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Abstract In this research, several plant compounds were in vitro evaluated against *Salmonella enterica* ser. Saintpaul and *Escherichia coli* O157:H7 through disc's method. Among them *Panax ginseng* (PG) showed the highest antimicrobial activity in comparison to other natural antimicrobials. Combinations of PG (2 % v/v) with malic acid (MA) (0.5 % v/v) and/or potassium sorbate (PS) (0.05 % v/v) were made for evaluating their effects on *S.* Saintpaul and *E. coli* O157:H7 populations in sterile and fresh mango and orange juices stored at 5 °C. The best combination of antimicrobial compounds on native flora (during 21 days) as well as sensory attributes (0 day) of fresh juices were evaluated. The antimicrobial compounds added into mango and orange juices were more effective against *S.* Saintpaul than against *E. coli* O157:H7. The combination of PG (2 % v/v), MA (0.5 % v/v) and PS (0.05 % v/v) showed the highest antimicrobial effectiveness against both pathogenic microorganisms in both juices, in addition to a higher microbiological inhibition during storage (21 days). Sensory attributes such as aroma, color and taste were enhanced, but acidity was notably affected in both juices. In conclusion, the combination of PG, MA and PS could be an effective method in the food industry for ensuring the microbial safety and quality in mango and orange juices.

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1 Introduction

Consumption of unpasteurized or fresh fruit juices has significantly increased over the last years, mostly due to the high demand for healthy and convenient foods with fresh-like characteristics (Rico et al. 2007). There are some scientific evidences that demonstrate that consumption of raw fruits and vegetables helps to prevent many degenerative diseases such as cardiovascular problems and several cancers (Kaur and Kapoor 2001). However, these new consumption patterns may increase the risk of foodborne microbial diseases to the consumers, if those fresh juices are improperly handled, processed or stored (Beuchat 1996; Díaz-Cinco et al. 2005). In the last decades, the number of documented cases and outbreaks of foodborne illnesses caused by pathogenic microorganisms due to consumption of unpasteurized fruit juices has increased (Harris et al. 2003; Raybaudi-Massilia et al. 2009a; CDC 2010; Strawn et al. 2011). Some outbreaks from *Salmonella* (ser. Saintpaul, Typhimurium, Newport, Muenchen, Enteritidis, amongst others) and *E. coli* O157:H7 associated to consumption of fresh orange and mango juices and freshly cut fruits have been reported by Raybaudi-Massilia et al. (2009a) and Strawn et al. (2011).

Thermal pasteurization has been the most commonly used treatment to preserve the fruit juices which might affect texture and fresh-like characteristics (Mosqueda-Melgar et al. 2008). Several alternative methods to the thermal treatment are

being tested in the food industry and institutions of research. One of them is the use of natural antimicrobials from plant origin such as plant extracts, essential oils and spices, which have demonstrated to have antimicrobial activity against several pathogenic and spoilage microorganisms (Ejechi et al. 1998; Raybaudi-Massilia et al. 2009a). The majority of these natural antimicrobials are GRAS (generally recognized as safe) substances. According to USFDA (2011), there are no quantitative restrictions for the use of the majority of GRAS substances.

The objectives of this research were to determine the in vitro antimicrobial activities of *Panax ginseng, Ginkgo biloba, Aloe vera* and red grapes skin and seed extracts, as natural plant compounds, against *Salmonella enterica* ser. Saintpaul and *Escherichia coli* O157:H7; as well as to evaluate the effectiveness of the most powerful plant compound in combination with malic acid and potassium sorbate (commonly used in commercial juices), as potential hurdle technology for ensuring the microbial safety and quality of orange and mango juices. Sensory evaluation on attributes such as aroma, color, taste and acidity of the treated juices was conducted also.

2 Materials and methods

2.1 Fruits

Whole mangoes (*Mangifera indica* L.) and oranges (*Citrus sinensis* L.) at commercial ripeness were acquired from a distributer of tropical fruits placed in Caracas, Venezuela. A characterization of the fruits was made following the COVENIN norms 924-83 and 1151-77 (COVENIN 1977, 1983). Titratable acidity, pH (Microprocessor pH-meter model 211, Hanna Instruments, Cluj, Romania) and soluble solids (Digital Refractometer PR-101, Atago Co., Ltd., Tokyo, Japan) were the evaluated parameters (Table 1).

Table 1 Characterization of mango (*Mangifera indica* L.) var. "Haden" and orange (*Citrus sinensis* L.) var. "Valencia" juices

Fruit	Evaluated parameters		
	pH[a]	Titratable acidity (g malic or citric acid/100 g fruit)[a]	Soluble solids (%)[a]
Mango	4.02 ± 0.36	0.10 ± 0.21	12.03 ± 0.61
Orange	3.52 ± 0.02	0.90 ± 0.25	11.10 ± 0.17

[a] Values are the mean of four determinations ± SD (n = 4)

2.2 Microorganisms

Strains of *Salmonella enterica* ser. Saintpaul (CVCM 488 isolated from bean sprouts) and *Escherichia coli* O157:H7 (CVCM 442/ATCC 35150 isolated from human feces from hemorrhagic colitis) were provided by the "Centro Venezolano de Colecciones de Microorganismos (CVCM)" of the Institute of Experimental Biology of the Central University of Venezuela, Caracas-Venezuela.

2.3 Antimicrobial agents

Aloe vera (AV) (ArcoIris Laboratorio Naturista, Maracay, Venezuela), *Panax ginseng* (PG) (ArcoIris Laboratorio Naturista), *Ginkgo biloba* (GB) (ArcoIris Laboratorio Naturista) and red grapes skin and seed extracts (RGSSE) (Uvagen, Laboratorio Italiano Biochimico Farmaceutico Lisapharma, S.P.A, Erba, Italy) were acquired in drugstores placed in Caracas, Venezuela. Potassium sorbate (PS) (Scharlau Chemie S.A., Barcelona, Spain) and malic acid (MA) (Scharlau Chemie S.A.) were also used as antimicrobials. Powders of each antimicrobial agent were mixed with a magnetic stirrer in sterilized warm water (35–40 °C) for 10 min; and then sterilized in cold through Millipore filtration (0.45 μm).

2.4 Disc assay method

The method used in this experiment was the suggested by Kirby-Bauer (Davidson and Parish 1989). Filter-paper discs sized 1 cm in diameter were dipped in solutions of AV, PG, GB and RGSSE individually prepared and sterilized by filtration at different concentrations (0.0, 1.0, 1.5, 2.0, 2.5 and 3.0 % v/v) to evaluate the antimicrobial effect of each agent against *Salmonella enterica* ser. Saintpaul and *E. coli* O157:H7. The agent with the highest bactericidal effect (highest zone of inhibition) will be chosen for in vivo studies in orange and mango juices. The discs impregnated with natural antimicrobials were put on Müller-Hinton agar (Himedia, Mumbai, India) plates previously inoculated with *Salmonella* Saintpaul or *E. coli* O157:H7 at 10^7 colony forming units/milliliter (CFU/ml), and incubated at 37 °C for 24 h. The zone of inhibition was measured using a vernier caliper (Hauptner, Solingen, Germany).

2.5 Juice preparation

2.5.1 Sterile juice

Whole mangoes and oranges were washed, dried, peeled, cut into pieces, pit and made juices through in an extractor machine (Juice extractor model Pro 3168, Oster, Boca Raton, USA). The juices were then centrifuged to 2,000 rpm for 20 min at 5 °C in a centrifuge model CRU-500 (International Equipment Company, Massachusetts, USA) to obtain pulp-free mango and orange juices. The supernatant juice was filtered, bottled and sterilized at 121 °C for 15 min in an autoclave (American Sterilizer, Pennsylvania, USA) to obtain juices free from microorganisms. Once cooled, PG (at 2 % v/v), PS (at 0.05 % v/v) and MA (at 0.5 % v/v) alone or combined were immediately added to the juices. The concentrations of PS and MA were selected according to the permitted limits for processed fruits and juices (Davidson and Taylor 2007; USFDA 2011). Control samples of juices without added antimicrobials were also evaluated.

2.5.2 Fresh juice

Fresh juices were prepared in the same way as sterile juices but without treatment by autoclave or sterilization. Before processing, the extractor machine, table and knifes were carefully washed first with warmed tap water and then with abundant sterile water to minimize the presence of spoilage microorganisms of the environment. The fresh juices were used to evaluate the effect of the combination of PG, MA and PS against the pathogenic microorganisms in presence of the native flora. Fresh juices were also used to evaluate the effect of that combination of antimicrobial compounds on the native flora (microbiological shelf-life) and sensory attributes.

2.6 Preparation of microbial culture and inoculation into juices

Strains of *Salmonella enterica* ser. Saintpaul and *E. coli* O157:H7 were grown in tryptone soy broth (TSB) (Himedia) and TSB plus yeast extract (Himedia) at 0.6 %, respectively. Cultures were incubated at 37 °C for 18 h to obtain cells in stationary growth phase. The maximum population reached by both microorganisms in the growth medium was approximately 10^9 CFU/ml. Fifty milliliters of each juice (mango or orange) containing the antimicrobial agents were separately inoculated with 500 μl of each culture of microorganism to reach a final concentration of 10^7 CFU/ml. Finally, the juices were incubated at 5 °C for 72 h or 21 days.

2.7 Recovery and enumeration of *S*. Saintpaul and *E. coli* O157:H7 viable cells

A recovery for 20 min in phosphate buffer (pH 7.2) and enumeration of cells in tryptone soy agar (TSA) (Himedia) by pour plate method was made at both 0 and 72 h after refrigerated storage (5 °C) for sterile juices inoculated with *Salmonella enterica* ser. Saintpaul and *E. coli* O157:H7. Whereas, by spread plate method in selective agars of Hektoen (Himedia) for *Salmonella enterica* ser. Saintpaul and McConkey Sorbitol (Himedia) for *E. coli* O157:H7 for fresh juices inoculated with the same microorganisms. The plates were incubated at 37 °C for 24 h for enumeration. The recovery time (20 min) was selected according to generation time of each microorganism from growth curves previously made in the laboratory (data not shown).

2.8 Microbiological shelf-life of fresh juices

Counts of native microorganisms such as mesophilic, psychrophilic, yeast and mould populations in fresh mango and orange juices treated with antimicrobials or not were measured at 0, 1, 3, 7, 14 and 21 days of storage at 5 °C. A pair of samples for each juice and treatment was taken out. Counts were made in duplicates (n = 4) and reported as $\log_{10}$ CFU/ml.

Counts of mesophilic and psychrophilic populations were made according to the ISO 4833: 1991 guideline using Plate Count Agar (PCA) (Himedia) by the pour plate method. The plates of psychrophilic microorganisms were incubated at 5 °C for 10–14 days; whereas mesophilic populations were incubated at 35 °C for 48 h. Counts of yeast and mould populations were made according to the ISO 7954: 1987 guideline using Chloramphenicol Glucose Agar (CGA) (Himedia) by the spread plate method. The plates were incubated at room temperature for 3–5 days.

2.9 Sensory evaluation

Untreated mango and orange juices as well as those treated with a combination of PG (2 % v/v), PS (0.05 % v/v) and MA (0.5 % v/v) were given to the panelists for sensory evaluation. The procedure performed for this evaluation was similar to that described by Mosqueda-Melgar et al. (2008) and Raybaudi-Massilia et al. (2009b). A total of 30 non-trained panelists belonging

to the Institute of Food Science and Technology of the Central University of Venezuela participated in the sensory tests. Fifteen milliliters of each sample were served in a disposable plastic cup coded with three digits randomly numbered. A water glass and a piece of non-salted cracker were provided to the panelists between the samples to eliminate the residual taste. The panelists were asked to rate their preference of aroma, color, taste, acidity and overall acceptability in a hedonic scale from 0 (dislike extremely) to 10 (like extremely). Values are means of 30 determinations.

2.10 Statistical analysis

A multilevel factorial analysis of variance was carried out to evaluate the effects of PG, PS and MA alone or in combination on the microbial inactivation of *S*. Saintpaul and *E. coli* O157:H7 individually inoculated on the sterilized and fresh mango and orange juices. Multiple range tests were then applied to determine which combinations were significantly different ($p \leq 0.05$). The experiments were carried out twice and microbial counts were made in duplicate; therefore, means and standard deviations of 4 measurements were calculated for each treatment ($n = 4$). For sensory evaluation an analysis of variance with multiple range tests was made to determine significant differences ($p \leq 0.05$) between juices samples treated with antimicrobials or not. The analyses were carried out using the statistical software Statgraphics Centurion XVI (StatPoint Technologies, Inc., Virginia, USA).

3 Results

3.1 Antimicrobial activity of plant compounds

All compounds of plant origin (AV, PG, GB and RGSSE) evaluated in vitro through disc assay method showed antimicrobial effects against *Salmonella enterica* ser. Saintpaul and *E. coli* O157:H7 (Table 2). The inhibition zone increased when higher concentrations of each natural antimicrobial were used. The effectiveness of each antimicrobial agent was depending on the target microorganism, being PG > RGSSE > GB > AV for *S. enterica* ser. Saintpaul, and PG > AV > GB > RGSSE for *E. coli* O157:H7. A higher antimicrobial effect of GB, PG and RGSSE was observed on *S. enterica* ser. Saintpaul than *E. coli* O157:H7; AV showed a contrary effect. Of all the studied compounds, PG showed the highest antimicrobial effect in comparison with the others tested for both pathogenic microorganisms (Table 2).

3.2 Antimicrobial activity in fruit juices

3.2.1 Effect on sterile juices inoculated

In sterile mango and orange juices with or without antimicrobial agents added, inoculated with *S*. Saintpaul and *E. coli* O157:H7, separately, and incubated at 5 °C during 72 h, reductions of both pathogenic microorganisms were observed; being lower in those juices without antimicrobials (Figs. 1, 2). The antimicrobial agents chosen for this study, PG at 2 % v/v, PS at 0.05 % v/v, and MA at 0.5 % v/v showed antimicrobial activity against both pathogenic microorganisms just after added, being MA more effective than PS and PG when applied separately (Figs. 1, 2). *S*. Saintpaul was more sensible to MA than *E. coli* O157:H7 after 72 h of storage at 5 °C in both fruit juices. The combinations which contained MA were more effective against *S*. Saintpaul than those without this acid. Nonetheless, a higher antimicrobial effect against both pathogenic microorganisms was reached after 72 h of storage at 5 °C in comparison with those treated juices just prepared ($t = 0$ h). PG (2 % v/v), MA (0.5 % v/v) and PS (0.05 % v/v) was the combination of antimicrobials with the highest bactericidal activity for inactivating both pathogenic microorganisms in each juice after 72 h at 5 °C. The antimicrobial agents added into mango and orange juices were more effective against *S*. Saintpaul than against *E. coli* O157:H7.

3.2.2 Effect on fresh juices inoculated

The most effective antimicrobial combination—PG (2 % v/v) + MA (0.5 % v/v) + PS (0.05 % v/v)—obtained in the previous experiment (sterile juice inoculated), was used in the fresh orange and mango juices experiment. These juices were also inoculated with *Salmonella* ser. Saintpaul and *E. coli* O157:H7, separately, at a level of 7 log to evaluate the effect of those compounds against the pathogenic microorganisms in presence of the native flora during 21 days of refrigerated storage (5 °C). Populations of *Salmonella* ser. Saintpaul and *E. coli* O157:H7 were able to survive in orange and mango juices without antimicrobials (controls) during storage time (Table 3). About 1.7 log reductions of both *Salmonella* ser. Saintpaul and *E. coli* O157:H7 populations were found in orange juice without antimicrobials (controls). Reductions of 0.7 and 0.1 log in mango juice without antimicrobials

Table 2 Antimicrobial activity of *Ginkgo biloba*, *Aloe vera*, *Panax ginseng* and red grape skin and seed extracts on *Salmonella enterica* ser. Saintpaul and *Escherichia coli* O157:H7 through the disc's method

Microorganism	Plant extract	Antimicrobial concentration					
		Inhibition zone (mm)[a]					
		0.0 %	1.0 %	1.5 %	2.0 %	2.5 %	3.0 %
Salmonella Saintpaul	GB	NV	0.40 ± 0.01	0.60 ± 0.02	1.00 ± 0.01	1.10 ± 0.01	1.20 ± 0.01
	AV	NV	0.40 ± 0.02	0.50 ± 0.01	0.60 ± 0.00	0.80 ± 0.03	1.20 ± 0.01
	PG	NV	0.50 ± 0.01	1.00 ± 0.01	1.20 ± 0.01	1.50 ± 0.01	2.00 ± 0.01
	RGSSE	NV	0.50 ± 0.03	0.80 ± 0.01	1.10 ± 0.02	1.20 ± 0.01	1.50 ± 0.00
E. coli O157:H7	GB	NV	0.30 ± 0.00	0.30 ± 0.02	0.50 ± 0.01	0.80 ± 0.01	1.00 ± 0.01
	AV	NV	1.00 ± 0.05	1.00 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	1.30 ± 0.02
	PG	NV	1.00 ± 0.03	1.00 ± 0.01	1.30 ± 0.01	1.30 ± 0.00	1.60 ± 0.02
	RGSSE	NV	0.30 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.30 ± 0.02	0.60 ± 0.04

GB, *Ginkgo biloba*; AV, *Aloe vera*; PG, *Panax ginseng*; RGSSE, red grape skin and seed extracts; NV, not visible

[a] Values are the mean of three determinations ± SD (n = 3)

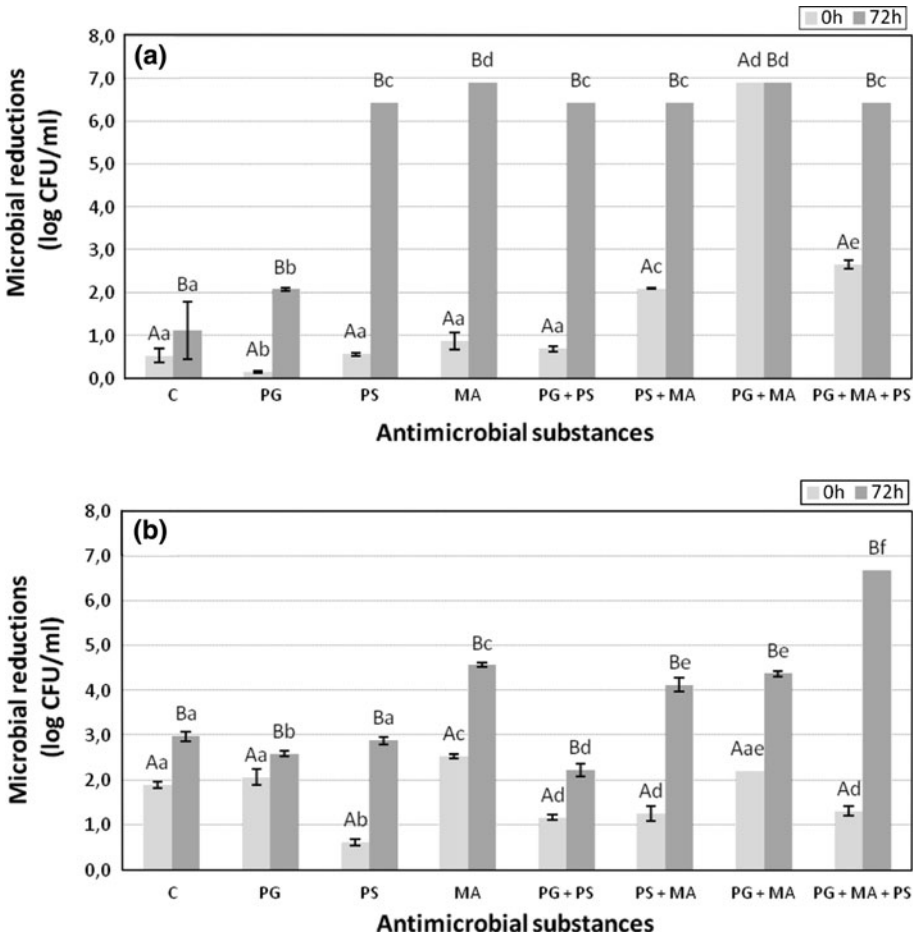

Fig. 1 Reductions of *Salmonella enterica* var. Saintpaul (**a**) and *Escherichia coli* O157:H7 (**b**) in sterile mango (*Mangifera indica* L.) var. "Haden" juice by antimicrobial substances just after being added (0 h) and after 72 h of incubation at 5 °C. Control (C), *Panax ginseng* (PG) at 2 %, potassium sorbate (PS) at 0.05 % and malic acid (MA) at 0.5 %—combined or not. *Different capital letters* (*A, B*) *above bars* indicate significant differences between storage times. *Different lowercase letters* (*a, b, c, d, e*) *above bars* indicate significant differences between antimicrobial agents by each storage time. Values are the mean of two determinations in duplicates ± standard deviation (n = 4)

(controls) for *Salmonella* ser. Saintpaul and *E. coli* O157:H7 populations, respectively, were detected. Higher reductions of these populations of pathogenic microorganisms were observed when antimicrobial agents were added (Table 3). Where *Salmonella* ser. Saintpaul populations were inactivated by more than 5 log from 0 to 1 days of storage at 5 °C in orange and mango juices, respectively; and populations of *E. coli* O157:H7 were inactivated by more than 5 log after 3 days of storage at 5 °C in both kinds of juices (Table 3). Populations of *Salmonella* ser. Saintpaul in orange and mango juices without antimicrobials

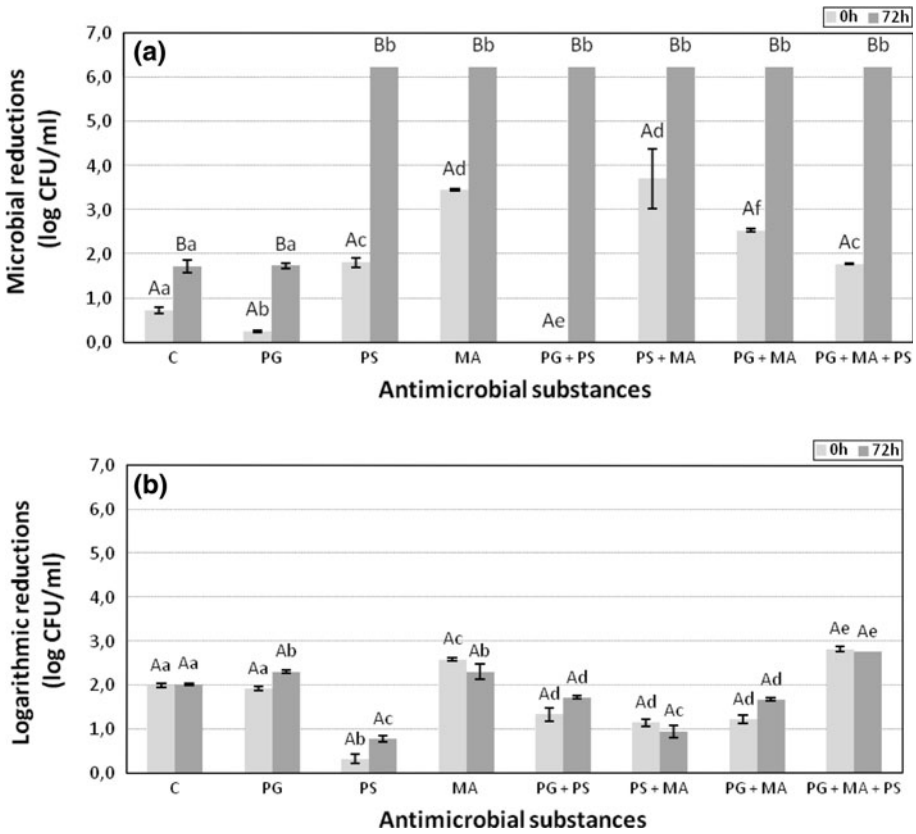

Fig. 2 Reductions of *Salmonella enterica* var. Saintpaul (**a**) and *Escherichia coli* O157:H7 (**b**) populations in sterile orange (*Citrus sinensis* L.) var. "Valencia" juice by antimicrobial substances just after being added (0 h) and after 72 h of incubation at 5 °C. Control (C), *Panax ginseng* (PG) at 2 %, potassium sorbate (PS) at 0.05 % and malic acid (MA) at 0.5 %—combined or not. *Different capital letters* (*A, B*) *above bars* indicate significant differences between storage times. *Different lowercase letters* (*a, b, c, d, e*) indicate significant differences between antimicrobial agents by each storage time. Values are the mean of two determinations in duplicates ± standard deviation (n = 4)

were decreasing throughout storage time; whereas, populations of *E. coli* O157:H7 were kept in orange juice, and decreased in mango juice throughout storage time (Table 3).

3.2.3 Effect on fresh juices non-inoculated

The same antimicrobial combination (PG + MA + PS) which is effective against pathogenic microorganisms was used to evaluate the effect of those compounds on the native flora of fresh mango and orange juices during 21 days of storage (5 °C). Populations of mesophilic, psychrophilic, moulds and yeast increased significantly during the storage time in fresh orange and mango juices without antimicrobial substances added (Fig. 3). The maximum populations in orange juice reached 6.10, 6.24 and 6.10 log CFU/ml, respectively and 6.99, 5.26 and 3.04 log CFU/ml, respectively in mango juice. Whereas, in those juices with antimicrobial substances added the microbial growth was inhibited or delayed (Fig. 3). Populations of mesophilic microorganisms were inhibited from growing by 21 days of storage at 5 °C in both kinds of juices. Populations of psychrophilic microorganisms were inhibited from growing for 7 and 14 days in orange and mango juices, respectively. Moulds and yeasts populations in orange juice began to grow after 14 days of storage; in mango juice, those populations were inhibited throughout the 21 days of the experiment (Fig. 3).

3.3 Sensory evaluation

Sensory attributes of orange and mango juices with antimicrobials (PG + MA + PS) or without antimicrobials (controls) were significantly different ($p < 0.05$). The attributes color, aroma and taste were enhanced when antimicrobials were added in both kinds of juices in comparison to the control samples. The acidity had a noticeable impact on the global acceptance of the treated samples (Fig. 4). In general the panelists perceived the impacts of antimicrobials added to mango juice minor to those added to orange juice.

4 Discussion

In vitro studies demonstrated that the tested plant compounds had powerful antimicrobial activity against *Salmonella* ser. Saintpaul and *E. coli* O157:H7. The effectiveness of these plant compounds was

Table 3 Behavior of *Salmonella* Saintpaul and *Escherichia coli* O157:H7 inoculated into fresh orange and mango juices treated or not with antimicrobials and stored at 5 °C for 21 days

Time (days)	*Salmonella* Saintpaul (Log_{10} UFC/ml)[A]				*E. coli* O157:H7 (Log_{10} UFC/ml)[A]			
	Orange juice		Mango juice		Orange juice		Mango juice	
	WOA	WA[B]	WOA	WA[B]	WOA	WA[B]	WOA	WA[B]
I	7.00 ± 0.10[a]	7.00 ± 0.10[b]	7.00 ± 0.10[a]	7.00 ± 0.10[b]	7.00 ± 0.10[a]	7.00 ± 0.10[b]	7.00 ± 0.10[a]	7.00 ± 0.10[b]
0	5.31 ± 0.11[a]	<2.0[b]	6.34 ± 0.15[a]	4.31 ± 0.19[b]	5.37 ± 0.17[a]	4.77 ± 0.10[b]	6.90 ± 0.08[a]	6.06 ± 0.04[b]
1	5.14 ± 0.09[a]	<2.0[b]	6.41 ± 0.03[a]	<2.0[b]	6.01 ± 0.02[a]	4.49 ± 0.02[b]	6.48 ± 0.03[a]	5.40 ± 0.11[b]
3	4.87 ± 0.04[a]	<2.0[b]	4.98 ± 0.03[a]	<2.0[b]	5.69 ± 0.12[a]	3.72 ± 0.10[b]	5.46 ± 0.02[a]	4.85 ± 0.01[b]
7	4.08 ± 0.12[a]	<2.0[b]	2.74 ± 0.13[a]	<2.0[b]	5.74 ± 0.05[a]	<2.0[b]	5.12 ± 0.00[a]	<2.0[b]
14	2.39 ± 0.12[a]	<2.0[b]	3.32 ± 0.01[a]	<2.0[b]	5.72 ± 0.03[a]	<2.0[b]	3.02 ± 0.04[a]	<2.0[b]
21	2.00 ± 0.06[a]	<2.0[b]	2.45 ± 0.21[a]	<2.0[b]	5.71 ± 0.08[a]	<2.0[b]	3.01 ± 0.15[a]	<2.0[b]

I initial inoculum, *WOA* without antimicrobial, *WA* with antimicrobials

[A] Values are the mean of two determinations in duplicated ± SD (n = 4)

[B] A combination of potassium sorbate (0.05 % w/v), malic acid (0.5 % w/v) and *Panax ginseng* (2 % w/v) was added

Different lower-case letters (a, b) between columns by juice and microorganism indicate statistically significant differences between treatments ($p < 0.05$)

depending on the sensibility of each kind of microorganism and antimicrobial nature. Mathew et al. (2002) reported a higher resistance of *E. coli* to antibiotics than *Salmonella* Typhimurium in live swine. In contrast, Ahn et al. (2004) found a higher resistance of *Salmonella* Typhimurium to grape seed extract than *E. coli* O157:H7 in raw ground beef. The differences found between both microorganisms could be attributed to the sensibility of each one to different antimicrobials.

Of all the tested plant compounds, PG showed the highest antimicrobial activity against both pathogenic microorganisms; and therefore, it was used for inoculation studies in fruit juices. Just a few studies on the antimicrobial activity of PG have been carried out; and no research on real food systems has been found. Yasni (2007) showed that *E. coli* and *Salmonella* ser. Typhimurium had different sensibilities to *Panax ginseng* obtained by two ways of extraction (methanol or ethylacetate). Tan and Vanitha (2004) indicated that several compounds of *ginseng* such as acidic polysaccharides (uronic acids), pananotin, panaxagin and quinqueginsin seem to induce hemagglutination, leading to bacterial cytotoxicity and acting on genetic material. Others studies have demonstrated that the main active constituents of *ginseng* include ginsenosides, polysaccharides, peptides, polyacetylenic alcohols, and fatty acids; the former being mostly responsible of the antimicrobial effect (Attel et al. 1999). Ginsenosides are amphiphilic in nature, and have the ability to intercalate into the plasma membrane. This leads to changes in membrane fluidity, and thus affects membrane function, producing a cellular response. The same authors suggested that ginsenosides directly interact with specific membrane proteins. They are lipid-soluble signaling molecules, which can traverse the plasma membrane and initiate effects on genetic material.

In vivo studies demonstrated a higher bactericidal effectiveness of antimicrobial agents at 72 than 0 h at refrigeration temperature for *Salmonella* ser. Saintpaul. This could be attributed to the total or greater diffusion time of those antimicrobials through cellular membrane along the storage time; and to a low adaptation to an acidic environment of this pathogenic microorganism. No significant differences ($p > 0.05$) were found in populations of *E. coli* O157:H7 inoculated in orange juice at 0 and 72 h of storage at 5 °C (Fig. 2). This might be due to the fact that *E. coli* O157:H7 could activate a defense mechanism in media of low pH which makes it more resistant to others substances. Several researchers have reported and described the survival, acid-tolerance or acid resistance of *E. coli* O157:H7 and *Salmonella* populations in acidic environments during its storage (Benjamin and Datta 1995; Lin et al. 1996; Foster 2001; Yuste and Fung 2002; Al Tayib and Al-Bashan 2007). The actual protective mechanisms of acid tolerance have not been defined, but it is known that acid shock induces pH homeostasis and protein repair systems. This inducible pH homeostasis appears to be the result of preventing net proton movement across the membrane of adapted cells rather than increased internal buffer capacity (Foster 2001).

Populations of *Salmonella* ser. Saintpaul and *E. coli* O157:H7 were decreasing through 21 days of storage in both fresh orange and mango juices without

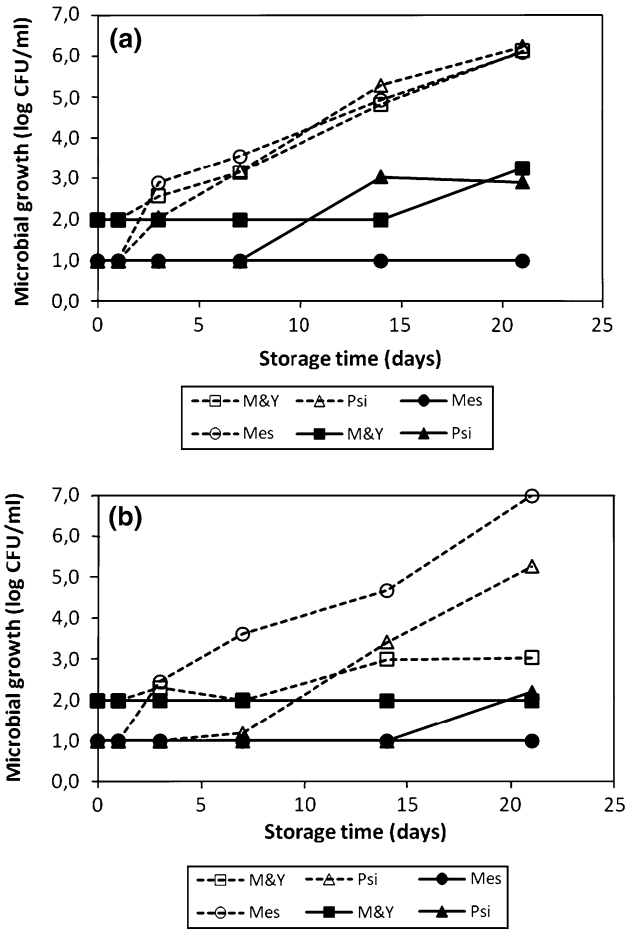

Fig. 3 Microbiological growth of mesophilic (Mes, *filled circles*, *open circles*), psychrophilic (Psi, *filled triangles*, *open triangles*), moulds and yeasts (M&Y, *filled squares*, *open squares*) populations in orange (**a**) and mango (**b**) juices with (*continuous lines*) or without (*dashed lines*) antimicrobial substances (*Panax ginseng* at 2 %, potassium sorbate at 0.05 % and malic acid at 0.5 %) during 21 days of storage at 5 °C. Values are the mean of two determinations in duplicates (n = 4)

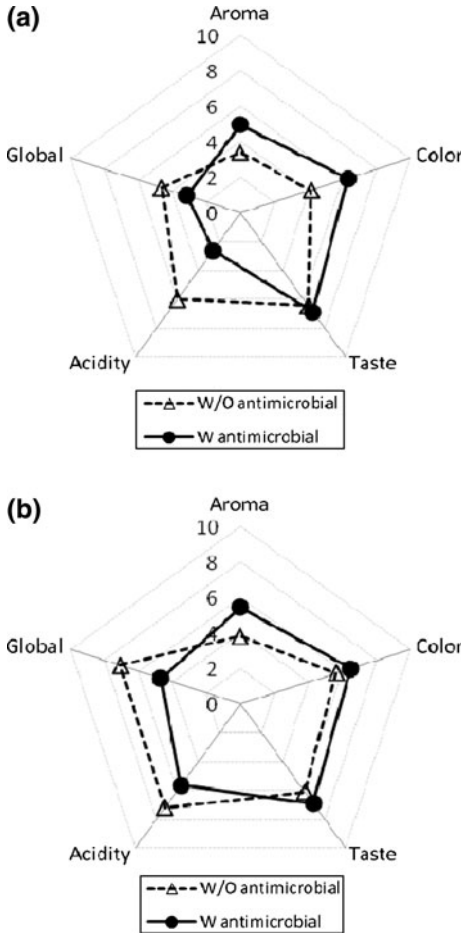

Fig. 4 Sensory evaluation of orange (**a**) and mango (**b**) juices with (*solid line with circle*) or without (*dashed line with triangle*) antimicrobial substances (*Panax ginseng* at 2 %, potassium sorbate at 0.05 % and malic acid at 0.5 %) just after added. Values are the mean of 30 determinations (n = 30)

antimicrobials added, with the exception of *E. coli* O157:H7 in orange juice. This fact could be attributed to the high acidity of the juices tested, which is a consequence of the amount of naturally occurring organic acids in the fruit, and/or to the effect of the storage temperature (5 °C). Since the growth of both pathogenic microorganisms is not favored at refrigeration temperatures. This same behavior has been reported by Raybaudi-Massilia et al. (2009b) in apple and pear juices. In general, *Salmonella* ser. Saintpaul was more sensitive to the antimicrobials agents and acidity of fruit juices than *E. coli* O157:H7. Therefore, the latter should be selected as target microorganism in those kinds of products.

Orange and mango juices alone (without antimicrobials added) exerted a bactericidal effect on the pathogenic microorganism populations; since significant reductions ($p < 0.05$) during storage were observed. However, pathogens were able to survive in both juices through the time of storage (21 days) at 5 °C. These results are in accordance with Parish et al. (1997), who indicated that, populations of *Salmonella* serovars Hartford, Rubislaw, Gaminara and Typhimurium can survive in orange juice (pH 3.5) stored at 4 °C by 27 days. This behavior also has been reported by Eblen et al. (2004), who observed that, populations of *E. coli* O157:H7 and *Salmonella* ser. Hartford were kept in fresh orange juice stored at 4 °C for 3 days. In this experiment, populations of *Salmonella* ser. Saintpaul were decreasing through time in both kinds of juices without antimicrobials. Similar results were observed by Raccach and Carlson (2010) in orange juice inoculated with *Salmonella* ser. Muenchen and stored at 6 °C during 11 days. These

results could be attributed to the low acid resistance of this microorganism in acidic environment.

Orange and mango juices with PG, MA or PS alone or combined showed a powerful antimicrobial activity against inoculated *Salmonella* ser. Saintpaul and *E. coli* O157:H7 populations. Raybaudi-Massilia et al. (2009b) reported that MA at 2 % was able to inactivate populations of *L. monocytogenes*, *E. coli* O157:H7 and *Salmonella* ser. Enteritidis by more than 5 log in apple, pear and melon juices stored at 5 °C. They indicated that MA can pass through water-filled channels formed by transmembrane proteins (porins) embedded into the lipid bilayer that permit hydrophilic transport, and produced damage in the cell cytoplasm of pathogens without apparent changes in the cell membrane. Ceylan et al. (2004) reported the reduction of populations of *E. coli* O157:H7 (up to 3.8 log) in apple juice stored at 8 °C by 14 days when 0.1 % of PS was added.

Growth of spoilage microorganisms in orange juice treated with PG + MA + PS were kept below 3 logs during experiment time at 5 °C, and inhibited by 7 (psychrophilic populations), 14 (mould and yeast populations) and more than 21 (mesophilic populations) days at 5 °C. Hodgins et al. (2002) reduced the microbial population in orange juice treated with PEF, nisin and/or lysozyme by more than 6 logs, extending the shelf-life of juice by more than 28 days at 4 °C. The spoilage flora in mango juice treated with antimicrobials were also kept below 3 logs during experiment time at 5 °C, and inhibited by 14 (psychrophilic populations) and more than 21 (mesophilic, mould and yeasts populations) days at refrigeration temperature (5 °C). Zhang and Mittal (2005) reduced the microbial load in mango juice by more than 4 logs using mild heat (<52 °C), nisin, lysozyme and PEF (pulsed electric fields). In this experiment, lower levels of microbial load were achieved in both juices treated with antimicrobials (PG + MA + PS) in comparison to control samples.

Sensory attributes of orange and mango juices were significantly ($p < 0.05$) affected by the antimicrobials added, especially malic acid (0.5 %), which could cause a significant decrease of the pH of the juices, was thus reducing its acceptance by the panelists. Further studies with hurdle technologies, using non-thermal methods in these kinds of fruit juices are needed for minimizing the impact on sensory attributes.

5 Conclusions

A combination of PG (2 %), MA (0.5 %) and PS (0.05 %) was effective as antimicrobial treatment to reduce the populations of pathogenic microorganisms inoculated, as well as to extend the shelf-life of orange and mango juices by inhibition of the growth of native flora. However, the acidity significantly affected the global acceptance of the juices tested.

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References

Ahn J, Grün IU, Mustapha A (2004) Antimicrobial and antioxidant activities of natural extracts in vitro and in ground beef. J Food Prot 67(1):148–155

Al Tayib YAM, Al-Bashan MM (2007) Experimental studies on the potential for acid tolerance, growth and survival of *Salmonella enterica* serovar *Typhimurium* and *E. coli* O157:H7 in orange juices. Adv Biol Res 1(3–4):99–107

Attel AS, Wu JA, Yuan CS (1999) Ginseng pharmacology. Biochem Pharmacol 58:1685–1693

Benjamin MM, Datta AR (1995) Acid tolerance of Enterohemorrhagic *Escherichia coli*. Appl Environ Microbiol 61:1669–1672

Beuchat LR (1996) Pathogenic microorganisms associated with fresh produce. J Food Prot 59:204–216

CDC: Annual Listing of Foodborne disease outbreak. United States, 1990–2004. Center for Disease Control and Prevention, Atlanta GA. http://www.cdc.gov/foodborneoutbreaks/outbreak_data.htn. Accessed 15 July 2010

Ceylan E, Fung DYC, Sabah JR (2004) Antimicrobial activity and synergistic effect of cinnamon with sodium benzoate or potassium sorbate in controlling *Escherichia coli* O157:H7 in apple juice. J Food Sci 69:FSM102–FSM106

COVENIN 1151-77 (1977) Frutas y productos derivados. Determinación de la acidez. Comisión Venezolana de Normas Industriales. Fondonorma, Caracas, Venezuela

COVENIN 924-83 (1983) Frutas y productos derivados. Determinación de sólidos solubles por refractometría. 1st. Revision. Comisión Venezolana de Normas Industriales. Fondonorma, Caracas, Venezuela

Davidson PM, Parish ME (1989) Methods for testing the efficacy of food antimicrobials. Food Technol 43:148–155

Davidson MP, Taylor MT (2007) Chemical preservatives and natural antimicrobial compounds. In: Doyle MP, Beuchat LR (eds) Food Microbiology. Fundamentals and Frontiers, 3rd edn. ASM Press, Washington DC, pp 713–745

Díaz-Cinco ME, Acedo-Félix E, García-Galaz A (2005) Principales microorganismos patógenos y de deterioro. In: González-Aguilar GA, Gardea AA, Cuamea-Navarro F (eds) Nuevas tecnologías de conservación de productos vegetales frescos cortados. CIAD AC, Sonora, pp 216–240

Eblen BS, Walderhaug MO, Edelson-Mammel S, Chirtel SJ, De Jesus A, Merker RI, Buchanan RL, Miller AJ (2004) Potential for internalization, growth, and survival of *Salmonella* and *Escherichia coli* O157:H7 in oranges. J Food Prot 67(8):1578–1584

Ejechi BO, Souzey JA, Akpomedaye DE (1998) Microbial stability of mango (*Mangifera indica* L.) juice preserved by combined application of mild heat and extracts of two tropical spices. J Food Prot 61:725–727

Foster JW (2001) Acid stress responses of *Salmonella* and *E. coli*: survival mechanisms, regulation, and implications for pathogenesis. J Microbiol 39(2):89–94

Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, Garrett EH, Busta FF (2003) Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. CRFSFS 2:78–141

Hodgins AM, Mittal GS, Griffiths MW (2002) Pasteurization of fresh orange juice using low-energy pulsed electric field. J Food Sci 67(6):2294–2299

ISO (International Standards Organization) 4833:1991 (1991) Microbiology-general guidance for the enumeration of microorganisms-Colony-count technique at 30 degrees C. Genève, Switzerland

ISO (International Standards Organization) 7954:1987 (1987) Microbiology-general guidance for the enumeration of yeast and mould-Colony-count technique at 25 degrees C. Genève, Switzerland

Kaur C, Kapoor HC (2001) Antioxidants in fruits and vegetables—the millennium's health. Int J Food Sci Tech 36:703–725

Lin J, Smith MP, Chapin KC, Baik HS, Bennett GN, Foster JW (1996) Mechanisms of acid resistance in Enterohemorrhagic *Escherichia coli*. Appl Environ Microbiol 62:3094–3100

Mathew AG, Jackson F, Saxton AM (2002) Effects of antibiotic regimens on resistance of *Escherichia coli* and *Salmonella* serovar Typhimurium in swine. J Swine Health Prod 10(1):7–13

Mosqueda-Melgar J, Raybaudi-Massilia RM, Martín-Belloso O (2008) Combination of high-intensity pulsed electric fields with natural antimicrobials to inactivate pathogenic microorganisms and extend the shelf-life of melon and watermelon juices. Food Microbiol 25:479–491

Parish ME, Narciso JA, Friedrich LM (1997) Survival of salmonellae in orange juice. J Food Saf 17:273–281

Raccach M, Carlson E (2010) Modeling the response of *Salmonella* Muenchen in orange juice. J Food Process Preserv 34(4):681–699

Raybaudi-Massilia RM, Mosqueda-Melgar J, Soliva-Fortuny R, Martín-Belloso O (2009a) Control of pathogenic and spoilage microorganisms in fresh-cut fruit and fruit juices by traditional and alternative natural antimicrobials. CRFSFS 8:157–180

Raybaudi-Massilia RM, Mosqueda-Melgar J, Martín-Belloso O (2009b) Antimicrobial activity of malic acid against *Listeria monocytogenes*, *Salmonella* Enteritidis and *Escherichia coli* O157:H7 in apple, pear and melon juices. Food Contr 20:105–112

Rico D, Martín-Diana AB, Barat JM, Barry-Ryan C (2007) Extending and measuring the quality of fresh-cut fruit and vegetables: a review. Trends Food Sci Tech 18:373–386

Strawn L, Schneider K, Danyluk M (2011) Microbial safety of tropical fruits. Crit Rev Food Sci Nutr 51(2):132–145

Tan BKH, Vanitha J (2004) Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: a review. Curr Med Chem 11:1423–1430

USFDA: Listing of Food Additive Status. Silver Spring MD: US Food and Drug Administration. http://www.fda.gov/Food/FoodIngredientsPackaging/FoodAdditives/FoodAdditiveListings/ucm091048.htm. Accessed 07 June 2011

Yasni S (2007) Antipathogenic and anti food spoilage activities of ethylacetate and methanol extract of *Panax ginseng* var. Notoginseng. Microbiol Indones 1(2):95–97

Yuste J, Fung DYC (2002) Inactivation of *Listeria monocytogenes* Scott A 49594 in apple juice supplemented with cinnamon. J Food Prot 65:1663–1666

Zhang Y, Mittal GS (2005) Inactivation of spoilage microorganism in mango juice using low energy pulsed electric field in combination with antimicrobials. Italian J Food Sci 17(2):167–176