Antimicrobial Activity of *Panax ginseng* and Traditional Antimicrobials on Pathogenic and Spoilage Microorganisms in Fresh-Cut Mangoes and Oranges

Rosa Raybaudi-Massilia*
Institute of Food Science and Technology, Central University of Venezuela, Caracas-Venezuela. Postal code 1041A, Postal zip 47097.

Patrizia D'Amore
Institute of Food Science and Technology, Central University of Venezuela, Caracas-Venezuela. Postal code 1041A, Postal zip 47097.

Abstract

The effectiveness of Panax ginseng (PG) combined with potassium sorbate (PS) and/or malic (MA) and citric (CA) acids against Salmonella enterica ser. Saintpaul, Escherichia coli O157:H7 and native microflora on fresh-cut mangoes and oranges was evaluated. A combination of PG 3% (w/v), MA 1% (w/v), PS 0.05% (w/v) achieved to reduce by more than 2.5 and 1.0 logs CFU/g Salmonella Saintpaul and E. coli O157:H7 populations, respectively, on fresh-cut mangoes and oranges just after added. Significant reductions ($p<0.05$) by more than 4 and 2 log CFU/g of Salmonella and E. coli O157:H7 populations, respectively, during 21 days of storage at 5ºC in fresh-cut mango and orange were also achieved. The native microflora growth was delayed with this combination of antimicrobials; and microbiological shelf-life of fresh-cut mangoes and oranges was extended by more than 20 days. Sensorial attributes of fresh-cut mango were not affected by the addition of antimicrobials.

Keywords: Fresh-cut fruits, antimicrobials, *Salmonella*; *E. coli* O157:H7; orange; mango; *Panax ginseng*.

"1. Introduction"

In recent years, the consumption of fresh-cut fruits has significantly increased as consequence of consumers' demand toward fresh-like, high quality and healthy products [1]. In addition, there are scientific evidences demonstrating consumption of raw fruits and vegetables can help to prevent several degenerative diseases such as cardiovascular problems and some cancers [2]. However, these new consumption patterns may also increase the risk of microbial diseases to the consumers, if those fresh and fresh-cut fruits are improperly handled, processed or stored [3]; because these products can result in foods that may carry some microorganisms, including pathogenic bacteria such as *Salmonella* spp. and *E. coli* O157:H7 [4, 5].

Balla and Farkas [6] mentioned that the shelf-life of fresh-cut fruits is limited by deteriorative processes such as browning, softening and microbial decay. Therefore, minimal processing of fresh products to control, reduce or inactivate spoilage and pathogenic microorganisms without significantly affecting their physicochemical and nutritionals attributes is still required.

The use of natural compounds from plant origin as plant extracts, essential oils and spices as substitutes of traditional antimicrobials for controlling the growth of pathogenic and spoilage microorganisms in fresh-cut fruits have recently awakened the interest of the researchers and consumers. Several "*in vitro*" studies about the atimicrobial activity of some natural substances such as *Ginkgo biloba*, *Aloe vera*, *Panax ginseng* and grape seed extract have been carried out [7-12]. However, studies about their applications on fresh product such as fresh-cut fruits have not been reported.

Among them, the *Panax ginseng* (*PG*) is one of the best-known Chinese medicinal herbs and it has been widely used in China for thousands of years. Several studies have indicated that this herb cans positively influence the cardiovascular, nervous, endocrine and immune systems [9]. In addition, it may possess some anti-cancer, anti-inflammation and anti-aging effects [13]. Empirically, *PG* has been used as a supplement to cure fatigue (energizing), to control the blood pressure and to accelerate the small intestine transit [14].

The main objective of this research was to determine the antimicrobial activity of *PG* as natural plant compound alone or in combination with other antimicrobial agents such as potassium sorbate, malic and citric acids, to control *Salmonella enterica* ser.

Saintpaul and *Escherichia coli* O157:H7 in fresh-cut oranges and mangoes at time 0 day and during 21 days of storage at 5°C. In addition, microbiological shelf-life and sensory attributes of fresh-cut mangoes and oranges with antimicrobials added were also evaluated.

"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 "Comisión Venezolana de Normas Industriales (COVENIN)" 1151-77 and 924-83 [15, 16]. Titratable acidity, expressed as g citric or malic acid/100g fruit, was determined as follow: 400 g of fresh-cut fruit was homogenized in a stomacher Lab-blender type 80 (Model BA 6020, London, UK); then 300 g of this was added to a volumetric flask of 2000 ml and diluted with distilled water (approximately 800 ml). Afterwards, this solution is boiled by 1 h and then cooled. The remains fraction was adjusted with distilled water up to 2000 ml and then filtered. From this solution, an aliquot of 25 ml is diluted with distilled water up to 100 ml and then is titrated with 0,1 N sodium hydroxide, using 0,3 ml phenolphthalein solution, as indicator. The pH (Microprocessor pH-meter model 211, Hanna Instruments, Cluj, Romania), soluble solids, expressed as ºBrix (Digital Refractometer PR-101, Atago Co., Ltd., Tokyo, Japan), color (ColorFlex Spectrocolorimeter model 45°/0°, HunterLab, Virginia, USA) and firmness (Texture Analyzer TA.XT2i, Stable Micro Systems, Godalming, UK) were also evaluated (Table 1).

"Table 1. Physical and chemical characteristic of the fresh-cut mangoes (*Mangifera indica* L.) and oranges (*Citrus sinensis* L.)"

Fresh-cut fruit	Parameters *			
	pH	Titratable acidity (g *MA* or *CA* / 100g fruit)	Soluble solids (°Brix)	Firmness (g)
Mango	4.02 ± 0.36	0.10 ± 0.21[a]	12.03 ± 0.61	5.64 ± 0.12
Orange	3.52 ± 0.02	0.90 ± 0.25[b]	11.10 ± 0.17	15.51 ± 1.37

*****pH, titrable acidity, soluble solid and firmness are mean of four determinations ± standard deviation; ***MA***: malic acid; ***CA***: citric acid; [a]Values expressed as g *MA*/100g fruit; [b]Values expressed as g *CA*/100g fruit.

2.2. Microorganisms and culture preparation

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 supplied by the "Centro Venezolano de Colecciones de Microorganismos (CVCM)" of the Institute of Experimental Biology of the Central University of Venezuela, Caracas-Venezuela for this study. Strains of *Salmonella* Saintpaul and *E. coli* O157:H7 were grown in tryptone soy broth (TSB) (Himedia, Mumbai, India) and TSB plus yeast extract (Himedia) at 0.6%, respectively. Those cultures were incubated at 37ºC for 18h without agitation to obtain cell in early stationary growth phase. These conditions were obtained from growth curves previously made in the Laboratory (data not shown). The maximum population reached by both microorganisms in the growth medium was approximately 10^9 Colony Forming Unit (CFU)/ml

2.3. Antimicrobial and stabilizing agents and preparation

Powder of *Panax ginseng* (*PG*) (ArcoIris Laboratorio Naturista, Maracay, Venezuela), Potasium sorbate (*PS*) (Scharlau Chemie S.A., Barcelona, Spain), DL-malic acid (*MA*) (Scharlau Chemie S.A.) and citric acid (*CA*) (Scharlau Chemie S.A.) were used as antimicrobial agents; whereas, calcium lactate (*CL*) (Sigma-Aldrich, Saint Louis, Missouri, USA) was used as stabilizing agent of firmness. Powders of each compound (*PG*, *PS*, *MA*, *CA*, *CL*) were individually prepared in sterilized warming water (35-40ºC) and mixed by 10 min using a magnetic stirrer, and then sterilized in cold through Millipore filtration (0.45 µm) to obtaining free-microorganisms solutions. For that, different concentrations of each solution were prepared as suggested by Raybaudi-Massilia et al. [17] in the following way: *PG* (0%, 1%, 2%, 3%; w/v), *PS* (0%, 0.05%; w/v), *MA* (0%, 0.5%; w/v), *CA* (0%, 0.5%; w/v) and *CL* (0, 0.75%; w/v).

2.4. Fruit processing and inoculation

Mangos and oranges were sanitized by immersion in sodium hypochlorite solution (300 ppm); then washed with potable water and finally dried with absorbent paper. Mangoes were cut in slices of 2 cm of thick with a knife of stainless steel and then cut in pieces of 2 cm of wide x 2 cm of height with a rectangular hollow instrument of stainless steel; whereas oranges were cut in slices of 1 cm of thick

approximately with a knife of stainless steel. Fresh-cut mangoes and oranges were dipped during 1 min into a solution containing *PG* (0%, 1.5%, 2%, or 3% (w/v)), and/or *MA* / *CA* (0 or 1% (w/v)), and/or *PS* (0 or 0.05% (w/v)), and/or *CL* (0 or 0.75% (w/v)) following a multilevel factorial design (Table 2). Afterwards, fresh-cut fruits were naturally wrung out with a plastic strainer for 2 min. Fifty grams of mango or orange pieces were placed into the polypropylene trays of 173×129×35 mm and separately inoculated with 500 µl of each culture of pathogenic microorganism on the fresh-cut fruit surface using a micropipette to obtain a final concentration of approximately 10^7 CFU/g. The samples inoculated were then dried at room temperature (23 ± 3ºC) by 30 min. The trays were then sealed with a plastic film (water vapor permeability of 142.86 fmol $s^{-1}.m^{-2}.kPa^{-1}$ at 38°C and 90% HR; oxygen permeability of 52.38 fmol $s^{-1}.m^{-2}.kPa^{-1}$ at 23°C and 0% HR; and carbon dioxide permeability of 2.38 fmol $s^{-1}.m^{-2}.kPa^{-1}$ at 23°C and 0% HR) using a thermo-sealing machine ILPRA Food Pack Basic model FP400 V/G (Vigenovo, Italy). The more effective combination of antimicrobials (*PG* at 3% (w/v), *PS* at 0.05% (w/v), and *MA* at 0.5% (w/v)), and firmness stabilizing (*CL* at 0.75% (w/v)) obtained from multilevel factorial design was used to study the behavior of both pathogenic microorganisms throughout the storage at 5ºC by 21 days.

2.5. Recovery and enumeration of viable cells.

A recovery for 20 min in phosphate buffer (pH 7.2) and enumeration of cells by spread plate method in selective agars of Hektoen (Himedia) (for *Salmonella* Saintpaul) and McConkey Sorbitol (Himedia) (for *E. coli* O157:H7) was made just 1h after processed the pieces of mango and orange inoculated, and after 3, 7, 14, 18 and 21 days of storage at 5ºC. The plates were incubated at 37ºC by 24h for enumeration. Experiments were carried out twice and counts were made in duplicated (n=4), and reported as log CFU/g. The recovery time (20 min) was selected according to the generation time of each microorganism from growth curves previously made in the laboratory (data not shown).

2.6. Effect of antimicrobials on the behavior of the native microflora in fresh-cut mango and orange

The more effective combination of antimicrobials (*PG* at 3% (w/v), *PS* at 0.05% (w/v), and *MA* at 0.5% (w/v)), and firmness stabilizing (*CL* at 0.75% (w/v)) for inactivating populations of pathogenic microorganisms was used to study the behavior of the native microflora in fresh-cut mangoes and oranges. Counts of native mesophilic, psychrotrophic, yeast and mould populations in fresh cut mangoes and oranges treated or not with the optimum combination of antimicrobials were performed at 0, 3, 7, 14, 18 and 21 days of storage at 5ºC. Counts of mesophilic and psychrotrophic populations were made according to the ISO 4833:1991[18] guideline using Plate Count Agar (PCA) (Himedia) by the pour plate method. The plates of psychrotrophic microorganisms were incubated at 5ºC for 10-14 days; whereas mesophilic populations were incubated at 35ºC for 48h. Counts of yeast and

"Table 2. Multilevel factorial design* of antimicrobials used to observe the effect on populations of *Salmonella* Saintpaul and *E. coli* O157:H7 inoculated on fresh-cut mangoes and oranges"

Antimicrobials[a]				*Microorganisms*[b]	
PS	*MA / CA*	*CL*	*PG*	*SS*	*EC*
0.05	1	0.75	3.0	X_1	X_1
0.05	1	0.75	2.0	X_2	X_2
0.05	1	0.75	1.5	X_3	X_3
0.05	1	0.75	0.0	X_4	X_4
0.05	0	0.75	3.0	X_5	X_5
0.05	0	0.75	2.0	X_6	X_6
0.05	0	0.75	1.5	X_7	X_7
0.05	0	0.75	0.0	X_8	X_8
0.00	1	0.75	3.0	X_9	X_9
0.00	1	0.75	2.0	X_{10}	X_{10}
0.00	1	0.75	1.5	X_{11}	X_{11}
0.00	1	0.75	0.0	X_{12}	X_{12}
0.05	0	0.00	3.0	X_{13}	X_{13}
0.05	0	0.00	2.0	X_{14}	X_{14}
0.05	0	0.00	1.5	X_{15}	X_{15}
0.05	0	0.00	0.0	X_{16}	X_{16}
0.00	1	0.00	3.0	X_{17}	X_{17}
0.00	1	0.00	2.0	X_{18}	X_{18}
0.00	1	0.00	1.5	X_{19}	X_{19}
0.00	1	0.00	0.0	X_{20}	X_{20}
0.00	0	0.00	3.0	X_{21}	X_{21}
0.00	0	0.00	2.0	X_{22}	X_{22}
0.00	0	0.00	1.5	X_{23}	X_{23}
0.00	0	0.00	0.0	X_{24}	X_{24}

Based on statistic analysis run in Statgraphics Centurion XVI. ***PS: potassium sorbate, ***PG***: *Panax ginseng*, ***MA***: malic acid, ***CA***: citric acid, ***CL***: calcium lactate; ***SS***: *Salmonella* Saintpaul; ***EC***: *E. coli* O157:H7. [a]Values are applied concentrations (%) of antimicrobials. [b]Values (X_1, X_2,… X_{23}, X_{24}) are microbial reductions (log UFC/g). The experiment was carried out two times in duplicate (n = 4).

mould populations were made according to the ISO 7954:1987 [19] guideline using Chloramphenicol Glucose Agar (CGA) (Himedia) by the spread plate method. The plates of molds and yeasts were incubated at room temperature for 3-5 days. The experiment was carried out twice and counts were made in duplicated (n=4), and expressed as log CFU/g.

2.7. Sensory evaluation

Fresh-cut mangoes and oranges dipped in a solution containing *PG* (3% w/v), *MA* (1% w/v), *PS* (0.05% w/v) and *CL* (0.75% w/v) or distilled water only (as control) were used to carry out the sensory analyses as suggested by Raybaudi-Massilia et al. [20]. An affective proof was applied to 30 volunteers panelist aged among 20 and 50 years, who frequently like and eat mangoes and oranges. The samples were coded with three-digit numbers and randomly given to the panelists for scoring acceptability of flavor, color, taste, firmness and global characteristics on a structured 10-cm hedonic scale labeled from "extremely unpleasant" (0) to "extremely pleasant" (10). In addition, potable water and pieces of non-salted cracker were provided to panelists for eliminating the residual taste between samples.

2.8. Predictive model

Growth of mesophilic, psychrotrophic, yeasts and molds populations in non-inoculated fresh-cut mangoes and oranges were modeled according to the Gompertz's equation modified by Zwietering *et al.* [21] (Equation 1) using the statistic package Statgraphics Centurion XVI (StatPoint Technologies, Inc., Virginia, USA):

$$Y = k + A.\exp\{-\exp[(\mu_{max}.e/A)(\lambda - t) + 1]\} \quad \text{Eq. 1}$$

where, *Y*, is the current number of microorganisms present in the fresh-cut fruit; *k*, is the initial level of microorganisms (log CFU/g); *A*, is the difference in log (CFU/g) of microorganisms found between t = 0 days (initial population) and the maximum population density achieved at the stationary phase; μ_{max}, is the maximum growth rate (Δlog (CFU/g)/day); λ, is the lag phase time (days); *t*, is the time (days); and *e*, is a constant of 2.7182 value.

The microbiological shelf-life (*SL*) was calculated as suggested by Lanciotti et al. [22], considering as maximum limit of mesophilic aerobic total count at expiry date 10^7 CFU/g according to the Spanish regulation for hygienic processing, distribution and commerce of prepared meals [23]. This limit was also considered for psychrotrophic and yeast and mold populations [24, 25].

$$SL = \lambda - \frac{A\left\{ln\left[-\ln\left(\frac{\log(10^7)-K}{A}\right)\right]-1\right\}}{\mu_{max} * 2.7182} \quad \text{Eq. 2}$$

where, *SL* is the shelf-life time; λ, is the count of microorganisms (log CFU/g) for a given time; *k*, is the microorganisms initial count estimated by the model (log CFU/g); *A*, is the maximum microorganism growth attained at the stationary phase ($\log_{10}$ CFU/g), μmax, is the maximal growth rate (Δlog (CFU/g)/day); λ, is the lag time (days); and *t*, is the storage time (days).

2.9. Statistical analysis

A multilevel factorial analysis of variance (ANOVA) was carried out to evaluate significant ($p<0.05$) effects of *PG* at 0%, 1%, 2% and 3% (w/v), with or without *PS* at 0% and 0.05% (w/v), *MA* or *CA* at 0% and 1% (w/v) and/or *CL* at 0% and 1.5% (w/v) just after added, on the inactivation of *Salmonella* Saintpaul and *E. coli* O157:H7 separately inoculated on fresh-cut mangoes and oranges. Likewise, significant ($p<0.05$) differences on the microbial reductions of *Salmonella* Saintpaul and *E. coli* O157:H7 obtained during storage (0, 3, 7, 14, 18 and 21 days) and between treatments (with or without antimicrobials), in addition to sensory attributes among samples treated or not with antimicrobials were also analyzed. A multiple range tests, using the Fisher's LSD method, were then applied to determine which levels of each factor were significantly ($p< 0.05$) different. All 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. All the analyses were made using the statistic package Statgraphics Centurion XVI (StatPoint Technologies).

"3. Results and Discussion"

Analytical characteristics such as pH, titratable acidity, soluble solids and firmness were measured to offer detailed information about fresh-cut mangoes and oranges used in this study (Table 1). The quality of fresh-cut fruits depends directly on the quality of the raw material and others factors such as firmness, size, variety, and ripeness at processing [26]. The pH value and titratable acidity (defined as content of organic acid by gram) are an indicative of the microflora that can to be present in the food. In spite of than fresh-cut

mangoes have a relative low pH (4.0±0.4), these produce may support the survival and growth of *Salmonella* Saintpaul and *E. coli* O157:H7 populations. In such sense, Strawn and Danyluk [27] reported that fresh-cut mangoes (pH 4.2) may be potential vectors for *E. coli* O157:H7 and *Salmonella* spp. transmission, because they could survive by 28 days at 4 °C on this produce. Likewise, Pao et al. [28] indicated that *E. coli* O157:H7 and *Salmonella* spp. could stay alive on peeled oranges (pH 3.8) during 14 days at 4ºC. The firmness and soluble solids are others important parameters, because these indicate the ripeness state of fruit. Martín-Belloso et al. [26] indicated that ripening have an important influence on sensorial featured related to flavor and texture of fruit. The values obtained in this experiment are corresponding to commercial or intermediate ripeness. Texture fewer firm, is an indicative that enzymes are degrading the cell wall of vegetative cells, and as consequence, a higher amount of nutrients is available for spoilage and/or pathogenic microorganisms. On the other hand, a higher amount of soluble solids would indicate a higher available of sugars in the fruit.

Significant ($p<0.05$) reductions of *Salmonella* Saintpaul and *E. coli* O157:H7 populations on fresh-cut mangoes and oranges treated with *PG* at 1% (w/v) in dipping treatment was observed; being greater the antimicrobial effect when higher concentrations of this compound were used (Table 3). Tan and Vanitha [9] reported that compounds of ginseng such as acidic polysaccharides (uronic acids), pananotin, panaxagin and quinqueginsin may induce hemagglutination, leading to bacterial cytotoxicity and acting on genetic material. Others studies have demonstrated that ginsenosides, main active constituent of ginseng, could mostly be responsible of the antimicrobial effect [29]. 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. 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.

Studies about antimicrobial effects from *PG* on fresh-cut fruits or real food systems have not been reported; nonetheless, *in vitro* studies have pointed out that *E. coli* and *Salmonella* Typhimurium had different sensibilities to *PG* obtained from two ways of extraction (methanol or ethylacetate) [14]. Tan and Vanitha [9] and Lee et al. [30] indicated that *Staphylococcus aureus*, and *Helicobacter pylori* by the induced hemagglutination and macrophage stimulation method, respectively; and *Bacillus cereus*, *Salmonella* Enteritidis, *Escherichia coli* O157:H7 and *Listeria monocytogenes* by agar well diffusion assay, have been suppressed or inactivated by the addition of *PG* extracts.

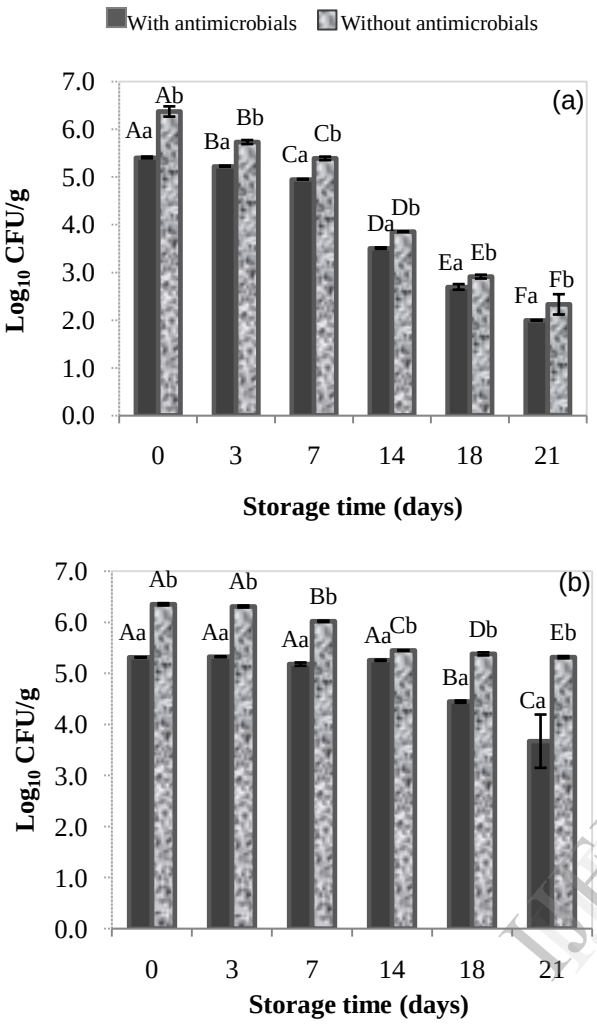
Malic acid was more effective alone as antimicrobial against *Salmonella* Saintpaul than *PG*, *PS* and *CA*; whereas *E. coli* O157:H7 was more sensible to *PG* (3% w/v) or *PS*. When antimicrobials were combined a higher antimicrobial effect against *Salmonella* Saintpaul and *E. coli* O157:H7 in fresh-cut mangoes and oranges was observed (Table 3).

"Table 3. Microbial reductions of *Salmonella* Saintpaul and *E. coli* O157:H7 inoculated on fresh-cut mangoes and oranges treated with antimicrobials just after added"

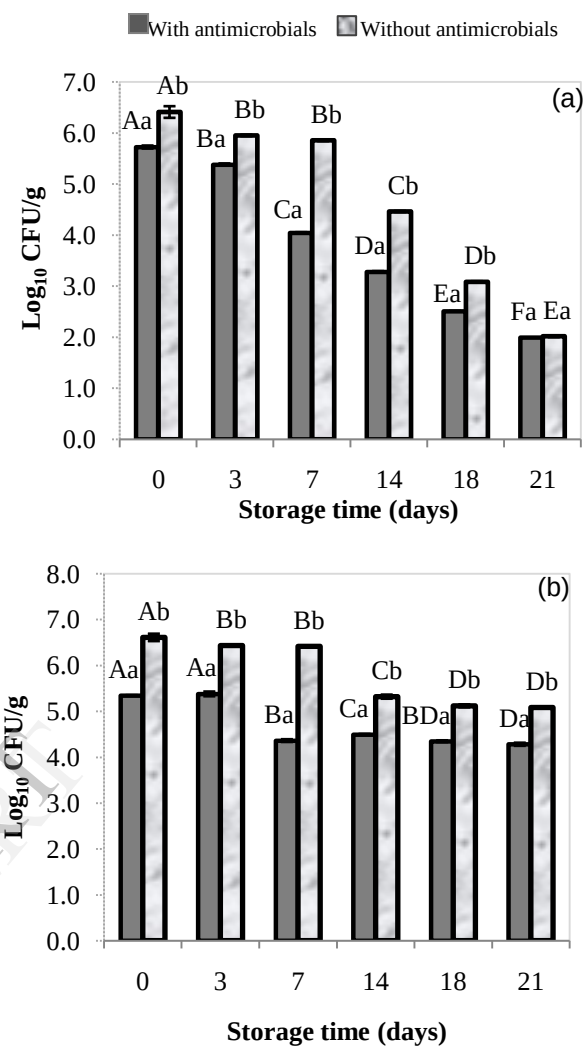
Treatment	***Salmonella* Saintpaul*** (log CFU/g)		***E. coli* O157:H7*** (log CFU/g)	
	Fresh-cut Mangoes	**Fresh-cut Oranges**	**Fresh-cut Mangoes**	**Fresh-cut Oranges**
PG (1%)	0.68 ± 0.02^{a}	0.68 ± 0.71^{a}	1.25 ± 0.05^{bc}	0.97 ± 0.05^{a}
PG (2%)	$0.95 \pm 0,48^{al}$	$1.64 \pm 0,03^{b}$	$1.27 \pm 0,03^{bc}$	1.02 ± 0.05^{ab}
PG (3%)	1.04 ± 0.29^{b}	1.78 ± 0.00^{c}	1.29 ± 0.05^{bc}	1.13 ± 0.05^{cd}
MA (1%)	2.18 ± 0.28^{d}	2.24 ± 0.16^{de}	1.12 ± 0.05^{a}	1.00 ± 0.05^{ab}
CA (1%)	0.99 ± 0.03^{b}	2.35 ± 0.00^{e}	1.08 ± 0.03^{a}	0.93 ± 0.04^{a}
PS (0.05%)	0.39 ± 0.51^{a}	0.92 ± 0.01^{a}	1.22 ± 0.02^{b}	1.18 ± 0.01^{d}
PG (3%) + MA	2.24 ± 0.00^{d}	2.73 ± 0.09^{f}	$1,21 \pm 0,03^{b}$	$1.05 \pm 0,00^{b}$
PG (3%) + CA	1.58 ± 0.23^{c}	2.19 ± 0.00^{d}	$1.20 \pm 0,03^{ab}$	$1.14 \pm 0,02^{c}$
PG (3%) + PS	$1.96 \pm 0.56^{c\alpha}$	1.65 ± 0.01^{b}	$1.30 \pm 0,03^{c}$	$1.19 \pm 0,05^{cd}$
PG (3%) + PS + MA	$2,51 \pm 0,36^{d}$	$2.74 \pm 0,13^{f}$	$1.30 \pm 0,05^{c}$	$1.01 \pm 0,08^{abc}$
PG (3%) + PS + CA	$2.37 \pm 0,49^{d}$	$2.28 \pm 0,01^{d}$	$1.24 \pm 0,03^{bc}$	$0.97 \pm 0,01^{a}$

PG: *Panax ginseng*, **MA**: malic acid, **CA**: citric acid, **PS**: potassium sorbate. *****Values are mean of two determinations ± standard deviation in duplicate (n = 4). Different letters in a same column indicate significant difference ($p<0.05$) among treatments by microorganism and fresh-cut fruit.

Combinations of *PG* (3% w/v) with *PS* and *MA* or *PG* (3% w/v) with *MA* were more effective for achieving the higher reductions of *Salmonella* Saintpaul in the two fresh-cut fruits. Populations of *E. coli* O157:H7 were more sensible when combinations of *PG* (3% w/v) with *PS* and *MA* or *PG* (3% w/v) alone were applied on both fresh-cut fruits. In general, populations of *Salmonella* Saintpaul were more sensible to antimicrobials than populations of *E. coli* O157:H7 under same conditions. This fact may be attributed to the sensibility or resistance of each strain to the kind of antimicrobial and medium [17]. In addition, Lin et al. [31] indicated that some strains of enterohemorrhagic *E. coli* can create several mechanisms of resistance under acid stress conditions and induce mechanisms of protection, which may also serve as barrier against some antimicrobial substances.



"Figure 1. Behavior of (a) *Salmonella enterica* ser. Saintpaul and (b) *E. coli* O157:H7 populations in fresh-cut mango treated or not with *Panax ginseng* (3% w/v), malic acid (1% w/v), potassium sorbate (0.05% w/v) and calcium lactate (0.75% w/v) during storage by 21 days at 5ºC. Values are the mean of two determinations ± standard deviation in duplicated (n = 4). Capital letters indicate significant differences (p<0.05) between microbial reductions for each microorganism with respect to storage time. Lower-case letters indicate significant differences (p<0.05) between treatments by day"

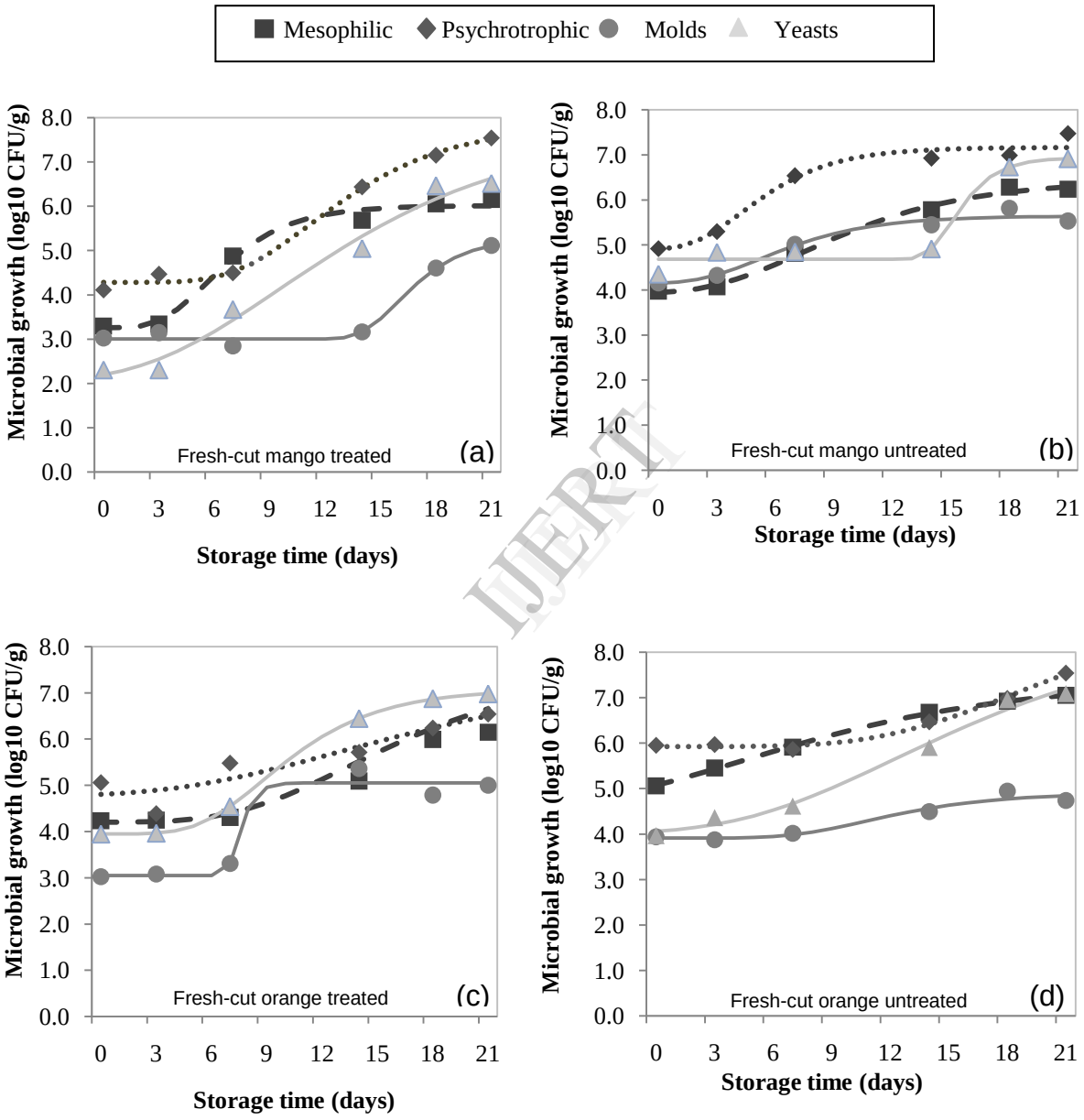


"Figure 2. Behavior of (a) *Salmonella enterica* ser. Saintpaul and (b) *E. coli* O157:H7 populations in fresh-cut oranges treated or not with *Panax ginseng* (3% w/v), malic acid (1% w/v), potassium sorbate (0.05% w/v) and calcium lactate (0.75% w/v) during storage by 21 days at 5ºC. Values are the mean of two determinations ± standard deviation in duplicated (n = 4). Capital letters indicate significant differences (p<0.05) between microbial reductions for each microorganism with respect to storage time. Lower-case letters indicate significant differences (p<0.05) between treatments by day"

Malic acid was generally more effective than *CA* against both pathogenic microorganisms in both fresh-cut fruits (Table 3). It could be explained by the molecular size of each acid. Eswaranandam et al. [32] reported that smaller undissociated molecules of *MA* (134.09 Dalton) may enter more easily into the bacterial cells in comparison with *CA* (192.13 Dalton), with a higher molecular size, which may not effectively enter toward inside of the cell. Raybaudi-Massilia et al. [33] reported 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.

From these results, *PG* (3%), *MA* (1%), *PS* (0.05%) and calcium lactate (0.75%) were selected as the optimum combination for studying the behavior of both pathogenic microorganisms on fresh-cut mangoes and oranges during 21 days at 5ºC (Figures 1 and 2). Significant reductions ($p<0.05$) of *Salmonella* Saintpaul and *E. coli* O157:H7 populations in fresh-cut mangoes and oranges treated or not with the optimum combination were found (Figures 1 and 2). Both pathogenic populations were decreasing through the storage time in both fresh-cut fruits, irrespective of the



"Figure 3. Behavior of the mesophilic, psychrotrophic, molds and yeast populations in mango (a, b) and orange (c, d) treated or not with a combination of *Panax ginseng* (3% w/v), malic acid (1% w/v), potassium sorbate (0.05% w/v) and calcium lactate (0.75%) at 5ºC. Solid symbols are mean of four determinations. Lines are values modeled by the Gompertz's equation"

treatment applied (with or without antimicrobials); however, higher reductions in those treated samples were found. On the other hand, populations of *Salmonella* Saintpaul were more sensitive to the inactivation than populations of *E. coli* O157:H7 during storage time at 5ºC (Figures 1 and 2).

The observed reductions in untreated fresh-cut fruits throughout storage time could be attributed to the storage temperature (5ºC) and/or the high acidity of fruits tested. In this sense, Liao and Sapers [34] and Strawn and Danyluk [27] reported a slight reduction of *Salmonella* spp. on fresh-cut apples and mangoes stored at 8ºC and 4ºC after 3 and 7 days of storage, respectively. Similar results were reported by Raybaudi-Massilia et al. [20, 35], who indicated that storage temperature (5ºC) was the main factor that caused reductions of the populations of *Salmonella* Enteritidis and *E. coli* O157:H7 in fresh-cut apples and pears dipped in distilled water by 30 days. Alegre et al. [36] reported a decreasing of *E. coli* O157:H7 and *Salmonella* spp. on processed minimally peaches during 14 days of storage at 5ºC. Fisher and Golden [37] indicated that *E. coli* O157:H7 was able to survive and slightly reduce its population during 18 days at 4ºC on ground Golden Delicious, Red Delicious, Rome and Winesap. All these results to indicate that populations of *E. coli* O157:H7 and *Salmonella* spp. on fresh-cut fruits could be reduced under conditions of refrigerated storage (3-5ºC), or by the high amount of naturally occurring organic acids in these fruits. In the last case, a decrease in cytoplasm pH of the cell caused by the internalization of the undissociated organic acid molecules throughout storage time could explain the inactivation of those microorganisms studied. Lou and Yousef [38] indicated that the antimicrobial action of organic acids is attributed to cytoplasm acidification. The antimicrobial compounds added into dipping treatments exerted a significant ($p<0.05$) bactericidal effect on the populations of *E. coli* O157:H7 and *Salmonella* Saintpaul in fresh-cut mangoes and oranges in comparison with the control samples (Figures 1 and 2). An initial inactivation (0 day) of both populations of pathogenic microorganisms about 1 log CFU/g in fresh-cut mangoes and oranges was observed. The antimicrobial effects of *PS*, *CL* and *MA* on pathogenic microorganisms in fresh-cut fruits have been previously reported by Raybaudi-Massilia et al. [5]. Davidson and Taylor [39] indicated that one of the primary targets of sorbic acid in vegetative cells seem to be the cytoplasmic membrane by inhibiting amino acid uptake, affecting the proton motive force through nutrient depletion. Likewise, Alakomi et al. [40] reported that lactic acid can cause permeabilization of the outer membrane of the gram-negative bacteria such as *E. coli* O157:H7 and *Salmonella* Typhimurium.

"Table 4. Gompertz's parameters to describe the growth of mesophilic, psychrophilic, yeasts and molds populations and to predict the microbiological shelf-life of non-inoculated fresh-cut mangoes and oranges treated with different antimicrobials and a firmness stabilizer"

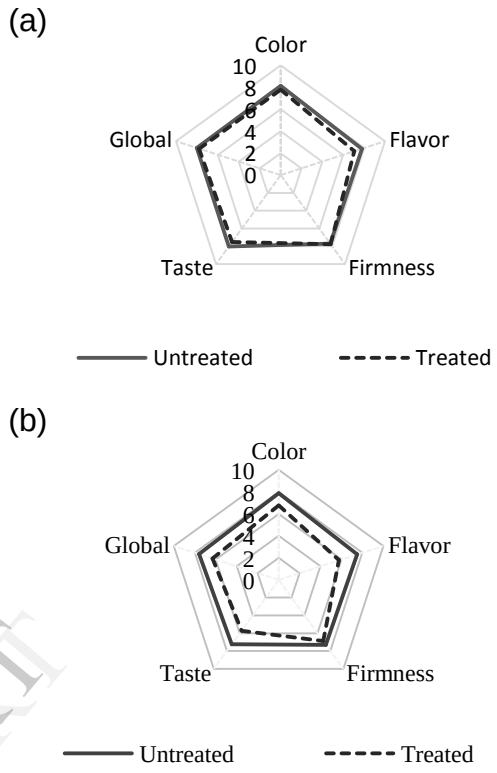
Fresh- cut fruit	Population	Dipping condition	R^2	K (log CFU/g)	*A* (log CFU/g)	λ (Days)	μ_{max} (Δlog [CFU/g]/day)	Shelf-life (days)
Mango	Mesophilic	Control (water)	99.42	3.92±0.08[a]	2,51±0.16[a]	6,44±0.45[a]	0,19±0.02[a]	> 21
	Psychrophilic		96.81	4,89±0.16[a]	2,28±0.20[a]	3,31±0.60[a]	0,32±0.07[a]	12,59
	Molds		97.22	4,14±0.10[a]	1,50±0.14[a]	4,24±0.68[a]	0,18±0.04[a]	> 21
	Yeast		97.34	4,68±0.08[a]	2,25±0.19[a]	14,03±0.50[a]	0,67±0.21[a]	> 21
	Mesophilic	*PG* (3%) MA (1%) PS (0.05%) CL (0.75%)	98.94	3.25±0.11[b]	2.76±0.14[a]	4.59±0.39[b]	0.41±0.07[b]	> 21
	Psychrophilic		99.19	4.28±0.08[b]	3.57±0.26[b]	10.21±0.56[b]	0.31±0.04[a]	19,94
	Molds		98.89	3.00±0.05[b]	2.28±0.17[b]	14.95±0.35[b]	0.42±0.05[b]	> 21
	Yeast		98.32	2.05±0.39[b]	5.66±0.28[b]	8.55±1.43[b]	0.28±0.04[b]	> 21
Orange	Mesophilic	Control (water)	99.92	4.61±0.17[a]	2.77±0.24[a]	3,535±0.75[a]	0,13±0.00[a]	> 21
	Psychrophilic		99.19	5.93±0.03[a]	3.41±1.60[a]	17.92±3.60[a]	0.17±0.02[a]	> 21
	Molds		95.28	3.92±0.06[a]	0.99±0.15[a]	9.68±1.32[a]	0.10±0.03[a]	>21
	Yeast		98.82	4.02±0.17[a]	4.49±1.06[a]	11.32±1.81[a]	0.20±0.02[a]	> 21
	Mesophilic	*PG* (3%) MA (1%) PS (0.05%) CL (0.75%)	98.04	4.20±0.01[b]	3.32±0.81[a]	12.53±1.68[b]	0.19±0.03[b]	> 21
	Psychrophilic		83.78	4.76±0.50[b]	2.64±3.18[a]	12.06±9.68[a]	0.11±0.04[a]	> 21
	Molds		96.99	3.05±0.10[b]	2.00±0.15[b]	6.38±0.58[b]	1.38±1.43[a]	> 21
	Yeast		99.98	3.95±0.01[a]	3.13±0.02[b]	7.76±0.07[b]	0.33±0.01[b]	> 21

K= microorganisms initial count estimated by the Gompertz's model (log CFU/g); ***A***= maximum microorganism growth attained at the stationary phase (log CFU/g); μ_{max}= maximal growth rate (Δlog [CFU/g]/day); λ= Lag time (days). Different lower-case letters (a, b) indicate significant ($p < 0.05$) statistically differences between dipping conditions by parameter and fresh-cut fruit.

This optimum combination of antimicrobials was also used to study the microbiological shelf-life of fresh-cut mangoes and oranges during 21 days of storage at 5ºC. In the Figure 3 is shown the behavior of mesophilic, psychrotrophic, and mold and yeast populations in presence or not of antimicrobial substances applied by dipping treatment on fresh-cut mangoes and oranges stored at 5ºC. These curves were modeled by the Gompertz's equation, and parameters describing their microbial growth are shown in the Table 4.

Significant ($p < 0.05$) statistically differences among the initial microbial counts (k values) from treated and untreated fresh-cut mangoes and oranges stored at 5ºC were found, with the exception of yeasts in fresh-cut oranges, where initial populations were not affected (Table 4). These results to indicate that k values of spoilage microorganisms in both fresh-cut fruits was significantly ($p<0.05$) affected by the combination of antimicrobials just after added; where the k values of treated fresh-cut mangoes and oranges were lower than in those without treatment (Table 4). The lag phase of psychrotrophic and molds populations in treated fresh-cut mangoes was delayed in comparison with control samples; whereas the lag phase of mesophilic populations in treated fresh-cut oranges was also prolonged (see λ values in Table 4). Likewise, the maximal microbial growth rate (μ_{max} values) of mesophilic and mold populations in treated fresh-cut mangoes was delayed in comparison with those untreated samples. In the same way, mesophilic and yeast populations in fresh-cut oranges had higher values of the maximal growth rate than control sample. The shelf-life of treated and untreated fresh-cut mangoes was limited by psychrotrophic populations. However, the shelf-life of treated fresh-cut mangoes was extended by more than 7 days in comparison with untreated samples (Table 4). On the other hand, the shelf-life of treated or untreated fresh-cut oranges was not limited by any microorganisms during the 21 days of the experiment. Similar results have been reported by Raybaudi-Massilia et al. [20, 41], who evaluated the shelf-life extension of fresh-cut "Fuji" apples and "Flor de Invierno" pears using natural antimicrobial substances, among them, *MA* (2.5%, w/v) as dipping treatment.

Sensory evaluations indicated that significant statistically differences ($p<0.05$) among the values assigned by the panelist to flavor, taste and global acceptance on treated and untreated fresh-cut oranges were found (Figure 4b); nonetheless, treated samples were accepted by panelists. On the other hand, no significant ($p>0.05$) differences between treated and untreated fresh-cut mangoes were observed (Figure 4a). The antimicrobials added in fresh-cut mango did not affect significantly ($p<0.05$) the sensory attributes.



"Figure 4. Sensory attributes of fresh-cut mangoes (a) and oranges (b) treated or not with a combination of *Panax ginseng* (3% w/v), malic acid (1% w/v), potasium sorbate (0.05% w/v) and calcium lactate (0.75% w/v). Values are mean of 30 determinations"

"4. Conclusions"

A combination of PG (3% w/v), *MA* (1% w/v), PS (0.05% w/v) and CL (0.75% w/v) was effective to reduce populations of *Salmonella* Saintpaul and *E. coli* O157:H7, as well as to delay the growth of the native microflora in fresh-cut mangoes and oranges. In addition, fresh-cut mangoes with antimicrobials added had a good acceptation for the panelists. This method can ensure the safety and quality of fresh-cut fruits and represents an alternative technology to physical treatments; in addition to its feasibility for food industry. Further studies using hurdle technologies are needed for reducing the negative impacts of these compounds on some kind of fresh-cut product such as orange.

"5. Acknowledgments"

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