Growth Inhibition of Bacteria by Salsa Mexicana

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ABSTRACT: Antimicrobial activity of salsa mexicana against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* was studied. The pressed salsa juice suppressed completely the growth of *E. coli, B. subtilis*, and *S. aureus. K. pneumoniae* and *B. cereus* were suppressed partly but *Saccharomyces cerevisiae* was not suppressed at all by the juice. Three types of microbicidal or microbiostatic components have been found: (1) H^+, (2) volatile compounds, and (3) non-ionized carboxylic acids or analogous compounds. It is proposed that the supplementation of stink bugs in the salsa seen widely in the south of Mexico is used to intensify the herbal odor of the salsa.

Keywords: carboxylic acids, growth inhibition, hexenal, microorganisms, salsa mexicana, stink bug

Introduction

SALSA MEXICANA (MEXICAN SAUCE) IS ADDED TO MOST MEXICAN dishes such as steak, tacos, tamales, and quesadillas. Fresh vegetables such as tomato, onion, coriander, and green chili are mashed by a blender or cut into tiny pieces by a cooking knife. Restaurants supply any amount that customers want without extra charge. The salsa gives a comfortable sour taste and herbal aroma to food.

Recently, the odor compounds of plants have been intensively studied. The herbal odors are caused by C_6-C_{10} aldehydes and alcohols such as *trans*-2-hexenal and *trans*-3-hexenol, which are synthesized from α-linolenic and linoleic acids by the complex action of lipoxygenase-hydroperoxide liase (Noordermeer and others 2001). The production of these compounds is related to the defense systems of plants (De Bruxelles and Roberts 2001; Norin 2001), plant–plant communications (Chamberlain and others 2000; Bruin 2001; Karban 2001), and plant–insect interactions (Tromans 2000; Kessler and Baldwin 2001; Van Poecke and others 2001; Varassin and others 2001). Naturally occurring volatile compounds such as aldehydes are used to sterilize food materials (Hammond and Kubo 2001). Archbold and Hamilton-Kemp (2000) reviewed the use of hexenal to disinfect berries.

Salsa mexicana, which presents a strong herbal odor, could possess a strong bactericidal or bacteriostatic function. Foodborne diseases are very common in a tropical environment in Mexico.

Stink bugs, locally called jumiles, are sold in many markets in the south of Mexico and a proper species is used in different provinces, for example, *Euschistus taxcoensis*, at Taxco, Guerrero, Mexico. The insects are especially favored for use in the preparation of salsa mexicana (Ramos-Elorduy and Pino 1989).

Materials and Methods

Preparation of fresh salsa mexicana

Saladet tomato (*Lycopersicum esculentum*), green chili (*Capsicum annum*), white onion (*Allium cepa*), and coriander (*Coriandrum sativum*), in the proportion of 60:18:18:4 (% w/w) were washed with running water and then mixed with a blender. The pressed juice of the salsa was obtained by use of a zero-headspace extractor (Gelman, purchased from VWR Scientific Products).

Preparation of the control media, glucose-peptone to pH 5.7 (GP5.7) and pH 4.8 (GP4.8)

The control media GP5.7 was prepared by dissolving 2.13 g of glucose and 11.3 g of peptone made from gelatin in 100 mL of distilled water. The pH value of the resultant medium was 5.7. GP4.8 copying the pH of salsa mexicana was prepared by 2.13 g of glucose and 11.3 g of peptone from gelatin in 100 mL of distilled water containing 7.52 m*M* of citric acid that results in a pH of 4.8.

Microbiological assay of pressed juice, SV4.8

Microbiological assays were performed using *Escherichia coli* (ATCC 11303), *Bacillus subtilis* (ATCC 6051), *Bacillus cereus* (ATCC 11778), *Klebsiella pneumoniae* (ATCC 33495), *Staphylococcus aureus* (ATCC 25923), and *Saccharomyces cerevisiae* (ATCC 834). All of the assays were carried out in triplicate.

The pressed juice was sterilized by filtering it through a Millipore filtration cartridge (Billerica, Mass., U.S.A.) and then filtered through a 0.22-μm membrane filter (SV4.8). The control media GP5.7 and GP4.8 were sterilized at 121 °C for 20 min. The juice samples were inoculated with 20 μL (1500×10^6 colony forming units [CFU]/mL) of each microorganism at logarithmic phase and incubated stationary at 36 °C. The growth was monitored up to 35 h measuring turbidity at 750 nm with a single beam spectrophotometer, Hach, DR 2000 (Hach Company, Loveland, Colo., U.S.A.). The purity of the cultures was verified by froth with Gram tincture.

Microbiological assay of the fresh salsa mexicana juice without volatile components, SNV4.8 and SNV5.7

Approximately 50 mL of water was removed by evaporation from 250 mL of filtered fresh salsa mexicana juice using a rotary evaporator (Brinkman Instruments, Westbury, N.Y., U.S.A.) at 50 °C. The lost volume was refilled by adding distilled water and was followed by sterilization by filtering through a 0.22-μm membrane filter. The sterilized juice without volatiles was divided into 2 parts: one of them raised the pH to 5.7 with 0.1 *M* NaOH (SNV5.7) whereas the other part was maintained at its original pH of 4.8 (SNV4.8).

Stink bugs

Stink bugs (*Euschistus taxcoensis*) (Ramos-Elorudy 1989) were purchased in an open market at the rear of the cathedral of Taxco, Guerrero, Mexico, and kept in a clear plastic container (5 L) filled with fallen leaves collected from La Montana de Jumiles where the local residents collect the insects.

Twenty-six insects (3.4 g) were kept in a glass tube (20 cm

Table 1—Chemical composition of the salsa mexicana juice (%w/w)

Carbohydrates	2.13
Protein	1.68
Fat	0.5
Fiber	1.06
Moisture	93
Ash	1.62

long × 25 cm o.d.) and the both ends were stoppered by a rubber stopper that was put through a small glass tube (2.5 cm long × 1.2 cm o.d.). A constant gas-flow (30 mL/min) was applied for 1 h from one end, and the other end was connected to a plastic cartridge for solid-phase extraction packed with 1 g of C18 (Alltech Associates, Inc., Deerfield, Ill., U.S.A.). The glass tube was tapped occasionally with a wooden stick to stress the insects.

The cartridge was washed by 1 mL of cyclohexane. The eluente was analyzed by gas chromatograph, using a Perkin-Elmer Autosystem (Wellesley, Mass., U.S.A.) equipped with a flame ionization detector (FID), a glass capillary column, and AT Wax (15 m × 0.53 i.d., 1-μm film thickness) from Alltech Associates. The oven temperature was 100 °C, isotherm. The temperatures of the injector and detector were 190 °C and 250 °C, respectively. The flow rate of He carrier gas was 3.0 mL/min.

Results and Discussion

THE PRESSED SALSA MEXICANA JUICE WAS A TRANSPARENT AND SLIGHTLY yellow liquid with a penetrating odor characteristic of salsa mexicana. The color of the juice did not interfere with the measurement of the growth of microorganisms using the change of absorbance at 750 nm.

Table 1 shows the chemical composition of the pressed salsa mexicana juice. A culture medium (GP5.7) composed of glucose (2.13 g/100 mL) and peptone made of gelatin (11.3 g/100 mL) equalizing carbohydrates and proteins in the juice was used as the control medium for pressed juice adjusted to pH 5.7.

The pressed salsa juice was titrated by 0.1 *M* NaOH as seen in Figure 1. It was found that a glucose–peptone medium containing 7.52 m*M* citric acid behaved similarly to the juice on titrating with HCl, as shown in Figure 1, especially in the region of pH less than 7 where this study had been done. Thus, the glucose–peptone medium containing 7.52 m*M* citric acid (GP4.8) was used as a control medium at a pH to that of the pressed juice.

Six microorganisms were inoculated to the sterile salsa juice and monitored spectrophotometrically. Figure 2 shows OD (optical density with arbitrary scale) compared with time curves for *E. coli* on various media monitored for 35 h. The bacteria in filtered salsa juice (SV) barely grew. Volatile components were removed from SV to obtain SNV, in which the growth was still inhibited completely.

It is well accepted that the growth of microorganisms in the steady state can be represented by the following exponential formula (Elsworth and Telling 1956):

$$N = N_0 e^{kt}, \quad (1)$$

where N is the number of a microorganism at time, t, initiating with the number N_0 at t = 0, and k is a constant. Eq. 2 can be derived from Eq. 1:

$$\log N = kt - \log N_0 \quad (2)$$

When a certain amount of a microorganism is added to a salsa juice, the initial stage of growth is complicated. However, a steady state will be achieved after a certain number of divisions of the microorganism. Then, the plot of *log*N against t gives a straight line with the slope *k*.

The relative slope, *K*, was obtained by dividing the observed *k* for a medium by that for gp4.8. Thus, *K*sv = *k*sv/*k*gp4.8, where the suffixes sv and gp4.8 indicate the medium sv (salsa juice) and gp4.8 (control medium), respectively. Table 2 summarizes the observed *K* values for the microorganisms in 5 media.

Bacillus subtilis and *Staphylococcus aureus*, as well as *E. coli*, did not grow at all in the salsa juice. However, *Saccharomyces cerevisiae* growth was not suppressed by the juice.

Since the growth rate for *E. coli* after removing the volatiles from the salsa juice (*K*snv) remains zero, there must be present a nonvolatile toxic compound in SNV. The role of [H+] can be see from Table 2 comparing GP5.7 and SNV5.7 with GP4.8 and SNV. *K. pneumoniae*, known as acidophilic bacteria, shows *K*gp > 1, that is, *k*gp5.7 is smaller than *k*gp4.8. However, all other microorganisms grow slower in GP4.8 than in GP5.7.

Figure 3 shows the effect on the growth of *E. coli* by the additions of 10 m*M* acetate (GP4.8A) or butyrate (GP4.8B) ion to GP4.8, and similarly the corresponding set based on GP5.7. All of the growths

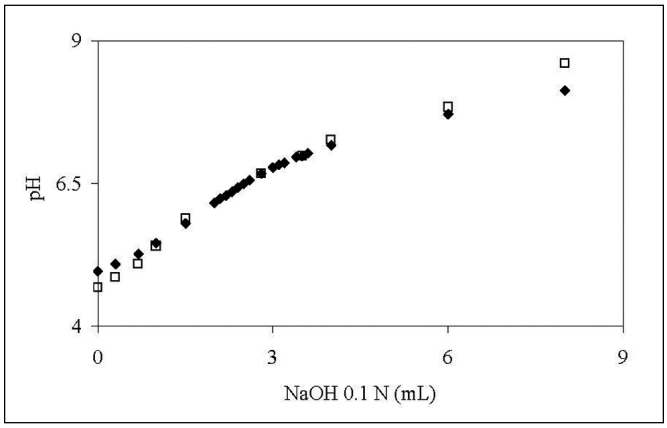

Figure 1—The pH behavior of salsa juice (◆) and glucose–peptone medium containing 7.52 m*M* (□) on titrating with 0.1 *M* NaOH.

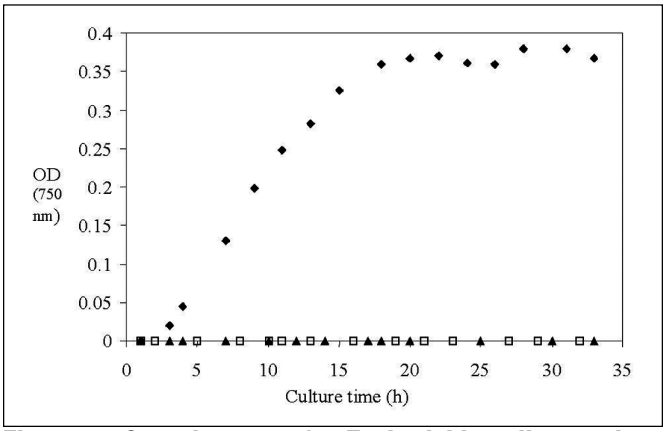

Figure 2—Growth curves for *Escherichia coli* on various media monitored for 35 h: (◆) control medium GP4.8, (□) salsa juice, and (▲) salsa juice without volatiles.

Table 2—Relative growth rates, *K*, of 6 microorganisms in salsa mexicana juice preparations and glucose-peptone media

		Microorganism					
K	Medium	bs[a]	ec[b]	sa[c]	kp[d]	bc[e]	sc[f]
*K*sv	salsa juice	0	0	0	0.21	0.77	0.99
*K*snv	salsa without volatiles	0.50	0	1.58	0.41	0.95	1.38
*K*snv5.7	SNV adjusted pH 5.7	2.90	0.80	1.79	0.39	0.80	1.55
*K*gp4.8	glucose–peptone, pH 4.8	1.00	1.00	1.00	1.00	1.00	1.00
*K*gp5.7	glucose–peptone, pH 5.7	2.72	1.93	2.53	0.60	1.93	1.04

[a]*Bacillus subtilis*; [b]*Escherichia coli*; [c]*Staphyloccus aureus*; [d]*Klebsiella pneumoniae*; [e]*Bacillus cereus*; [f]*Saccharomyces cerevisiae*

were reduced notably in the presence of the carboxylate ions, especially the butyrate ion, which suppressed almost completely in GP4.8.

The approximated concentrations of H^+, acetic acid, acetate ion, butyric acid, and butyrate ion in the media shown in Figure 3 were estimated assuming Ka = $[H^+] \times [RCOO^-]/[RCOOH] = 10^{-5}$ for the purpose of considering the concentration behavior of these species as seen Table 3.

The H^+ concentration of GP5.7, GP5.7A, and GP5.7B are 2 μM. Therefore, the increased toxicities by the supplementation of acetate or butyrate ion must be attributable to these ions or to acetic or butyric acid. The toxicity of GP5.7B, which is higher than that of GP5.7A, might be caused by the fact that the former is easier to pass through the cell membrane than the latter.

On the other hand, the H^+ concentrations of GP5.7, GP5.7A, and GP5.7B are 16 μM. A similar relation in the set GP4.8, GP4.8A, and GP4.8B is seen as of GP5.7, GP5.7A, and GP5.7B. The growth of *E. coli* in the media with pH 4.8 is suppressed more than those with pH 5.7. There might be more toxic carboxylate derivatives present in the salsa originated from the defense system of plants. Therefore, the stronger suppression at lower pH media must be caused by a combination of a proper toxicity of H^+ and residual carboxylic acids or similar compounds present in the starting material of the media. The real contribution to toxicity of H^+ cannot be estimated yet.

Since the growth rate for *E. coli* after removing the volatile from the salsa juice (*K*snv) remains zero, there must be present a nonvolatile toxic compound in SNV. The role of $[H^+]$ can be seen from Table 2 comparing GP5.7 and SNV5.7 with GP4.8 and SNV. *K. pneumoniae*, known as acidophilic bacteria, shows *K*gp > 1, that is, *k*gp5.7 is smaller than *k*gp4.8. However, all other microorganisms grow slower in GP4.8 than in GP5.7.

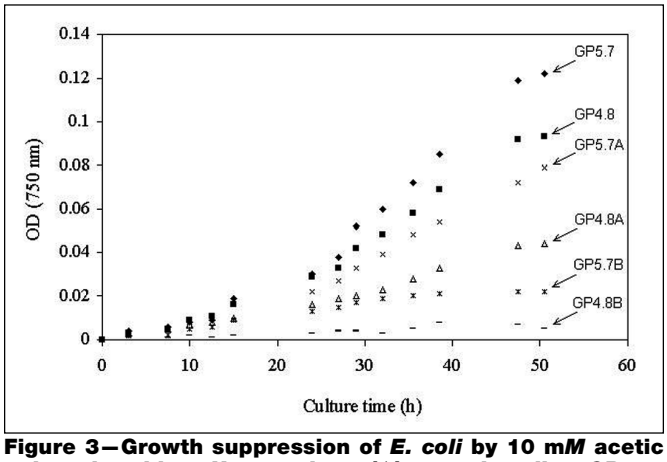

Figure 3—Growth suppression of *E. coli* by 10 m*M* acetic or butyric acid at pH 4.8 and 5.7: (◆) control medium GP5.7, (■) control medium GP4.8, (×) acetate ion in GP5.7 (GP5.7A), (△) acetate ion in GP4.8 (GP4.8A), (*) butyrate ion in GP5.7 (GP5.7B), (—) butyrate ion in GP4.8 (GP4.8B).

Table 3—Estimated concentrations of H^+, carboxylic acids, and carboxylate ions added to GP5.7 and GP4.8 assuming Ka = 10^{-5}

	[H^+]	[RCOOH]	[$RCOO^-$]
GP5.7	2 μM	0 m*M*	0 m*M*
GP5.7 + 10 m*M* acetate	2 μM	1.25 m*M*[a]	8.75 m*M*[b]
GP5.7 + 10 m*M* butyrate	2 μM	1.25 m*M*[c]	8.75 m*M*[d]
GP4.8	16 μM	0 m*M*	0 m*M*
GP4.8 + 10 m*M* acetate	16 μM	6 m*M*[a]	4 m*M*[b]
GP4.8 + 10 m*M* butyrate	16 μM	6 m*M*[c]	4 m*M*[d]

[a]acetic acid; [b]acetate ion; [c]butyric acid; [d]butyrate ion

The stink emitted by stink bugs when they are molested is well-known. It is well-documented that the main odorous principles are alkenal and alkenol (Stransky and others 1998; Krall and others 1999; Ho and Millar 2001). In the case of jumiles, 2-hexenal, 2-octenal and nonanal were found in the ratio of 2:1:1. Thus, the stink has a similar composition of the aldehydes as an herbal odor. The lipoxygenase-hydroperoxide liase system in plants to produce volatiles is activated when a tissue is injured. Therefore, the process of making salsa mexicana such as liquefying or grinding vegetables intensifies the formation of herbal odor. It is concluded that the stink bugs are supplemented to increase the herbal odor in the salsa.

The constituents of herbal flavor are destroyed during a heat process; therefore, all of the canned or bottled salsa mexicana products have no herbal flavor. The supplementation of 2-hexenal, which is permitted as a food additive by the FDA (2002), to canned or bottled salsa mexicana products might be beneficial to compensate the herbal flavor destroyed on heating and may also act as preservative (Beltran-Garcia and others 1997).

Conclusions

THE PRESSED JUICE OF SALSA MEXICANA SUPPRESSED COMPLETELY THE growth of *B. subtilis, E. coli,* and *S. aureus* and slowed the growth of *K. pneumoniae* and *B. cereus* but does not affect any of the growth of *S. cerevisiae.* The toxic components for the bacteria are classified as (1) volatile compounds, (2) H^+, and (3) undissociated compounds such as protonated carboxylic ion. As an example of undissociated acetic and butyric acids have been shown to be toxic to *E. coli*. The supplementation of hexenal, which is permitted for use as a food additive by the FDA, to canned or bottled salsa mexicana products might compensate for the destroyed herbal flavor in the heating process and also serve as a preservative.

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