Lemon Juice as a Natural Biocide for Disinfecting Drinking Water[1]

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The natural biocidal activity of lemon juice was studied in order to explore its possible use as a disinfectant and inhibitor of Vibrio cholerae *in drinking water for areas lacking water treatment plants.*

From January through July 1993, water samples of varying alkalinity and hardness were prepared artificially, and underground and surface water samples were obtained from a number of different rural and urban areas in Argentina's Buenos Aires Province. After measuring the latter samples' hardness and alkalinity, a range of concentrations of lemon juice and other acidifiers were added to each sample, and the resulting pH as well as the samples' ability to destroy V. cholerae *were determined.*

The results show that lemon juice can actively prevent survival of V. cholerae *but that such activity is reduced in markedly alkaline water. For example, treatment of underground drinking water, which is characterized as having the greatest degree of alkalinity in our area, will typically destroy* V. cholerae *if the alkalinity of the water is the equivalent of that produced by 200 mg $CaCO_3$ per liter, if enough lemon juice is added to bring the lemon juice concentration to 2%, and if the lemon juice is allowed to act for 30 minutes. All this points up the need to determine the alkalinity of water from any local source to be treated in the process of assessing the minimum concentration of lemon juice required.*

The reappearance of cholera in various regions of Latin America has spawned a search for new compounds to disinfect drinking water, the prime vehicle for transmission of the cholera agent, *Vibrio cholerae,* which is expelled in the excreta of infected individuals and survives best in an aquatic environment. (It should also be noted, however, that seafoods, vegetables, and other foods may also constitute sources of *V. cholerae* infection when hygiene and cooking procedures are inadequate) (*1–3*).

The compounds commonly used to disinfect domestic water are chlorine-based and are added to water until a chlorine concentration of 0.5 mg/l or greater is obtained (*4*). However, efforts are currently being made to minimize the intake of chlorinated compounds because of the potential health risk posed by chlorine's ability to form trihalomethanes in the presence of organic vegetable remains such as humus (*5–7*). This is one reason why chemicals are being sought that can substitute for chlorine derivatives. Moreover, in many places chlorinated compounds are unavailable, making it necessary to resort to other more accessible and cheaper substances.

One such substance is lemon juice, which is a natural biocide. The aim of the work reported here was to study the disinfectant activity of lemon juice in water contaminated with *V. cholerae,* together with certain water features (notably pH) that influence this disinfectant activity.

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MATERIALS AND METHODS

The study, which took place from January to July 1993, employed samples of natural surface and underground water obtained at different locations in Argentina's Buenos Aires Province. These water samples were taken from the domestic potable water distribution network, rivers, and (especially) wells, because all of these sources are accessible to both rural and urban populations and because they respond differently to the acid substances that were being tested, probably as a result of the varying characteristics (salt content, alkalinity, and hardness) of the aquifers involved.

Natural underground water samples, designated "a" through "e", and natural surface water samples, designated "f" through "i", were obtained from the following sources:

- Underground water: a—from the marine coastal area of Buenos Aires Province; b—from a rural area (Dolores) of Buenos Aires Province; c—from another marine coastal area of Buenos Aires Province located along the coast 10 km away from the source of sample a; d—from an urban area (Castelar) of Buenos Aires Province; e—from another urban area (Munro) of Buenos Aires Province.
- Surface water: f—from a domestic potable water distribution system supplied by the River Plate, this being soft water with no residual chlorine and a hardness equivalent to 63 mg/l of calcium carbonate; g—from the River Plate (5 km in from the coast); h—from the Luján River; i—distilled water used for control purposes that was obtained from the domestic potable water distribution system and distilled with a Barnstead gas distiller, model GLO.H2.

In addition, the following were made up:

- a standard hard water solution, theoretically equivalent to a solution of 340–360 mg/l of calcium carbonate ($CaCO_3$), was prepared using $MgCl_2$ (0.11 g/l) and $CaCl_2$ (0.27 g/l) (*8*);
- a standard alkaline water solution was prepared with sodium carbonate (Na_2CO_3) to attain an alkalinity approximately equivalent to 400 mg/l $CaCO_3$ (*9*);
- a standard solution of hard and alkaline water was prepared by adding sodium bicarbonate ($NaHCO_3$) to the standard hard water solution until an alkalinity approximating that of a solution of 400 mg/l of $CaCO_3$ was obtained.

The hardness and alkalinity of the water from the various natural and artificial sources were determined using standard methods (*10*). Subsequently, water from each source was mixed with one of three acid substances to produce a series of dilutions of varying strength, 10 ml portions of these dilutions being placed in a series of test tubes. The acid substances mixed with the water samples were as follows: (1) juice from natural lemons grown in Argentina's Tucumán and Entre Ríos provinces; (2) bottled commercial lemon concentrate; and (3) 7% citric acid (Merck) at a 7% strength prepared in our laboratory, this strength being equivalent to that found in the natural lemon juice and also in the reconstituted concentrate employed (*11*). The pH of the various dilutions prepared with water from each source was then determined using a Cole Palmer measuring gauge, model 5985-80.

A suspension of *V. cholerae*, biotype El Tor, serotype Inaba, that had been cultured for 24 hours in soy trypticase agar broth (Difco) was prepared in a physiologic solution to obtain a concentration of 2–3 × 10^9 bacteria per ml.

To assess the bactericidal action of the various acidic substances, 10 ml dilutions of lemon juice and citric acid were again prepared with each of the various natural and artificial water samples, the dilutions again being placed in a series of test tubes. Each test tube was then inoculated with 0.1 ml of the *V. cholerae* suspension and placed in a water bath maintained at 20 °C, and the resulting solution was examined for *V. cholerae* after varying periods of time.

This examination was performed by removing 0.1 ml of the solution and placing it in 10 ml of a soy trypticase culture broth (Difco) with 0.5% Tween 80 to permit proliferation of the microorganisms. The culture tubes were then incubated at 32–33 °C for 48 hours, after which the presence or absence of bacterial growth was recorded. The contents of each culture tube where bacterial growth was observed were cultured on agar plates containing trypteine, citrate, bile, and sucrose. The presence of *V. cholerae* in positive cultures was then confirmed by means of serologic tests performed with polyvalent agglutinating antiserum obtained from the Dr. Carlos G. Malbrán National Institute of Microbiology for diagnosing *V. cholerae* 01 (Inaba and Ogawa serotypes).

RESULTS

The hardness and alkalinity of the natural surface and underground water samples (and of the distilled water that served as a control) are shown in Table 1 in terms of equivalent mg $CaCO_3$ per liter. Similar data on the artificially prepared solutions of alkaline and hard water appear in Table 2, which also shows how hardness and alkalinity were affected by varying dilutions of lemon juice. As was to be expected, the degree of hardness did not significantly influence the acid pH produced by the lemon juice, while the degree of alkalinity obviously did.

Table 1. Hardness and alkalinity (expressed in mg/l of $CaCO_3$) of underground and surface water samples. Water samples "a" through "e" came from underground sources, and samples "f" through "h" came from surface sources. Sample "i" consisted of distilled water and served as a control.

Water	Hardness	Alkalinity
a	245.0	249.80
b	283.0	326.03
c	290.0	305.33
d	175.0	401.06
e	170.0	429.52
f	63.0	51.75
g	43.7	77.62
h	42.0	77.00
i	N/A	N/A

The results obtained with reconstituted commercial concentrated lemon juice are not shown in Table 2 or any of the subsequent tables, as they were generally very similar to those obtained with unconcentrated lemon juice. For the same reason, the results obtained with 7% citric acid are shown in Table 3 only.

Table 3 shows the various pH readings produced by adding different amounts of lemon juice and 7% citric acid to the samples of natural underground water until the indicated dilutions were obtained. Similarly, Table 4 shows the pH readings yielded by adding varying amounts of lemon juice to the natural surface water samples and the distilled water control sample.

Various dilutions of lemon juice were also prepared using the artificial hard water that had been rendered alkaline in order to simulate the hardness and alkalinity of natural water. As Table 5 shows, when relatively hard and alkaline artificial water samples were used the dilutions yielded pH readings similar to those obtained with the natural underground water samples, all of which had an average alkalinity exceeding the equiv-

Table 2. pH readings obtained by using water samples artificially adjusted to specific levels of hardness or alkalinity (both expressed in mg/l of $CaCO_3$) to prepare the indicated lemon juice dilutions. (The control samples shown received no lemon juice.)

	Dilutions of lemon juice						Untreated
	1/10	1/20	1/50	1/100	1/500	1/1 000	control
Water hardness:							
360.0	2.58	2.59	2.73	2.88	3.16	3.52	6.86
180.0	2.60	2.63	2.78	2.86	3.13	3.17	6.88
90.0	2.63	2.66	2.83	2.92	3.15	3.17	6.88
45.0	2.64	2.70	2.85	2.95	3.13	3.13	6.64
22.5	2.64	2.72	2.86	2.94	3.12	3.50	6.70
Alkalinity:							
396.0	2.90	2.95	3.80	4.82	9.66	10.12	10.29
198.0	2.88	2.93	3.31	3.90	7.14	9.14	9.88
99.0	2.84	2.86	3.08	3.28	5.71	6.42	9.49
49.5	2.95	2.84	2.92	3.05	3.87	5.12	9.12
24.8	2.96	2.86	2.93	3.13	3.40	3.97	8.12

Table 3. pH readings obtained by using natural underground water samples "a" through "e" to prepare the indicated lemon juice and 7% citric acid dilutions. (The control samples shown received no lemon juice or citric acid.)

Acidifier and	Dilutions of lemon juice or 7% citric acid						Untreated
water sample	1/10	1/20	1/50	1/100	1/500	1/1 000	control
Lemon juice:							
a	2.75	2.94	3.36	3.95	6.55	6.67	7.46
b	2.85	3.05	3.60	4.35	6.47	6.67	7.26
c	2.86	3.01	3.60	4.36	6.61	6.76	7.29
d	2.83	3.07	3.91	4.95	6.75	6.93	7.33
e	2.82	3.09	3.97	5.18	6.65	6.72	6.88
7% citric acid:							
a	2.68	2.71	3.29	3.88	6.31	6.37	7.46
b	2.60	2.78	3.34	4.12	6.43	6.45	7.26
c	2.69	2.80	3.38	4.06	6.41	6.52	7.29
d	2.70	3.00	3.83	4.80	6.70	6.96	7.33
e	2.71	2.98	3.85	4.87	6.60	6.63	6.88

Table 4. pH readings obtained by using natural surface water samples "f" through "h" and distilled water sample "i" to prepare the indicated lemon juice dilutions. (The control samples shown received no lemon juice.)

Water	Dilutions of lemon juice						Untreated
sample	1/10	1/20	1/50	1/100	1/500	1/1 000	control
f	2.85	3.02	3.30	3.47	3.72	5.47	7.40
g	2.86	3.10	3.40	3.67	4.10	6.24	7.23
h	2.84	3.10	3.38	3.62	3.93	6.18	7.20
i	2.75	2.85	3.10	3.25	3.35	3.78	6.36

Table 5. pH readings obtained by using water samples simultaneously adjusted to the indicated levels of hardness and alkalinity (expressed in mg/l of $CaCO_3$) to prepare the indicated lemon juice dilutions. (The control samples shown received no lemon juice.)

Hardness/ alkalinity	Dilutions of lemon juice						Untreated control
	1/10	1/20	1/50	1/100	1/500	1/1 000	
406/312	2.88	3.08	3.80	4.55	7.19	7.29	7.50
203/155	2.89	2.92	3.26	3.97	6.40	6.71	7.61
102/77	2.87	2.85	3.08	3.33	5.37	6.08	7.51
51/39	2.89	2.86	2.99	3.22	4.33	5.27	7.40
25/19	2.88	2.88	2.99	3.20	3.88	4.14	7.10

alent of 200 mg/l of $CaCO_3$ (see Table 1).

Table 6 shows whether or not *V. cholerae* were detected in lemon juice dilutions made with underground, surface, and distilled water samples that were kept for 15 and 30 minutes after inoculation with the disease agent. As may be seen, lemon juice concentrations of 1/100 (1%) or lower did not prevent survival of *V. cholerae* in dilutions made with the underground water samples, presumably because the resulting pH was too high. In general, 15 to 30 minute *V. cholerae* survival was not prevented by any of the dilutions with a pH of 3.9 or higher.

DISCUSSION AND CONCLUSIONS

Obviously, the initial alkalinity of a water sample will influence the ultimate

Table 6. Presence (+) or absence (−) of *Vibrio cholerae* in water acidified with lemon juice (see pH levels shown) 15 and 30 minutes after inoculation with the vibrios. Water samples "a" through "e" came from underground sources, and samples "f" through "h" came from surface sources. Sample "i" consisted of distilled water and served as a control.

Water source		Dilutions of lemon juice/action time (minutes)											
		1/20		1/30		1/40		1/50		1/100		1/1 000	
		15m	30m	15m	30m	15m	30m	15m	30m	15m	30m	15m	30m
Hard (standard)	+/−	−	−	−	−	−	−	−	−	+	+	+	+
	pH	3.1	3.1	3.2	3.2	3.5	3.5	3.8	3.8	4.5	4.5	7.3	7.3
a	+/−	−	−	−	−	−	−	−	−	+	+	+	+
	pH	2.7	2.7	2.9	2.9	3.0	3.0	3.3	3.3	3.9	3.9	6.4	6.4
b	+/−	−	−	−	−	−	−	−	−	+	+	+	+
	pH	2.9	2.9	3.0	3.0	3.3	3.3	3.5	3.5	4.4	4.4	6.6	6.6
c	+/−	−	−	−	−	−	−	−	−	+	+	+	+
	pH	3.0	3.0	3.0	3.0	3.2	3.2	3.4	3.4	4.2	4.2	6.7	6.7
d	+/−	−	−	−	−	−	−	−	−	+	+	+	+
	pH	3.0	3.0	3.2	3.2	3.5	3.5	3.8	3.8	4.8	4.8	7.0	7.0
e	+/−	−	−	−	−	−	−	+	−	+	+	+	+
	pH	3.0	3.0	3.1	3.1	3.6	3.6	3.8	3.8	4.9	4.9	6.6	6.6
f	+/−	−	−	−	−	−	−	−	−	−	−	+	+
	pH	3.0	3.0	3.0	3.0	3.2	3.2	3.3	3.3	3.5	3.5	5.5	5.5
g	+/−	−	−	−	−	−	−	−	−	−	−	+	+
	pH	3.1	3.1	3.1	3.1	3.4	3.4	3.4	3.4	3.7	3.7	6.2	6.2
h	+/−	−	−	−	−	−	−	−	−	−	−	+	+
	pH	3.1	3.1	3.1	3.1	3.3	3.3	3.4	3.4	3.6	3.6	6.2	6.2
i	+/−	−	−	−	−	−	−	−	−	−	−	−	−
	pH	2.8	2.8	2.9	2.9	2.9	2.9	3.1	3.1	3.2	3.2	3.8	3.8

pH obtained by adding to it an acidic substance such as lemon juice. In the case of the relatively alkaline underground water samples tested, the low pH induced by relatively high concentrations of lemon juice and 7% citric acid (see Table 3) was found to rise when the concentrations fell as low as 1 part in 100 (1%) and to approach neutrality at lower concentrations. These pH increases were much less marked in water samples whose alkalinity was artificially set at lesser levels (see Tables 2 and 5). Furthermore, when the various acid dilutions were prepared using distilled water (see Table 4), very little pH increase was observed at low lemon juice concentrations, owing to the slightly acid pH of the water itself (the result of absorption of atmospheric CO_2 during the distillation process) and its extremely poor buffering capacity. If one combines this circumstance with *V. cholerae*'s preference for some degree of salinity in water, the result of combining distilled water (see item i in Table 6), a low concentration of lemon juice, and the disease agent is large-scale destruction of *V. cholerae*.

Based on the above, it may be inferred that when lemon juice or lemon juice substitutes are added to alkaline underground water in concentrations lower than those indicated by this study, effective destruction of *V. cholerae* is not achieved. It should be remembered that alkalinity and hardness are characteristics of natural underground water. Of course the hardness of water, in and of itself, does not influence the pH produced through addition of an acid substance, although alkalinity obviously does; nor do hardness and alkalinity act synergistically.

Prior experience, acquired under laboratory conditions, has shown that much higher concentrations of lemon juice (greater than 10% and 25%) can disinfect water contaminated with *V. cholerae* in a relatively short time (5 to 10 minutes). It is recommended, however, that specific tests be conducted with the various types of water accessible to the general public, taking into consideration all of the various factors that may affect the results. Otherwise, an excessively low dose of the acidic substance in question might be recommended for disinfecting drinking water in connection with cholera prophylaxis activities. This error could have dangerous consequences if people became convinced they were being protected by what was in fact an inadequate disinfecting action.

From the above, it can be deduced that lemon juice can have an unquestionably beneficial effect as an affordable and accessible disinfectant capable of destroying *V. cholerae* in drinking water consumed by both rural and urban populations. It should be noted, however, that not all underground water will be disinfected by the same concentration of lemon juice. To judge by our tests, underground water with a mean alkalinity equivalent to 200 mg $CaCO_3$ per liter may be disinfected for the purpose of destroying *V. cholerae* with a minimum 2% concentration of lemon juice—equivalent to roughly 2 tablespoons per liter of water—if it is allowed to act for at least 30 minutes, a concentration and action time that would provide an even greater margin of safety if less alkaline water were treated.

Generally speaking, these results are applicable only to drinking water. Neither the proposed lemon juice concentration or action time would necessarily be applicable for disinfecting vegetables or other foods contaminated with *V. cholerae*; indeed, it seems likely that the minimum concentration of lemon juice would need to be much greater in such cases because of the possible presence of substances serving to protect the microorganism (*12*).

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World AIDS Day 1994

The theme for World AIDS Day 1994, celebrated on 1 December, was "AIDS and the Family." It was doubly appropriate, since 1994 was the International Year of the Family, and traditional as well as nontraditional families play a crucial role in addressing the HIV/AIDS pandemic.

Since its inception in 1988, World AIDS Day has been an annual focus of efforts to raise public awareness about HIV/AIDS. Each year, the WHO Global Program on AIDS (GPA) has chosen a different theme for events and activities leading up to World AIDS Day. The concepts emphasized by GPA in 1994 were that a family can be any group of people linked by trust, mutual support, and shared destinies and that a family takes care of its members—protecting them from HIV infection and giving compassionate support to those affected by HIV or AIDS.

The families of AIDS victims share the burden of the disease. Some are left destitute by the illness of the breadwinner; others are torn apart by the emotional toll and stigma of the disease. But families also are the first line of defense against HIV through teaching young people about safe behavior, and they are among the best sources of care for AIDS patients.