Bactericidal Activity of Lemon Juice and Lemon Derivatives against Vibrio Cholerae

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Food products can be possible vectors of the agent responsible for cholera epidemics, because some of these products allow Vibrio cholerae O1 to develop to concentrations above the dangerous level. This study deals with the behaviour of essential oils, natural and concentrated lemon juice and fresh and dehydrated lemon peel against V. cholerae O1 biotype Eltor serotype Inaba tox+ . Our aim was to evaluate whether these products, used at different dilutions, exhibit bactericidal or bacteriostatic activity against the microorganism, when present at concentrations of 10⁶, 10⁷, 10⁸ and 10⁹ colony forming units (CFU) ml⁻¹, and after different exposure times. 10⁶ CFU ml⁻¹ was considered an infectious dose. Concentrated lemon juice and essential oils inhibited V. cholerae completely at all studied dilutions and exposure times. Fresh lemon peel and dehydrated lemon peel partially inhibited growth of V. cholerae. Freshly squeezed lemon juice, diluted to 10⁻¹, showed complete inhibition of V. cholerae at a concentration of 10⁸ CFU ml⁻¹ after 5 min of exposure time; a dilution of 2 × 10⁻² produced inhibition after 15 min and a dilution of 10⁻³ after 30 min. It can be concluded that lemon, a natural product which is easily obtained, acts as a biocide against V. cholerae, and is, therefore, an efficient decontaminant, harmless to humans.

Key words Vibrio cholerae; lemon product; bactericide; bacteriostatic

Cholera has been an endemic disease in India for centuries, although there have been devastating epidemics in other parts of the world throughout history. In January 1991, a choleric epidemic broke out in Peru, and through 1993 over 70000 cases were notified along the Pacific coast with approximately 4200 deaths. Despite the variety of measures taken by neighbouring countries, the epidemic extended to several other Latin-American countries, and, until January 1994, 50000 cases were reported in Brazil, 9000 in Bolivia and over 2000 cases in the North of Argentina. In Argentina, Salta and Jujuy provinces were most affected with 95.5% of all cases. In our province, Tucumán, 11 isolates were detected from 1992 until 1994.

Cholera infection is produced by ingestion of Vibrio cholerae by means of the same direct and indirect transfer mechanisms that occur in other enteric infections. The propagation mode has not yet been well-defined, but it is reasonable to assume that it is caused by water uptake (e.g. contaminated drinking water), personal contact and by the indirect transmission of contaminated food. Probably the most important vectors of cholera are water, ice cubes, kitchen tools and fruits and vegetables, which are irrigated or washed with sewage water.

In areas where cholera is endemic or where the risk of an outbreak exists, it is important to implement simple measures to prevent its transmission.

The compounds normally used as disinfectants are based on chlorine (hypochlorite). At present, ingestion of chlorinated products is being reduced to a minimum, due to their potentially harmful effects on human health due to the formation of trichloromethanes. Moreover, chlorinated products are not always available and, therefore, other substances, such as natural biocides and organic acids which are more accessible and cheaper, might serve as alternatives.4-6

The objective of this paper is to evaluate the efficacy of lemon juice, essential oils extracted from lemon peel and derivatives from the lemon industry, as bactericidal or bacteriostatic agents against V. cholerae. This study was carried out in association with the Tucumán Citrus Association.

MATERIALS AND METHODS

Microorganism A strain of Vibrio cholerae Serogroup O1 tox+, originally isolated from patients in the North of Argentina, was grown for 24 h in Tryptic Soy Broth (TS-Broth, Difco). Suspensions of this strain were made in physiological saline to prepare concentrations of 10⁶, 10⁷, 10⁸ and 10⁹ colony forming units (CFU) ml⁻¹. The last was considered an infectious dose.

Culture Media Alkaline Meat Extract Agar (MEA), pH 8, and TS-Broth were purchased from Difco Laboratories Inc., Detroit, MI.

Lemon Products Assayed Freshly squeezed lemon juice, fresh lemon peel, concentrated lemon juice (40% citric acid), essential oils and dehydrated lemon peel.

Assays Concentrated lemon juice and freshly squeezed lemon juice were diluted to 10⁻¹, 2 × 10⁻², 10⁻³, 2 × 10⁻⁴, 10⁻⁵ and 10⁻⁶, and only inoculated with an infectious V. cholerae dose. Dilutions of freshly squeezed lemon juice (2 × 10⁻², 10⁻³, 2 × 10⁻⁴ and 4 × 10⁻⁵) were also used after storage at 25 °C for 30 min, 6 h and 24 h, before inoculation with V. cholerae (10⁶ CFU ml⁻¹). Subcultures were made after 1, 5, 10, 15 20, 25 and 30 min of exposure at room temperature (approximately 25 °C). In all these cases, the pH was measured.

Essential oils, diluted to 10⁻¹ and 10⁻², dehydrated lemon peel (rehydrated for 15 min with continuous agitation; 1 g ml⁻¹, diluted to 10⁻¹ and 10⁻²) and fresh lemon peel (1 g ml⁻¹), were infected with all 4 V. cholerae concentrations, and then subcultured after 0, 1, 3, 6, 10 and 24 h.

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To determine the bactericidal action of the assayed products, the dilutions were plated onto MEA medium, and colonies were counted according to the most probable number technique. For all samples, the initial concentrations of V. cholerae were determined and, after exposure to each of the products assayed, the number of colonies was counted again.

For each plate, minimal biochemical assays and serological classification were carried out for the characterization of V. cholerae.

The results of CFU were used to compare the efficacy.

RESULTS

In general, a reduction in the concentration of V. cholerae was obtained with all the products assayed. Freshly squeezed lemon juice at dilutions of $10^{-1}$, $2 \times 10^{-2}$ and $10^{-3}$ inhibited bacterial growth completely at a concentration of $10^8$ CFU ml$^{-1}$.

In Fig. 1 the number of CFU ml$^{-1}$ after treatment with freshly squeezed lemon juice is given as a function of the exposure time.

A dilution of $2 \times 10^{-3}$ of this juice inhibited 75% of the microorganism growth after 5 min of exposure and 100% after 15 min. A dilution of $10^{-3}$ inhibited bacterial growth after 10 min of exposure, producing death of 75% of the cell population. After 30 min, this value was 100%, similar to a dilution of $10^{-4}$.

Dilutions of $2 \times 10^{-2}$, $10^{-2}$, $4 \times 10^{-3}$ and $2 \times 10^{-3}$ of freshly squeezed lemon juice, previously stored after squeezing, showed the following results:

Dilution $2 \times 10^{-2}$, pH 1.42 (Fig. 2), previously stored for 30 min, 6 h and 24 h, inhibited cell growth of V. cholerae completely after 5 min of exposure.

Dilution $10^{-2}$, pH 1.93, previously stored for 30 min and 6 h, inhibited cell growth of V. cholerae completely after 5 min of exposure but, after storage for 24 h it needed 15 min of exposure for complete cell growth inhibition.

Dilutions $4 \times 10^{-3}$, pH 2.00, and $2 \times 10^{-3}$, pH 2.10, both previously stored for 30 min, 6 h and 24 h, inhibited cell
growth of *V. cholerae* completely after 30 min of exposure.

Essential oils extracted from lemon peel showed inhibition of *V. cholerae* after all exposure times assayed and at all dilutions.

Fresh lemon peel without treatment produced inhibition of the microorganism at concentrations of $10^5$ and $10^6$ CFU ml$^{-1}$ after 1 h of exposure. Inhibition at a concentration of $10^6$ CFU ml$^{-1}$ occurred after 3 h of exposure. At a *V. cholerae*
concentration of $10^9$ CFU ml$^{-1}$, complete inhibition was not observed even after 24 h (Fig. 3).

Dehydrated lemon peel showed an inhibitory effect at a microorganism concentration of $10^2$ and $10^4$ CFU ml$^{-1}$ after all the exposure times studied, while, at concentrations of $10^6$ and $10^8$ CFU ml$^{-1}$ no inhibitory effect was observed and, as a consequence, microbial growth occurred (Figs. 4A and 4B).

DISCUSSION

Numerous microorganisms can cause intestinal disorders and, among them, representatives of the genus Vibrio are very important, principally from the viewpoint of public health as they can be transmitted by a large number of food products.\(^7,8\)

In industrialised countries the incidence of these diseases is extremely low, due to the rigid control of the bacteriological quality of drinking water, irrigation water and food by public health organisations.\(^9,10\) However, the explosive potential of this disease in developing countries such as those in Latin-America, should not be forgotten.

Besides all these control measures some authors have given special attention to the study of the action of different products on the development of this microorganism.\(^11\)–\(^14\)

Hoffman \textit{et al.}\(^15\) have demonstrated the inhibitory effect of 5 types of tea on microorganisms of \textit{V. cholerae}: black tea, natural maté tea, maté tea with lemon, black tea with cinnamon and maté with orange peel.

Tiburzi \textit{et al.}\(^16\) have examined the efficacy of organic acids as chemical inhibitors of the spread of \textit{V. cholerae}, achieving a reduction in the isolation of the vibron from chicken meat treated with lactic acid and acetic acid solutions with a pH of 4.8. The lemon derivatives in our study showed a similar inhibitory effect against the viability of \textit{V. cholerae}, when employed at a pH lower than 3.

Anderson \textit{et al.}\(^4\) and Woolthuis \textit{et al.}\(^17\) have considered the use of products harmless to public health, such as acetic acid and lactic acid.

Lemon is beneficial to human health and it is a natural product that can be easily acquired. Due to its inhibitory effect against the microorganism, it is an efficient and economical material, offering significant benefits to public health.

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\textbf{REFERENCES}