Antimutagenic Effect of Fruit and Vegetable Aqueous Extracts against *N*-Nitrosamines Evaluated by the Ames Test

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The inhibitory effect of nine fruit and vegetable aqueous extracts against the mutagenicity of *N*-nitrosodimethylamine (NDMA), *N*-nitrosopyrrolidine (NPYR), *N*-nitrosodibutylamine (NDBA), and *N*-nitrosopiperidine (NPIP) was evaluated by means of the Ames test. Onion extract (500 μg/plate) showed the greatest inhibitory effect (60%) against NDMA, and the mutagenicity of NPYR was inhibited markedly (54%) by apple extract (50 μg/plate). The antimutagenic effect of carrot extract (250 μg/plate) was remarkable (49%) against NDBA, and the mutagenicity of NPIP was also strongly inhibited (65 and 50%) by garlic and kiwi extracts (2000 μg/plate), respectively. Vegetable and fruit extracts that exhibited an antimutagenic effect in decreasing order against NDMA and NPYR were as follows: onion > licorice > kiwi = apple > carrot > garlic > pineapple > broccoli; and apple > broccoli > kiwi > onion = pineapple, respectively. Decreasing orders against NDBA and NPIP were, respectively, carrot > garlic > broccoli > onion > kiwi and garlic > kiwi > broccoli > green pepper > pineapple > carrot > onion = apple.

Keywords: *Vegetables; fruits; N-nitrosamines; mutagenicity; Ames test*

INTRODUCTION

Because nitrite is an important and widely used additive in human food, particularly in the curing of meat, poultry, and fish, there has been an upsurge of interest in the possible occurrence of trace amounts of *N*-nitroso compounds in human foods (Hotchkiss, 1987; Gangolli and Phillips 1985). *N*-Nitroso compounds, including *N*-nitrosamines, are known to be strong mutagens and carcinogens, which are formed by N-nitrosation reactions between various secondary or tertiary amines and nitrite or other nitrosating agents (Lijinsky, 1992). It has been shown that volatile *N*-nitrosamines occur in a wide variety of foods, particulary cured meat products, smoked fish, dried malt, and beer (Spiegelhalder et al., 1980; Scanlan, 1983; Havery and Fazio, 1985; Österdahl, 1988). Although no clear causal association has yet been rigorously established between exposure to low levels of *N*-nitrosamines and the occurrence of cancer in humans, accumulated evidence makes it probable that human beings would be susceptible to cancer induction by nitrosamines if exposed to sufficient amounts long enough (Magee, 1996).

Several factors present in the diet can modify levels of *N*-nitrosamines by acting as catalysts or inhibitors (Shenoy and Choughuley, 1992). Recent research has confirmed that many common foods contain nonnutritive components that may provide protection against chronic disease including some forms of cancer. From a nutritional standpoint, particular attention must be paid to the inhibition of *N*-nitrosamines in foods. Naturally occurring compounds, which possess anticarcinogenic, antimutagenic, and other beneficial properties, are referred to as chemopreventers. The majority of these chemopreventers are available in and consumed from vegetables, fruits, grains, tea, etc. (Stavric, 1994).

Epidemiological studies have already established that fruits and vegetables have been associated with prevention against diseases, including cancer and cardio- and cerebrovascular diseases (Frei, 1995; Potter and Steinmetz, 1996; Steinmetz and Potter, 1996), attributed to the various antioxidants, such as vitamin C, vitamin E, or β-carotene, and chlorophyll and polyphenols, contained in them (Ziegler, 1993; Weisburger et al., 1996). It is well-known that vegetables such as broccoli, beets, chives, horseradish, rhubarb, spinach, green beans, and tomatoes have antimutagenic activity against 2-amino-3-methyl[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazomethyl[4,5-*f*]quinoline (MeIQ) (Edenharder et al., 1994). Onion, garlic, leek, and other vegetables belonging to the *Allium* genus contain a wide variety of specific compounds that act as antimutagens in vitro in laboratory experiments and seem to be anticarcinogens in vivo (Dorant et al., 1995; Ip and Lisk, 1994; Malaveille et al., 1996; Tsai et al., 1996; Ernst, 1997). The major water soluble constituent of licorice, glycyrrhizin, has been shown to inhibit the mutagenicity of benzo[*a*]pyrene, 2-aminofluorene, and aflatoxin B_1 (Wang et al., 1991) as well as granuloma angiogenesis in vivo and tube formation in vitro (Kobayashi et al., 1995).

Thus, a great variety of naturally occurring compounds or mixtures have been shown to inhibit the mutagenicity or carcinogenicity of several chemicals. However, a few studies have demonstrated the effect of fruit and vegetable extracts on the mutagenicity and carcinogenicity of *N*-nitrosamines. As fruits and vegetables are dietary components, it is more pertinent to evaluate their antimutagenic effect against *N*-nitro-

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samines (food mutagens), which are likely to be consumed simultaneously with fruits and vegetables.

We report in this paper the antimutagenic effect of fruit and vegetable aqueous extracts against *N*-nitrosodimethylamine(NDMA), *N*-nitrosopyrrolidine(NPYR), *N*-nitrosodibutylamine (NDBA), and *N*-nitrosopiperidine (NPIP) using the Ames test.

MATERIALS AND METHODS

Materials. Samples of the six vegetables and three fruits under investigation, onion (*Allium cepa*), garlic (*Allium sativum*), green pepper (*Capsicum annuum*), broccoli (*Brassica oleracea*), carrot (*Dacus carota*), licorice (*Glycyrrhiza glabra*), apple (*Malus domestica* Golden Delicious), kiwi (*Ananas sativus*), and pineapple (*Actinidia diasinensis*), were purchased from a local food market in Madrid, Spain. These fruits and vegetables were selected on the basis of preliminary in vitro assays described by several investigators (Helser et al., 1992; Bronzetti, 1994; Dorant et al., 1995).

Preparation of Aqueous Extracts of Fruits and Vegetables. Standard amounts of 100 g of fruits and vegetables were sliced and further homogenized at 4 °C in a homogenizer (Sorvall, Norwalk, CT). Homogenization was performed with distilled water, and the resulting homogenate was filtered with suction; the filtrate was centrifuged at 10000*g* for 30 min to remove any fruit or vegetable debris. The supernatant was sterilized by filtration through two Millipore filters (0.45 and 0.22 μm). Aqueous extracts were lyophilized and stored at −20 °C until use (Martínez et al., 1998b).

Chemicals. NDMA, NPYR, NDBA, and NPIP were purchased from Sigma Chemical Co. (St. Louis, MO). Standard solutions of NDMA and NPYR (5 mg/100 μL) were prepared in PBS (pH 7.4), and NDBA (0.5 mg/100 μL) and NPIP (2 mg/100 μL) were prepared in 12% dimethyl sulfoxide (DMSO; Merck, Darmstadt, Germany). *N*-Nitrosamines are potent carcinogenic agents; safety precautions must be taken for proper handling and disposal of the chemicals. The N-nitrosamines tested were chosen because they are the most frequently occurring volatile nitrosamines in foods.

Antimutagenicity Assay. The antimutagenic effect of each fruit and vegetable aqueous extract was assayed according to the Ames method with a 20 min preincubation at 37 °C (Maron and Ames, 1983). The histidine-requiring strain of *Salmonella typhimurium* TA100 was kindly supplied by Dr. B. N. Ames (University of California, Berkeley). The S9 mix (IFFA CREDO; Institut Francais de la Fièvre Aphteuse−Centre de Recherche et d'Elevage du Domaine des Oncins, Lyon, France) was prepared from Sprague−Dawley male rats treated with Aroclor 1254 according to the method of Ames et al. (1975). The indirect mutagens used for *S. typhimurium* TA100 were NDMA, NPYR, NDBA, and NPIP, which required S9 mix for metabolic activation. The mutagen (0.1 mL) was added to the mixture of *S. typhimurium* TA100 strain (0.1 mL) and each concentration of fruit and vegetable aqueous extracts (0.1 mL) with S9 mix (0.1 mL; 30% S9). The entire mixture was preincubated while shaking at 37 °C for 20 min before molten top agar (2 mL) was added; the mixture was poured onto a minimal medium agar plate. The his^+ revertant colonies were counted after incubation at 37 °C for 48 h. Each sample was assayed using triplicate plates, and the data presented are mean ± standard error of three independent assays. Plates without *N*-nitrosamines and without fruit and vegetable aqueous extracts were considered as negative controls and plates with *N*-nitrosamines as positive controls. NDMA and NPYR (5 mg/100 μL) gave 233.0 ± 12.7 and 573.0 ± 31.0 colonies, respectively. NDBA (0.5 mg/100 μL) and NPIP (2 mg/100 μL) gave 349.0 ± 8.4 and 531.0 ± 33.9 coloniess, respectively. Antimutagenic effect is expressed as percentage of inhibition (% inhibition) = $100 - (R_1/R_0 \times 100)$, where R_1 is the number of his^+ revertants/plate of plates exposed to *N*-nitrosamines and fruit or vegetable extracts and R_0 is the number of his^+ revertants/plate of the positive control. The number of spontaneous revertants was subtracted from the numerator and denominator. The mutagenicity of *N*-nitrosamines (positive control) in the absence of fruit and vegetable aqueous extracts is defined as 0% inhibition.

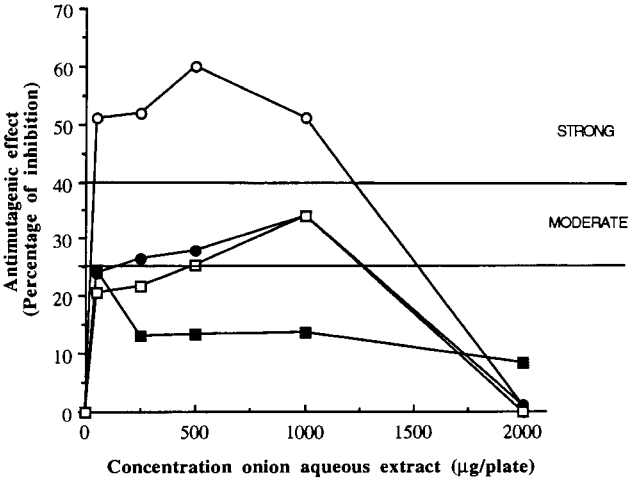

Figure 1. Inhibitory effect of onion (*A. cepa*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

Antimutagenic effect was considered moderate when the inhibitory effect of fruit and vegetable extracts was in the range of 25−40% and strong when the inhibitory effect was >40%. Inhibitory effect <25% was considered weak, and it was not recognized as a positive result.

Data Analysis. Simple regression analyses were used to determine the relationship between the percentage of inhibition versus extract concentration. Analyses were carried out using a StateView program (1985, Dan Feldman and Jim Gagnon) in an Apple Macintosh Performa 5260/120 computer.

RESULTS

The antimutagenic effect of fruit and vegetable aqueous extracts on four *N*-nitrosamines, including NDMA, NPYR, NDBA, and NPIP, was evaluated by means of the Ames test. No mutagenicity and toxicity have been previously described with the fruit and vegetable extracts used under the conditions tested (Martínez et al., 1998b). In the range of concentrations used, all aqueous extracts studied showed a moderate or strong inhibitory effect on the mutagenicity of the *N*-nitrosamines tested. Data points are the mean of values of triplicate plates from three independent assays. Standard error was in the range of 2−10%.

The inhibitory effect of onion (*A. cepa*) aqueous extract on the mutagenicity of *N*-nitrosamines appears in Figure 1. Onion extract showed a strong inhibitory effect (51−60%) on the mutagenicity of NDMA from 50 to 1000 μg/plate. The mutagenicity of NPYR and NDBA was slightly reduced (27−34 and 25−34%) by onion extract at dosages of 250−1000 and 500−1000 μg/plate, respectively.

Figure 2 shows the antimutagenic effect of garlic (*A. sativum*) aqueous extract to *N*-nitrosamines. The antimutagenic effect of garlic extract to NPIP ($R^2 = 0.99$; $p \leq 0.001$) increased with increasing concentration of this extract. Concentrations ≤500 μg/plate were required to observe a moderate inhibitory effect against NPIP (35−39%), whereas concentrations ≥1000 μg/plate showed a strong inhibitory effect (51−64.6%). The mutagenicity of NDBA was markedly reduced (44%) by garlic extract at the dosage of 1000 μg/plate and reduced to 28% for NDMA at the dosage of 250 μg/plate.

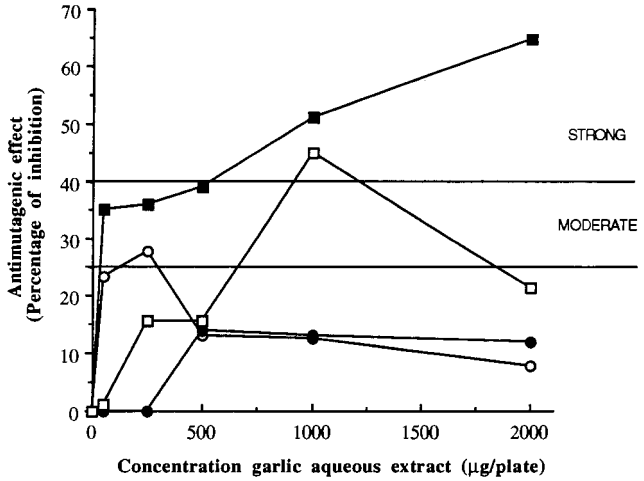

Figure 2. Inhibitory effect of garlic (*A. salivum*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

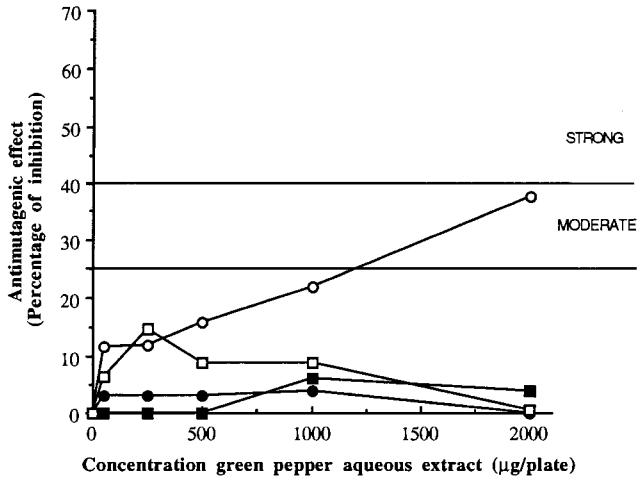

Figure 3. Inhibitory effect of green pepper (*C. annuum*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

The green pepper (*C. annuum*) aqueous extract showed inhibitory effect against mutagenicity of only NPIP (Figure 3). Antimutagenicity effect increased with increasing concentration ($R^2 = 0.99$; $p \leq 0.001$) of green pepper extract up to 2000 μg/plate (37.6%).

The inhibitory effect of broccoli (*B. oleracea*) aqueous extract against mutagenicity of *N*-nitrosamines is shown in Figure 4. Broccoli extract showed a strong antimutagenic effect against NPYR (50 μg/plate; 45.5%) and NDBA (1000 μg/plate; 43.5%), whereas it showed a moderate inhibitory effect (31−39%) against NPIP at the dosage of 50−1000 μg/plate.

Figure 5 shows the antimutagenic effect of carrot (*D. carota*) aqueous extract to *N*-nitrosamines. Carrot extract inhibited from 44 to 49% the mutagenicity of NDBA (50−250 μg/plate). A moderate inhibitory effect was observed against NDMA (≤1000 μg/plate; 26−31%), NPIP (250−500 μg/plate; 25−27%), and NDBA (500 μg/plate; 34%).

Results from Figure 6 show the inhibitory effect of licorice (*G. glabra*) aqueous extract against mutagenicity of NDMA, NPYR, NDBA, and NPIP. Antimutagenic effect was found only for NDMA (50−500 μg/plate; 34−38%), and the inhibitory effect decreased with increasing concentrations of licorice extract ($R^2 = 0.90$; $p \leq 0.01$).

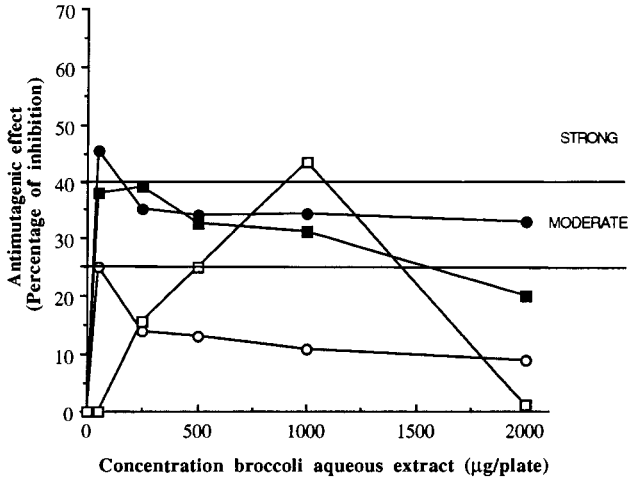

Figure 4. Inhibitory effect of broccoli (*B. oleracea*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

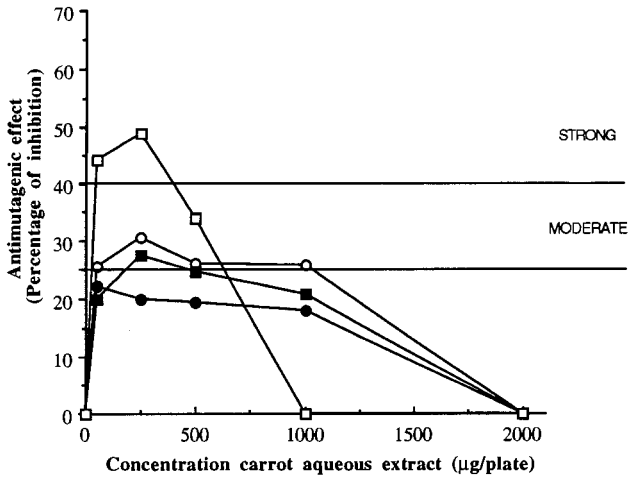

Figure 5. Inhibitory effect of carrot (*D. carota*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

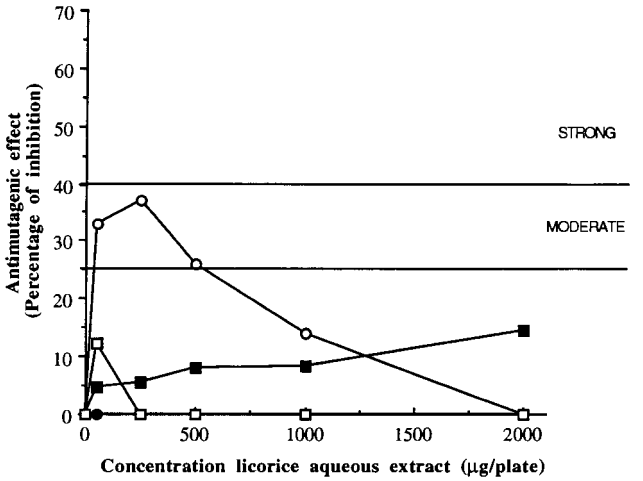

Figure 6. Inhibitory effect of licorice (*G. glabra*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

The antimutagenic effect of fruit aqueous extracts against the four *N*-nitrosamines tested is shown in Figures 7−9. Antimutagenic effect of apple (*M. domestica* Golden Delicious) aqueous extract against NPYR

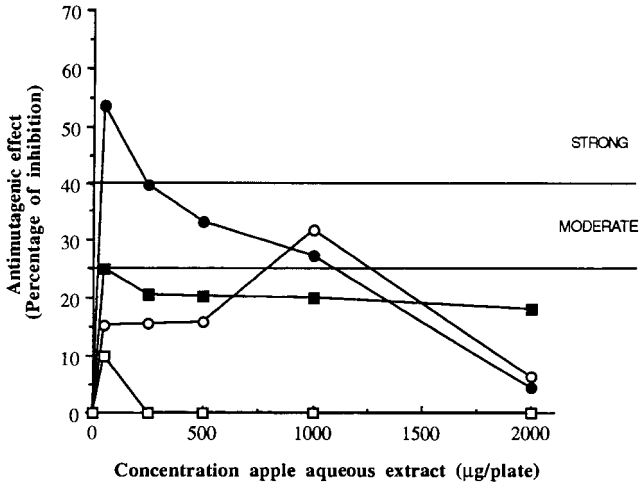

Figure 7. Inhibitory effect of apple (*M. domestica* Golden Delicious) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

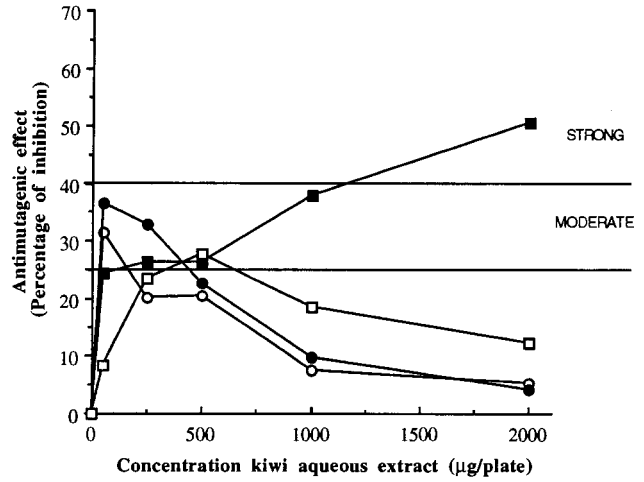

Figure 8. Inhibitory effect of kiwi (*A. sativus*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

(Figure 7) decreased with increasing concentrations ($R^2 = 0.90$; $p \leq 0.01$). Concentrations ≤250 μg/plate were required to observe a strong inhibitory effect (54%), whereas concentrations from 500 to 1000 μg/plate showed a moderate inhibitory effect (26−34%). Mutagenicity of NDMA was slightly inhibited (32%) at 1000 μg/plate.

Kiwi (*A. sativus*) aqueous extract (Figure 8) showed a strong inhibitory effect against the mutagenicity of NPIP (2000 μg/plate; 50.4%). Inhibitory effect increased with increasing concentrations of kiwi ($R^2 = 0.99$; $p \leq 0.005$). Mutagenicity of NDMA, NPYR, and NDBA was also reduced (32, 32−37, and 28%, respectively) by this fruit extract.

Figure 9 shows the inhibitory effect of pineapple (*A. diasinensis*) aqueous extract against *N*-nitrosamines. The results obtained indicate that pineapple extract showed a moderate inhibitory effect against mutagenicity of NDMA (50 μg/plate, 27%), NPYR (50−250 μg/plate, 28−35%), and NPIP (2000 μg/plate, 37%). Antimutagenic effect against NPYR decreased as concentrations of pineapple extract increased ($R^2 = 0.90$; $p \leq 0.05$).

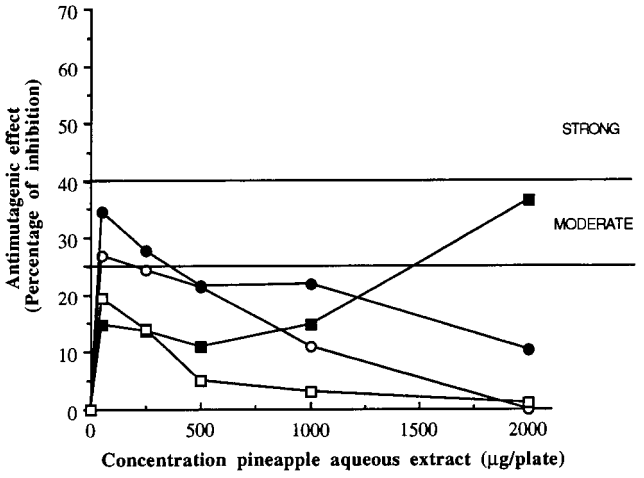

Figure 9. Inhibitory effect of pineapple (*A. diasinensis*) aqueous extract against mutagenicity of NDMA (○), NPYR (●), NDBA (□), and NPIP (■) to *S. typhimurium* TA100.

DISCUSSION

In an attempt to study the protective effect of fruit and vegetable extracts against cytotoxicity and mutagenicity of *N*-nitrosamines, their activities in several in vitro test have been assayed previously. The protective effect of fruit and vegetable extracts against cytotoxicity of *N*-nitrosamines has been tested in the MTT assay (Martínez et al., 1998a) as well as in the BrdU assay and by determination of total cellular DNA content (Martínez et al., 1998c). The aim of the present study was to evaluate the antimutagenic effect of fruit and vegetable extracts against *N*-nitrosamines by using the Ames test.

It has been reported that vitamin C, vitamin E, and phenols reduce the formation of nitrosamines and carotenoids, flavonoids, and related compounds and act as antioxidants, essentially disabling the carcinogenic potential of specific compounds (Potter and Steinmetz, 1996; Ferguson, 1994).

Several studies have shown the association between regular consumption of *Allium* vegetables and a lower risk of cancer (Lea, 1996; Ernst, 1997). In this sense, the ability of naturally occurring organosulfur compounds present in onion (*A. cepa*) and garlic (*A. sativum*) to reduce the formation and bioactivation of carcinogenic nitrosamines has been described (Shenoy and Choughuley, 1992; Dion et al., 1997). Diallyl sulfide, a thioether found in garlic, has been shown to have chemoprotective effects against mutagenesis induced by NDMA (Surh et al., 1995). On the other hand, organosulfur compounds in onion and garlic were found to inhibit the formation of mutagenic heterocyclic aromatic amines (Tsai et al., 1996), reduce the mutagenicity induced by benzo[*a*]pyrene, 4-nitro-1,2-phenylenediamine, and aflatoxin (Ishikawa et al., 1996; Soni et al., 1997), and prevent experimental carcinogenesis by exerting specific effects on carcinogen detoxification systems (Hatono et al., 1996). In agreement with these investigations, our results indicate that onion and garlic aqueous extracts exhibit the strongest inhibitory effect against mutagenicity of NDMA and NPIP, respectively, evaluated by the Ames test. Onion extracts also showed a moderate protective effect against the mutagenicity of NDBA and NPYR.

Green pepper has been shown to exert strong comutagenic activity against nitroarenes (Tang and Eden-

harder, 1997). However, green pepper juice was inactive against the mutagenic activities induced by heterocyclic amines (Edenharder et al., 1994). In the present study, green pepper (*C. annuum*) aqueous extract presented only a moderate inhibitory effect against mutagenicity of NPIP.

Epidemiological data concerning the cancer-preventive effect of *Brassica* vegetables, including all types of cabbages, broccoli, cauliflower, and Brussels sprouts, have been recently reported (Verhoeven et al., 1996). The protective effect of *Brassica* vegetables against cancer may be due to their relatively high content of isothiocyanates (mostly in the form of their glucosinolate precursors), some of which are very potent inducers of enzymes involved in chemical carcinogens detoxication (Verhoeven et al., 1997). Furthermore, cruciferous vegetables were found to have strong to moderate effects against the mutagenic activities induced by heterocyclic amines (Edenharder et al., 1994), and phenethyl isothiocyanate, a constituent of cruciferous extracts, has been shown to inhibit the genotoxic effects of *N*-nitrosodimethylamine (Kansmuller et al., 1996). In contrast to these findings, Kassie et al. (1996) have reported that glucosinolates and/or isothiocyanates from *Brassica* vegetables are potent genotoxins in bacterial and mammalian cells. Results obtained in this work showed the ability of broccoli aqueous extracts to exert an inhibitory effect (moderate to strong) against the mutagenicity of the four *N*-nitrosamines tested.

Numerous epidemiological studies support a strong inverse relationship between consumption of carotenoid-rich fruits and vegetables and the incidence of cancer (van Poppel, 1996). Carotenoids, including β-carotene, have been shown to reduce oxidative DNA damage, and this could be one of the mechanisms responsible for their cancer-protective effect (PoolZobel et al., 1997). However, in previous studies carrot (*D. carota*) was inactive against the mutagenic activities induced by heterocyclic amines (Edenharder et al., 1994) and has been shown to exert weak antimutagenic activity against nitroarenes (Tang and Edenharder, 1997). On the contrary, under our experimental conditions carrot aqueous extract exhibited a strong antimutagenic effect against NDBA as evaluated by the Ames test.

The antitumorigenic activity of licorice (*G. glabra*) has been evaluated in several investigations (Kobayashi, 1995; Agarwal et al., 1991). In this regard, glycyrrhizic acid, the main water soluble constituent of licorice, is shown to be a potent inhibitor of some enzymes involved in neoplasia (Duax and Ghosh, 1997). Furthermore, glycyrrhizic acid was found to inhibit the mutagenicity induced by benzo[*a*]pyrene, 2-aminofluorene, and aflatoxin B_1 in *S. typhimurium* TA100 (Wang et al., 1991). The results obtained in the present study demonstrate that licorice root aqueous extract showed moderate antimutagenic effect against NDMA and it did not show any activity against NPYR, NDBA, or NPIP.

Apple consumption has been associated with a reduction in the risk of colorectal cancer in humans (Deneo et al., 1996). Moreover, apple pectin significantly decreased the number and incidence of colon tumors in experimental rat colon carcinogenesis (Tazawa et al., 1997). On the other hand, apple juice was found to have a weak antimutagenic activity against heterocyclic amines (Edenharder et al., 1994). In the present study, apple (*M. domestica* Golden Delicious) aqueous extract had a strong inhibitory effect against mutagenicity of NPYR and a moderate effect against NDMA.

Juices from kiwi and pineapple exerted weak to moderate comutagenic activity with respect to mutagenicity induced by nitroarenes in *S. thyphimurium* TA98 (Tang and Edenharder, 1997). However, juices from kiwi and pineapple have shown moderate and strong antimutagenic effects, respectively, against heterocyclic amines (Edenharder et al., 1994). In our study, kiwi (*A. sativus*) aqueous extract was found to have strong antimutagenic effect against NPIP and slight inhibitory effect against NDMA, NDBA, and NPYR, whereas pineapple (*A. diasinensis*) had a moderate effect against NPIP, NPYR, and NDMA.

The presence of different antimutagenic activities in fruit and vegetable extracts tested against structurally different *N*-nitrosamines suggests that either a great variety of antimutagenic compounds of different structure do exist in fruit and vegetable extracts or that groups of similar compounds may be responsible for the direct interaction between the genotoxic reactive intermediates of *N*-nitrosamines and antimutagenic compounds. In most cases the structural identity of antimutagenic factors remains to be elucidated, and work is in progress to do so.

Most of the results obtained in this work have shown nonlinear dose responses. This is a common situation in work with complex mixture or combined treatments. The effect of the modifying chemical can be either enhancing or inhibiting depending on its mechanism of action (Wallum et al., 1990). The modifier agent(s) may act outside the bacteria by interfering with the bacterial metabolism. Other modifying effects inside as well as outside the bacteria may include chemical or enzymatic modifications of the mutagen. Our results clearly indicate that fruit and vegetable aqueous extracts have shown antimutagenicity against indirect mutagens (*N*-nitrosamines), requiring S9 activation. One feasible mechanism of antimutagenic action is that fruit and vegetable components may interact with the enzyme systems catalyzing the metabolic activation of the various promutagens, impeding the production of genotoxic intermediates. Because cytochrome P-450 is the major enzyme in S9 mix, whether the antimutagenic effect of fruit and vegetable extracts operates through the inactivation of enzyme activity or through other mechanisms such as enhancement of detoxification pathways and/or scavenging of the reactive intermediates to prevent their reaction with DNA is unclear; further investigation is needed.

Taken together, the results reported in this work indicate that fruit and vegetable aqueous extracts exert antimutagenic effect against *N*-nitrosamines evaluated by means of the Ames test. Further investigation is needed on the isolation of the antimutagenic compounds from fruits and vegetables and to understand the antimutagenic mechanism by which they may provide protection against mutagenic activities of *N*-nitrosamines.

CONCLUSION

This paper has discussed the inhibitory effect of fruit and vegetable extracts against the mutagenicity of NDMA, NPYR, NDBA, and NPIP using the Ames test. Vegetable and fruit aqueous extracts that exhibited an antimutagenic effect in decreasing order against NDMA and NPYR were onion > licorice > kiwi = apple > carrot

> garlic > pineapple > broccoli and apple > broccoli > kiwi > onion = pineapple. Decreasing orders against NDBA and NPIP were, respectively, carrot > garlic > broccoli > onion > kiwi and garlic > kiwi > broccoli > green pepper > pineapple > carrot > onion = apple. The results indicate that vegetable and fruit could be useful to humans for the purpose of mutation chemoprevention against *N*-nitrosamines.

ABBREVIATIONS USED

DMSO, dimethyl sulfoxide; NDMA, *N*-nitrosodimethylamine; NPYR, *N*-nitrosopyrrolidine; NDBA, *N*-nitrosodibuthylamine; NPIP, *N*-nitrosopiperidine.

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