



## Study of the effect of 'Ataulfo' mango (*Mangifera indica* L.) intake on mammary carcinogenesis and antioxidant capacity in plasma of *N*-methyl-*N*-nitrosourea (MNU)-treated rats

Pablo García-Solís<sup>a</sup>, Elhadi M. Yahia<sup>a,\*</sup>, Carmen Aceves<sup>b</sup>

<sup>a</sup>Laboratorio de Fitoquímicos y Nutrición, Facultad de Ciencias Naturales, Universidad Autónoma de Querétaro, Avenida de las Ciencias, Juriquilla, Querétaro, 76230 Qro., Mexico

<sup>b</sup>Instituto de Neurobiología, Universidad Nacional Autónoma de México, Campus UAQ-UNAM, Juriquilla, Querétaro 76230, Mexico

### ARTICLE INFO

#### Article history:

Received 3 October 2007

Received in revised form 12 March 2008

Accepted 18 March 2008

#### Keywords:

*Mangifera indica*

Fruit

Mammary cancer

Phytochemicals

Rat

### ABSTRACT

The effect of 'Ataulfo' mango consumption on chemically induced mammary carcinogenesis and plasma antioxidant capacity in rats treated with *N*-methyl-*N*-nitrosourea (MNU) was studied. Mango was administered in the drinking water (0.02–0.06 g/mL) during both short-term and long-term (LT) periods to rats treated or not with MNU. Rats treated with MNU showed no differences in mammary carcinogenesis or in plasma antioxidant capacity measured by both ferric reducing/antioxidant power (FRAP) and total oxyradical scavenging capacity assays. However, in animals not treated with MNU but with a LT intake of mango the plasma antioxidant capacity as measured by the FRAP assay tended to increase in a dose-dependent manner. This suggests that mango consumption by healthy subjects may increase antioxidants in plasma.

© 2008 Elsevier Ltd. All rights reserved.

### 1. Introduction

Breast cancer is the most common worldwide neoplasia in women, and the totality of its etiological factors is unknown; thus, an effective preventive strategy is important (Parkin, Bray, Ferlay, & Pisani, 2005). Risk factors associated with breast cancer can be grouped into three broad categories: (a) family history factors, (b) endocrine and reproductive factors, and (c) environmental and life-style factors including diet (Parkin et al., 2005). Several authors have postulated that high intake of fruits and vegetables could reduce cancer incidence because their phytochemical antioxidants prevent the oxidative damage produced by free radicals (Singletary, Jackson, & Milner, 2005). Free radicals oxidize lipids, proteins, and DNA, and they are involved in the initiation and promotion/progression of carcinogenesis (Brown & Bicknell, 2001). In 1997, an expert panel of the American Institute for Cancer Research concluded that there was convincing evidence that high intake of fruits and vegetables decreases the risk of cancers of the mouth and pharynx, oesophagus, lung, stomach, colon, and rectum, and probably decreases the risk of cancer of larynx, pancreas, bladder, and breast. However, several recent cohort studies have indicated no associations between fruit and vegetable consumption and risk of breast cancer and total cancer, and therefore epidemio-

logical research has not clarified this possible association (reviewed in Riboli & Norat, 2003). Animal studies have shown that several phytochemical compounds, such as the soy isoflavonoid genistein and isothiocyanates extracted from broccoli sprouts, prevent 7, 12-dimethylbenzo[*a*]anthracene (DMBA)-induced mammary carcinogenesis (Singletary et al., 2005). However, phytochemicals such as the carotenoid lycopene failed to prevent mammary cancer induced by *N*-methyl-*N*-nitrosourea (MNU) (Cohen, 2002). It has been proposed that the health benefits of fruits and vegetable may result from multiple combined effects of their phytochemicals rather than from the effects of a single active ingredient (Percival et al., 2006). Recent reports using more complex formulations of phytochemicals such as whole extracts of 'Red Delicious' apple or purple grape juice have reported the prevention of mammary carcinogenesis in DMBA-treated rats (Jung, Wallig, & Singletary, 2006; Liu, Liu, & Chen, 2005). Moreover, using *in vitro* approaches it has been shown that several whole extracts of fruits have antioxidant and antiproliferative activities on cancer cell lines (Percival et al., 2006; Sun, Chu, Wu, & Liu, 2002). Mango, one of the most consumed fruits, is rich in phytochemical antioxidants including vitamin C, vitamin E, carotenoids, and phenolics (Ornelas-Paz, Yahia, & Gardea-Bejar, 2007; Rocha-Ribeiro, Queiroz, Lopes, Campos, & Pinheiro, 2007). Botting, Young, Pearson, Harris and Ferguson (1999) showed that mango fruits have antimutagens using the salmonella typhimurium mutagenicity assay and the heterocyclic amine 2-amino-3-methylimidazo[4,5-*f*]quinoline.

\* Corresponding author. Tel.: +52 442 1921200x5354; fax: +52 442 2342951.  
E-mail address: [yahia@uaq.mx](mailto:yahia@uaq.mx) (E.M. Yahia).

Recently, Percival et al. (2006) showed that whole mango juice inhibited cell proliferation in the leukemic cell line HL-60 and also inhibited the neoplastic transformation of BALB/3T3 cells. The presence of antioxidant and antimutagenic activities in mango as well as its antineoplastic effects using mammalian *in vitro* systems suggested important anticancer activity *in vivo*. In this study we investigated the effect of the consumption of mango (*Mangifera indica* L., Cv Ataulfo) juice on the plasma antioxidant capacity and on the incidence of mammary carcinogenesis in rats treated with the carcinogen MNU.

## 2. Materials and methods

### 2.1. Animals

Virgin, female Sprague-Dawley rats, 3 weeks of age, were obtained from the vivarium of the Instituto de Neurobiología, UNAM-Juriquilla. Four rats per cage were housed in a temperature-controlled room ( $21 \pm 1$  °C) with a 12-h light/dark schedule. They were provided with food (Purina 5001, Laboratory Rodent Diet, which is a Constant Nutrition™ formulation recommended for rats, St. Louis, MO) and water *ad libitum*. All of the animal procedures and treatments followed the Official Mexican Norm NOM-062-ZOO-1999.

### 2.2. Mango preparation

Ripe fruit were purchased in a local market in Querétaro, México. All of the mangoes were free from physical and pathological defects and were processed immediately after their arrival at the laboratory. They were cleaned, dried, and analyzed objectively for external (peel) and internal (pulp) colour on the basis of the CIELAB colour system ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $C^*$ ,  $h^\circ$ ) with a Minolta CM-2002 spectrophotometer (Minolta Co., Japan) and the program Spectra Match 3.3.7. Within the uniform space CIELAB, two colour coordinates,  $a^*$  and  $b^*$ , as well as an index of lightness,  $L^*$ , are defined. The coordinate  $a^*$  indicates positive values for reddish colours and negative values for greenish ones, whereas  $b^*$  indicates positive values for yellowish colours and negative values for bluish ones (Melendez-Martinez, Vicario, & Heredia, 2003). From the uniform colour spaces, other parameters can be defined such as chroma ( $C^* = [(a^*)^2 + (b^*)^2]^{1/2}$ ) and  $h^\circ = \arctan(b^*/a^*)$ .  $C^*$  is considered as the quantitative attribute of colourfulness, whereas  $h^\circ$  is considered as the qualitative attribute of colour (Melendez-Martinez et al., 2003). External colour was longitudinally determined on three points of each flat side of the fruit (six points for each fruit). For internal colour a big slice from a flat side of each fruit was obtained, and colour was determined longitudinally on three equidistant points. After seed and peels were removed from the edible portion of mangoes, the pulp was cut, pureed, and stored in aliquots at  $-70$  °C. Samples of fresh purée pulp were placed in Petri dishes, and colour parameters were measured. Total soluble solids (°Brix) contents in fresh aliquots of purée pulp were also determined using a temperature compensation hand refractometer (Atago Co. Ltd., Osaka, Japan).

### 2.3. Mango treatment

Using a randomization process, 4 experimental groups were formed from 116 five-week-old rats. Mango was provided in the drinking water at three different concentrations by diluting the frozen mango purée pulp with purified water. The experimental groups were: (a) Control (water with 1.2% sucrose;  $n = 20$ ), (b) Mango-1 (0.02 g of mango/mL of water;  $n = 32$ ); (c) Mango-2 (0.04 g of mango/mL of water;  $n = 32$ ) and (d) Mango-3 (0.06 g of

mango/mL of water;  $n = 32$ ). All control and mango-containing fluids were adjusted with sucrose to have the same Brix degrees (sugar content). Controls and mango solutions were freshly prepared, changed and served in clean bottles every day, and both food and liquid intake were quantified. The quantity of liquid offered to the rats was adjusted according to body weight so that all groups consumed at least 90% of the available beverage every day. At the age of 7 weeks, 84 rats (12 control and 24 for each mango treatment) were anesthetized with a ketamine and xylazine (Cheminova, México) mixture (30 mg and 6 mg, respectively, per Kg of body weight [b. wt.]), and they were treated with a single intraperitoneal injection of 50 mg of MNU (Sigma, St. Louis, MO) per Kg b. wt. MNU was dissolved in 0.9% saline, pH 5.0, by warming the vial under hot tap water with vigorous shaking (Thompson, 2000). Negative control groups received only a saline injection. At the age of 8 weeks MNU-treated animals that consumed mango were divided; half continued the treatments (long-term intake of mango) while the other half received control treatment (short-term intake of mango). Also at this time, half of the MNU-uninjected rats of each treatment were sacrificed, and the other half continued with the treatment.

Total phenolic and carotenoid contents of the Mango-3 solution were monitored every 15 days for 4 months. In addition, total phenolic and carotenoid contents were analyzed in Mango-3 juice after 24 and 48 h incubation in the vivarium conditions described previously.

### 2.4. Evaluation of mammary gland carcinogenesis

Rats were weighed and palpated for tumours every week from 4 until 22 weeks after MNU administration. A tumour was defined as a discrete palpable mass recorded on at least 2 consecutive weeks. Tumour incidence for each treatment was calculated as the percentage of animals with one or more palpable tumours. Tumour multiplicity was calculated as the average number of tumours per animal in each treatment group. The mean latency of tumour onset for each treatment group was calculated as the mean time interval (in weeks) from MNU injection to the appearance of the first palpable tumour. When the tumours had grown to  $\geq 2.0$  cm in diameter, rats were anesthetized with the ketamine and xylazine mixture, and the tumours were surgically removed and fixed in 10% neutral buffered formalin. The tumours were measured using a caliper, and the volumes were calculated by the ellipsoid formula (Thompson, 2000). At the end of the experiment the rats from each experimental group were sacrificed by decapitation, and mammary tumours were fixed in 10% neutral buffered formalin.

### 2.5. Histopathological analysis

Fixed MNU-induced mammary tumours were embedded in paraffin blocks. Sections of 5- $\mu$ m thickness were cut from each block and placed on slides. Sections were deparaffinised in xylene, rehydrated in descending grades of ethanol, and stained with hematoxylin and eosin (H&E). Tumours were classified according to the criteria of Russo and Russo (2000).

### 2.6. Quantification of total carotenoid content in mango juice

The extraction and quantification of total carotenoids was done according to Soto-Zamora, Yahia, Brecht, and Gardea (2005) with some modifications. A 10-mL aliquot of mango-3 juice was added to 10 mL of hexane:acetone:toluene:ethanol (10:7:7:6 v/v) and homogenized; 1 mL of 20% KOH in methanol was added, and the mixture was homogenized again and shaken (80 rpm) for 16 h at 25 °C in darkness. The mixture was homogenized again with

10 mL of hexane, and then 10 mL of 10% Na<sub>2</sub>SO<sub>4</sub> was added. The mixture was shaken for 1 min, and was then held and the phases were allowed to separate for 30 min. The upper phase was removed, and the absorbance was read in a spectrophotometer at 450 nm. The results are the mean of at least three replicates. The carotenoid content in mango juice was quantified according to a standard curve of  $\beta$ -carotene dissolved in hexane, and the results were expressed as  $\mu\text{g}$  of  $\beta$ -carotene equivalents/mL.

### 2.7. Determination of total free phenolic content in mango juice

The content of total free phenolics in mango juice samples was analyzed by the Folin-Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventós, 1999) as modified in our laboratory. Mango-3 juice was centrifuged at 10,000g for 5 min, and then 30  $\mu\text{L}$  of mango-3 juice supernatant was oxidized with 150  $\mu\text{L}$  of Folin-Ciocalteu reagent; after 5 min the reaction was neutralized with 120  $\mu\text{L}$  sodium carbonate. The absorbance was measured at 620 nm after 2 h using a MRX Dynex plate reader (Dynex technologies, Inc., Chantilly, VA). Gallic acid was used as standard, and the results were expressed as  $\mu\text{g}$  of gallic acid equivalents/mL.

### 2.8. Total phenolic concentration in plasma

The phenolic content of plasma was measured by the Folin-Ciocalteu method modified to remove protein interference (Serafini, Maiani, & Ferro-Luzzi, 1998). Total phenolics were determined by extraction/hydrolysis, and precipitation of protein with 0.75 M metaphosphoric acid. To hydrolyze the conjugated forms of polyphenols, 500  $\mu\text{L}$  of 1 M HCl was added to 250  $\mu\text{L}$  of plasma, mixed vigorously for 1 min, and incubated at 37 °C for 30 min. Later, 500  $\mu\text{L}$  of 2 M NaOH in 75% methanol was added, and the resulting mixture was mixed for 3 min and incubated at 37 °C for 30 min. This step breaks the links of polyphenols with lipids and provides a first extraction of polyphenols. Then, 500  $\mu\text{L}$  of 0.75 M of metaphosphoric acid was added; after mixing for 3 min the sample was centrifuged at 1500g for 10 min to remove precipitated plasma proteins. The supernatant was removed and kept on ice in the dark, while polyphenols were extracted again by adding 500  $\mu\text{L}$  of a 1:1 (v/v) solution of acetone:water and centrifuged for 10 min at 2700g. The two supernatants were combined and filtered through 0.45  $\mu\text{m}$  nylon membrane. Samples of 30 mL were assayed for total polyphenols with the Folin-Ciocalteu reagent as described earlier.

### 2.9. Ferric reducing/antioxidant power (FRAP) assay

FRAP was measured using the method reported by Benzie and Strain (1999) with some modifications. Three reagents were used: (1) sodium acetate, acetic acid buffer (300 mM, pH 3.6); (2) 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) containing 40 mM HCl; (3) 20 mM ferric chloride hexahydrate prepared with HPLC-grade water. The FRAP reagent was prepared fresh prior to each analysis by combining 300 mM sodium acetate buffer, 10 mM TPTZ, and 20 mM ferric chloride in the ratio 10:1:1. Standard series of ascorbic acid were prepared fresh prior to analysis. The FRAP assay was carried out using MRX Dynex plate reader (Dynex technologies, Inc., Chantilly, VA), and absorbance was determined at 620 nm. After adding 10  $\mu\text{L}$  aliquots of sodium acetate buffer, ascorbic acid standard, or sample to the respective wells, 290  $\mu\text{L}$  of freshly prepared FRAP reagent was added, and readings were taken 1 h later.

### 2.10. Total oxyradical scavenging capacity (TOSC) assay

A modified rapid-screening of TOSC assay was used to determine total antioxidant capacity in plasma. The TOSC assay is based on the generation of peroxy radicals by thermal homolysis of

2-2'-azobis-(2-amidinopropane, ABAP) to oxidize  $\alpha$ -keto- $\gamma$ -methylbutyric acid (KMBA) to ethylene (Winston, Regoli, Dugas, Fong, & Blanchard, 1998). The final assay contained 0.2 mM KMBA and 20 mM ABAP in 100 mM potassium phosphate buffer, pH 7.4. The reactions were carried out in 10 mL rubber septa-sealed vials in a final volume of 1 mL and were initiated by injecting 100  $\mu\text{L}$  of 200 mM ABAP in water directly through the rubber septum. The vials were incubated for 90 min at 39 °C. Ethylene production was measured by the use of a gas chromatograph, from aliquots of 100  $\mu\text{L}$  taken directly from the headspace of the reactions vials. Analyses were performed in a HP 5890 Series II gas chromatograph (Agilent Technologies, CA) equipped with a HP-PLOT Q capillary column (30 m  $\times$  0.53 mm  $\times$  40  $\mu\text{m}$ ) (J&W Scientific, Agilent Technologies, CA) and flame ionization detector (FID). The oven, injection, and FID temperatures were 130, 180, and 220 °C, respectively. Nitrogen was used as the carrier gas at a flow rate of 24 mL/min. Plasma was diluted (1:20) in the potassium phosphate buffer, and 100  $\mu\text{L}$  was used for the assay. Ethylene production was measured at a single time point, as proposed by MacLean, Murr, and DeEll (2003).

### 2.11. Statistical analysis

The effects of dietary treatments on mammary cancer incidence were analyzed using 2  $\times$  2 contingency tables and a one-tail Fisher test. The effects of treatments on tumour multiplicity, tumour size, plasma antioxidant capacity, and total phenolic compounds in plasma were analyzed using one-way ANOVA. Tumour latency was analyzed using Kruskal-Wallis one-way ANOVA by ranks.

## 3. Results

### 3.1. Mango characterization

The mangoes tested had excellent external appearance with a weight of 225  $\pm$  38 g and a uniform yellow-orange peel and pulp colour. Table 1 shows the 'Ataulfo' mango colour as evaluated by the CIELAB colour system. This system provides an objective characterization of fruit colour, and it can be used to indicate physiological condition and chemical properties of the fruits (Ornelas-Paz et al., 2007). The colour values of 'Ataulfo' mango reported here are similar to the values reported previously by our group (Ornelas-Paz et al., 2007) in peel and pulp. After pureeing we noticed a reduction of the colour parameters  $L^*$ ,  $a^*$ ,  $b^*$ , and  $C^*$  of the pulp and an increase of the  $h^\circ$  value; these data indicate a slight darkening of the pulp. Additionally, the content of total soluble solids in fresh aliquots of purée pulp was 17.7  $\pm$  2.1 °Brix, which indicates that the mangoes used were ripe (Ornelas-Paz et al., 2007).

### 3.2. Total content of carotenoids and phenolic compounds in mango juice

All dilutions were made from the same mango purée pulp; therefore, we analyzed the total content of carotenoids and phenolic compounds only in Mango-3 juice. Mango-3 juice contained 2.3  $\pm$  0.3  $\mu\text{g}/\text{mL}$  of total carotenoids, whereas the phenolic compounds were 51.0  $\pm$  3.2  $\mu\text{g}/\text{mL}$ . The assays used to analyze carotenoids and phenolic compounds are not specific to distinguish between individual compounds; instead, both assays give a global quantity of phytochemicals present in each sample (Singleton et al., 1999; Soto-Zamora et al., 2005). The analysis every 15 days over the course of 4 months did not show any significant changes in the content of total carotenoids and phenolic compounds in Mango-3 juice freshly prepared from mango purée pulp that had been stored at -70 °C (data not shown). Mango-3 juice was also

**Table 1**  
Color characteristics of 'Ataulfo' mango

	$L^*$	$A^*$	$b^*$	$C^*$	$h^\circ$
Peel ( $n^a = 160$ )	66.7 ± 2.5	17.8 ± 2.7	42.3 ± 5.9	46.0 ± 5.8	67.1 ± 3.5
Pulp ( $n^a = 112$ )	63.7 ± 3.4	20.1 ± 3.4	50.5 ± 7.6	54.5 ± 7.4	68.0 ± 4.1
Pulp purée ( $n^b = 32$ )	57.3 ± 1.4	11.4 ± 1.3	34.7 ± 6.2	36.6 ± 5.9	71.3 ± 4.2

Colour characteristics were determined on the basis of the CIELAB colour system.  $n^a$  = number of mangoes used;  $n^b$  = number of observations. The values shown are the mean ± SD.

analyzed after 24 and 48 h of incubation in the vivarium conditions ( $23 \pm 1^\circ\text{C}$  with a 12-h light/dark schedule), and we noted a slight but significant ( $p < 0.05$ ) reduction with respect to fresh Mango-3 juice in both total carotenoids and phenolic compounds. After 24 h of incubation the reduction in total carotenoids was 6%

**Table 2**  
Animal weight and liquid intake of MNU-treated rats

Group	Initial weight (g)	Final weight (g)	Daily liquid intake (mL/rat)
Control	42.0 ± 6.7	265.9 ± 14.1	47.9 ± 7.3(21–61)
<i>STI</i>			
Mango-1	39.0 ± 10.1	264.1 ± 12.9	48.3 ± 7.7(21–64)
Mango-2	43.8 ± 10.1	264.5 ± 14.2	48.5 ± 7.8(19–62)
Mango-3	42.3 ± 8.5	271.1 ± 15.2	47.9 ± 6.8 (24–62)
<i>LTI</i>			
Mango-1	43.6 ± 5.7	265.4 ± 16.4	48.1 ± 7.5 (21–61)
Mango-2	43.1 ± 9.3	270.2 ± 16.0	46.5 ± 7.0 (24–61)
Mango-3	43.2 ± 8.5	269.5 ± 17.9	45.3 ± 6.5 (24–59)

Initial weight: 3 weeks of age ( $n = 12$ ); Final weight: 29 weeks of age ( $n = 9–12$ ). At 5 weeks of age, rats were treated with different quantities of mango in the drinking water. 2 weeks later, rats received a single i.p. injection of MNU (50 mg/Kg b. wt.), and the administration of mango was continued until 1 week (short-term intake: STI) or 22 weeks (long-term intake: LTI) had elapsed. The values shown are the mean ± SD (Range).

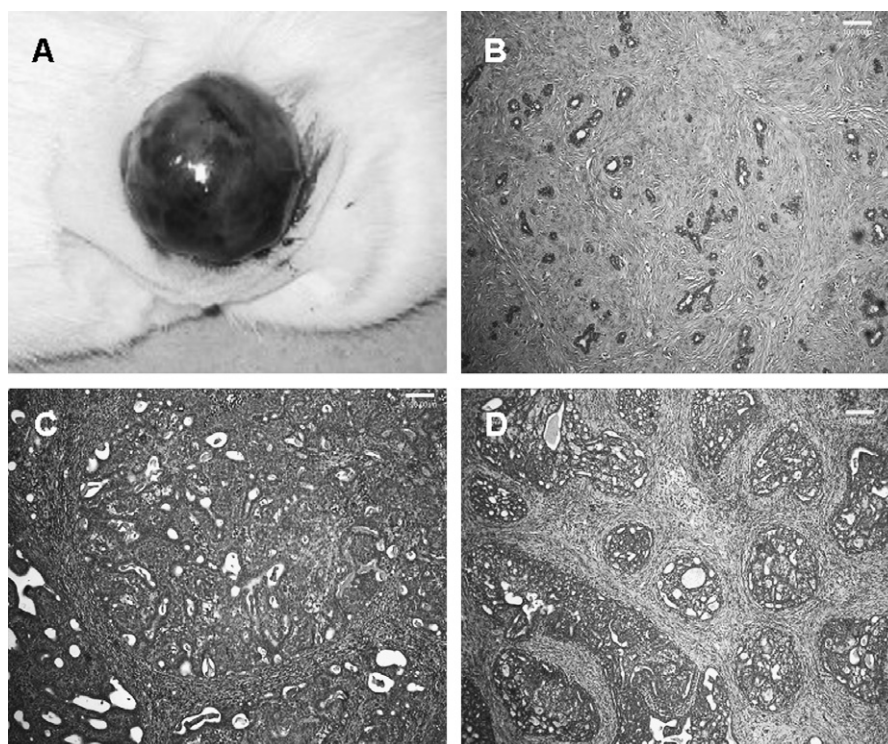
whereas after 48 h was 13%. In the case of phenolic compounds the reductions after 24 and 48 h were 11% and 14%, respectively.

### 3.3. Body weight and mango juice intake

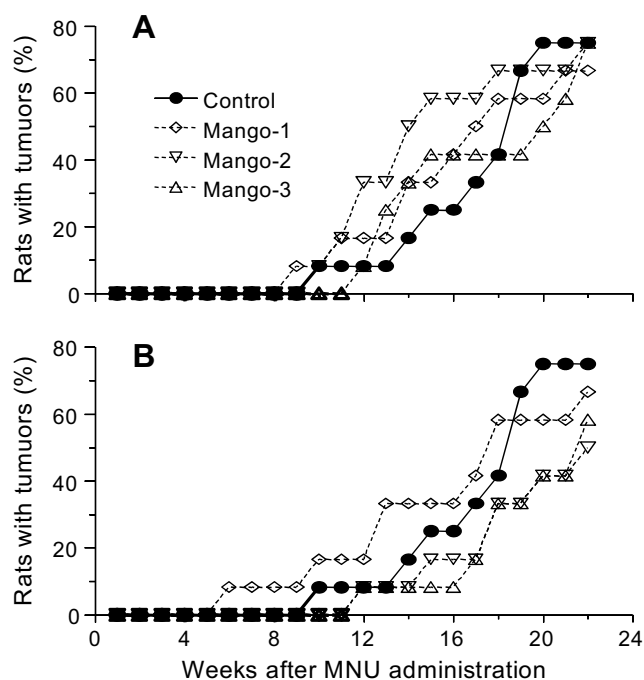
Animal weight gain and both liquid and food intake were similar in all groups studied. Table 2, for example, shows initial and final body weight and liquid intake of rats injected with MNU. Food intake was about 16 g per day per rat, with a minimum intake of about 12 g during the first weeks and a maximum of about 19 g in the last weeks (data not shown). On the other hand, mango intake was as follows: Mango-1 juice treated rats consumed a mean of  $4.5 \pm 1.0$  g of mango/Kg b. wt. (minimum: 3.7; maximum: 7.3), whereas the mean of mango intake of rats treated with Mango-2 and 3 juices were  $8.5 \pm 1.4$  g/Kg b. wt. (minimum: 7.2; maximum: 12.2), and  $12.7 \pm 2.8$  g/Kg b. wt. (minimum: 10.1; maximum: 21.9), respectively. Because the rats were small, the intake of mango per Kg of b. wt. was high during the first three weeks (maximum values). The mean, long-term consumption of mango by rats given the three different concentrations was comparable to human intake of two ( $\approx 293$  g of mango pulp per 70 Kg of b. wt.), four, and six mangoes per day, respectively. This level of mango intake could be considered physiological, because it is similar to recommendations for human consumption of fruit and vegetables such as in the 5-a-day programs (Havas et al., 1995).

### 3.4. Mammary gland carcinogenesis

Mammary glands were evaluated both macro- and microscopically for the presence of tumours. Fig. 1 shows a representative, macroscopic MNU-induced mammary tumour and H&E-stained sections of benign and malignant mammary tumours obtained in this work. We found no correlation between histological type of mammary tumours and treatments (data not shown). Fig. 2 shows cumulative percentages of animals that developed tumours in



**Fig. 1.** MNU-induced mammary tumours. (A) Macroscopic tumour removed by surgery; (B–D) H&E-stained sections of tumours, (B) fibroadenoma (benign) and (C–D) carcinomas (malignant). White bar in upper right corner represents 100  $\mu\text{m}$ . Magnification 10  $\times$ .



**Fig. 2.** Effect of mango intake on mammary cancer incidence. At 5 weeks of age, rats received different quantities of mango in the drinking water: (a) Control (1.2% of sucrose), (b) Mango-1 (0.02 g/mL); (c) Mango-2 (0.04 g/mL), and (d) Mango-3 (0.06 g/mL). Two weeks later, rats were i.p. injected with MNU (50 mg/Kg bw). At 8 weeks of age mango treatments were discontinued for half of the rats (short-term intake), and they were supplied with control beverage (A). The other half of the rats (long-term intake) continued with mango intake until the end of experiment (B).

**Table 3**

Effect of mango juice intake on mammary tumourigenesis after 22 weeks of MNU administration

Treatment	Number of rats with tumour	Tumour incidence (%)	Latency of tumours (weeks) <sup>a</sup>	Tumours per rat <sup>a</sup>	Size of tumours (cm <sup>3</sup> ) <sup>a</sup>
Control	9/12	75.0	16.8 ± 3.2	1.4 ± 0.5	0.8 ± 0.8
STI					
Mango-1 (0.02 g/mL)	9/12	75.0	16.9 ± 3.8	2.1 ± 0.9	0.8 ± 0.8
Mango-2 (0.04 g/mL)	9/12	75.0	14.2 ± 3.8	2.0 ± 0.9	1.8 ± 1.8
Mango-3 (0.06 g/mL)	8/12	66.7	15.4 ± 4.1	1.6 ± 0.7	1.2 ± 1.3
LTI					
Mango-1 (0.02 g/mL)	7/12	58.3	18.4 ± 3.5	1.9 ± 0.7	1.3 ± 2.3
Mango-2 (0.04 g/mL)	6/12	50.0	17.5 ± 3.6	1.5 ± 0.8	0.9 ± 1.2
Mango-3 (0.06 g/mL)	8/12	66.7	14.6 ± 5.1	1.8 ± 1.2	0.5 ± 0.7

At 5 weeks of age, rats were treated with different quantities of mango in the drinking water. Two weeks later, rats received a single i.p. injection of MNU (50 mg/Kg b. wt.), and the administration of mango was continued until 1 week (short-term intake: STI) or 22 weeks (long-term intakes: LTI) had elapsed.

<sup>a</sup> Result are expressed as the mean ± SD.

mango-fed and control groups after the 22-week experimental period, and Table 3 summarizes mammary tumourigenesis data in terms of incidence, latency, and both number and size of tumours for both control and mango-fed groups. In general, Fig. 2 and Table 3 show that short-term and long-term intake of mango by MNU-treated rats had no effect on mammary carcinogenesis.

### 3.5. Total phenolics and antioxidant capacity in plasma

Mango juice treatments did not modify total phenolic compounds and TOSC values in the plasma of rats fed with different levels of mango (Table 4). The FRAP assay indicated no differences in antioxidant capacity between the control group and the groups which consumed mango for three weeks (data not shown) for the MNU-treated rats (control versus Mango-3) (Table 4). However, in rats not treated with MNU but with long-term intake of mango, the FRAP values tended to increase in a dose-dependent manner (Table 4). The mango-3 group had a 3.1-fold increase in FRAP value with respect to the control, whereas Mango-1 and Mango-2 groups had 1.9- and 2.2-fold increases in FRAP values, respectively.

## 4. Discussion

The present study is, to our knowledge, the first to test *in vivo* antineoplastic effect of mango juice on mammary gland. Previous studies showed that mangoes have *in vitro* antioxidant, antimutagenic, and antineoplastic activities (Botting, Yong, Pearson, Harris, & Ferguson, 1999; Percival et al., 2006; Rocha-Ribeiro et al., 2007). However, we have shown here that short- and long-term mango consumption at physiological levels did not prevent mammary carcinogenesis in rats treated with the carcinogen MNU. This null effect of short-term and long-term intake of mango indicated that the kind and quantity of 'Ataulfo' mango phytochemicals do not inhibit the MNU-induced mammary cancer, neither at the initiation nor at the promotion/progression steps of carcinogenesis. Mango is a fruit rich in antioxidants such as carotenoids and phenolic compounds (Ornelas-Paz et al., 2007; Percival et al., 2006; Rocha-Ribeiro et al., 2007). Carotenoids are lipophilic pigments with antioxidant activity and some, such as  $\beta$ -carotene, have provitamin A activity (Lee et al., 1999). The main carotenoids of 'Ataulfo' mango are the provitamin A all-*trans*  $\beta$ -carotene and the non-provitamin A all-*trans* violaxanthin and 9-*cis* violaxanthin (Ornelas-Paz et al., 2007). Studies in humans have correlated the accumulation of carotenoids by breast adipose tissue with a reduced risk of breast cancer (Yeum et al., 1998). However, the protective effects of carotenoids against chemically induced, mammary carcinogenesis have been limited (Moon & Constantinou, 1997).  $\beta$ -Carotene has no effect on the prevention of mammary carcinogenesis using animal models (Moon & Constantinou A.I., 1997). This could be related to the fact that rats can transform provitamin A carotenoids to retinol with high efficiency (Lee et al., 1999), avoiding storage of carotenoids in mammary adipose tissue; therefore, they do not exert a protective effect against mammary carcinogenesis in rats. On the other hand, non-provitamin A carotenoids have different effects on mammary carcinogenesis: it is prevented by the consumption of canthaxanthin but not by lycopene (Cohen, 2002; Moon & Constantinou A.I., 1997). If fed diets containing supraphysiological levels of carotenoids ( $\geq 0.02\%$  of diet), rats can absorb and accumulate a variety of carotenoids, including  $\beta$ -carotene and lycopene, in tissues in a dose-dependent manner, but when normalized for body weight, consuming a diet containing 0.02% carotene is equivalent to a 70-Kg person eating 163 carrots a day (Cohen, 2002; Lee et al., 1999). Thus, the value of carotenoids in cancer prevention could be overestimated.

On the other hand, some whole extracts of fruits have been shown to have a protective effect on chemically induced mammary carcinogenesis. Previous studies using the DMBA rat model of mammary cancer showed that the consumption of whole extracts of red apple or purple grape juice inhibited carcinogenesis (Jung et al., 2006; Liu et al., 2005), but the mechanisms are unknown. Some of the main components which could participate in the protective effect of these fruits are phenolic compounds, mainly flavo-

**Table 4**  
Total phenolic compounds and antioxidant capacity in plasma of female rat with a long-term intake of mango (24 weeks)

Group	Phenolics ( $\mu\text{g}$ of GAE/mL)	TOSC value (mmol of AAE/L)	FRAP value ( $\mu\text{mol}$ of AAE/L)
Control ( $n = 4$ )	165.4 $\pm$ 7.2 (158–175)	405.8 $\pm$ 45.1 (346–455)	53.7 $\pm$ 20.7 (38–84)
Mango-1 ( $n = 4$ )	137.3 $\pm$ 12.0 (123–150)	366.1 $\pm$ 37.7 (323–636)	102.2 $\pm$ 73.8 (57–210)
Mango-2 ( $n = 4$ )	192.4 $\pm$ 21.0 (170–219)	430.3 $\pm$ 50.0 (370–478)	119.9 $\pm$ 68.9 (29–190)
Mango-3 ( $n = 4$ )	151.0 $\pm$ 9.5 (139–160)	443.6 $\pm$ 139.7 (325–415)	168.6 $\pm$ 76.0 (57–223)
<i>MNU-treated rats</i>			
Control ( $n = 6$ )	147.3 $\pm$ 32.7 (115–199)	421.8 $\pm$ 94.8 (306–530)	97.1 $\pm$ 1 26.0 (21–229)
Mango-3 ( $n = 6$ )	173.9 $\pm$ 33.5 (138–225)	468.2 $\pm$ 55.2 (406–529)	106.0 $\pm$ 104.4 (16–245)

AAE: Ascorbic acid equivalents; FRAP: Ferric reducing The values shown are the mean  $\pm$  SD ability of plasma; GAE: Gallic acid (Range), equivalents; TOSC: Total oxyradic scavenging capacity.

noids. Apple and purple grapes have a higher content of flavonoids than mango, and case-controlled studies have shown an inverse relation between flavonoid intake and breast cancer risk (Fink et al., 2007). Moreover, when rats were fed a diet containing up to 5% of quercetin, a flavonoid present in apples and grapes, the incidence of both DMBA- and MNU-induced mammary tumours was significantly reduced (Verma, Johnson, Gould, & Tanner, 1998). DMBA and MNU have different mechanisms of action; whereas DMBA requires metabolic activation by the mixed-function oxidase system to become carcinogenic, MNU is a direct-acting carcinogen that spontaneously decomposes into an alkylating agent at physiological pH (Thompson, 2000). These data suggest that bioactivation of the carcinogen DMBA is not essential for the protection provided by quercetin. Some authors have shown that the consumption of flavonoid-rich food causes a transient increase in plasma levels of phenolic compounds and antioxidant capacity (Serafini et al., 1998). However, other authors have suggested that circulating levels of flavonoids are too low (between 0.06 and 7.6  $\mu\text{M}$  for flavonols, flavanols, and flavanones, and less than 0.15  $\mu\text{M}$  for anthocyanidins) to exert an *in vivo* antioxidant activity (Lotito & Frei, 2006). Accordingly, we did not notice changes in total plasma phenolic compounds between control and mango-fed groups. Furthermore, mango intake in MNU-treated rats did not modify plasma antioxidant capacity as measured by both FRAP and TOSC assays. However, rats with a long-term intake of mango that did not receive a MNU-injection tended to increase plasma antioxidant activity in a dose-dependent manner as measured by the FRAP assay. Although the FRAP and TOSC assays measure global antioxidant capacity, they have different principles. On the one hand, the FRAP assay measures the ability of a compound or a mixture to reduce ferric to ferrous ions at low pH (Benzie & Strain, 1999). The FRAP assay gives a value of the combined reducing power of the electron-donating antioxidants present in the reaction mixture. In plasma, this ability is mainly due to ascorbic acid, uric acid, tocopherols, and other non-protein antioxidants (Benzie & Strain, 1999). On the other hand, the TOSC assay measures the ability of a compound or a mixture to scavenge peroxy radicals generated by thermal homolysis of azo compounds (Winston et al., 1998). The TOSC assay measures not only small antioxidant molecules such as ascorbic and uric acids but also the antioxidant capacity of proteins (Winston et al., 1998), which could mask the activity of small antioxidant molecules. This also could explain why plasma antioxidant activity measured by the FRAP assay is lower than with the TOSC assay. The increase of plasma antioxidant capacity in rats after a long-term consumption of mango could indicate the presence of large quantities of an antioxidant with reducing power, such as ascorbic acid; in fact, mango is a good source of this antioxidant (Rocha-Ribeiro et al., 2007). The lack of effect in MNU-treated rats could indicate that MNU increases the oxidative status as compared with normal counterparts. It has also been shown that, at least in mammary gland, MNU-treated animals have higher lipoperoxidation levels than normal rats (García-Solís et al., 2005).

## 5. Conclusion

In summary, we have shown that 'Ataulfo' mango, applied in drinking water at 0.02–0.06 g/mL for up to 24 weeks does not prevent mammary carcinogenesis or increase plasma antioxidant capacity in MNU-treated Sprague-Dawley rats. However, these findings of the present study should not be interpreted as a lack of health benefits from regular consumption of mango or other fruits and vegetables.

## Acknowledgments

The authors are grateful to Evelyn Flores for her support in the histological procedures and techniques, Nydia Hernandez for image advice, Felipe Ortiz and Martin García Servín for the animal care, and Brenda Anguiano, Guadalupe Delgado, Jesus Ornelas, Israel Hinogiante, Fernanda Gallegos, and Cristina Garcia for technical help. We also thank Dorothy Pless for proof-reading this manuscript. This work, including a postdoctoral fellowship to P. García-Solís, was supported by the grant CONACYT 47185. Partial support came from the Grant PAPIIT-UNAM IN201207.

## References

- Benzie, I. F. F., & Strain, J. J. (1999). Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, 299, 15–27.
- Botting, K. J., Yong, M. M., Pearson, A. E., Harris, P. J., & Ferguson, L. R. (1999). Antimutagens in food plants eaten by Polynesians: micronutrients, phytochemicals and protection against bacterial mutagenicity of heterocyclic amine 2-amino-3-methylimidazo[4,5-f]quinoline. *Food and Chemical Toxicology*, 37, 95–103.
- Brown, N. S., & Bicknell, R. (2001). Hypoxia and oxidative stress in breast cancer. Oxidative stress: Its effects on the growth, metastatic potential and response to therapy of breast cancer. *Breast Cancer Research*, 3, 323–327.
- Cohen, L. (2002). Review of animal model studies of tomato carotenoids, lycopene, and cancer chemoprevention. *Experimental Biology and Medicine*, 227, 964–969.
- Fink, B. N., Steck, S. E., Wolff, M. S., Britton, J. A., Kabat, G. C., Schroeder, J. C., et al. (2007). Dietary flavonoid intake and breast cancer risk among women on Long Island. *American Journal of Epidemiology*, 165, 514–523.
- García-Solís, P., Alfaro, Y., Anguiano, B., Delgado, G., Guzman, R., Nandi, S., et al. (2005). Inhibition of *N*-methyl-*N*-nitrosourea-induced mammary carcinogenesis by molecular iodine ( $\text{I}_2$ ) but not by iodide ( $\text{I}^-$ ) treatment. Evidence that  $\text{I}_2$  prevents cancer promotion. *Molecular and Cellular Endocrinology*, 236, 49–57.
- Havas, S., Heimendinger, J., Damron, D., Nicklas, T. A., Cowan, A., Beresford, S. A. A., et al. (1995). 5 a day for better health—nine community research projects to increase fruit and vegetable consumption. *Public Health Reports*, 110, 68–79.
- Jung, K. J., Wallig, M. A., & Singletary, K. W. (2006). Purple grape juice inhibits 7, 12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary tumorigenesis and *in vivo* DMBA-DNA adduct formation. *Cancer Letters*, 233, 279–288.
- Lee, C. M., Boileau, A. C., Boileau, T. W. M., Williams, A. W., Swanson, K. S., Heintz, K. A., et al. (1999). Review of animal models in carotenoid research. *The Journal of Nutrition*, 129, 2271–2277.
- Liu, R. H., Liu, J., & Chen, B. (2005). Apples prevent mammary tumors in rats. *Journal of Agricultural Food Chemistry*, 53, 2341–2343.
- Lotito, S. B., & Frei, B. (2006). Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radical Biology Medicine*, 41, 1727–1746.

- MacLean, D. D., Murr, D. P., & DeEll, J. R. (2003). A modified total oxyradical scavenging capacity assay for antioxidants in plant tissues. *Postharvest Biology and Technology*, 29, 183–194.
- Melendez-Martinez, A. J., Vicario, I. M., & Heredia, F. (2003). Application of tristimulus colorimetry to estimate the carotenoids content in ultrafrozen orange juices. *Journal of Agricultural Food Chemistry*, 51, 7266–7270.
- Moon, R. C., & Constantinou, A. I. (1997). Dietary retinoids and carotenoids in rodent models of mammary tumorigenesis. *Breast Cancer Research and Treatment*, 48, 181–189.
- Ornelas-Paz, J. De J., Yahia, E. M., & Gardea-Bejar, A. (2007). Identification and quantification of xanthophyll esters, carotenes and tocopherols in fruit of seven Mexican mango cultivars by liquid chromatography-atmospheric pressure chemical ionization-time-of-flight Mass Spectrometry [LC-(APCl(+))-MS]. *Journal of Agricultural Food Chemistry*, 55, 6628–6635.
- Parkin, D. M., Bray, F., Ferlay, J., & Pisani, P. (2005). Global cancer statistics, 2002. *CA: A Cancer Journal for Clinicians*, 55, 74–108.
- Percival, S. S., Talcott, S. T., Chin, S. T., Mallak, A. C., Lounds-Singleton, A., & Pettit-Moore, J. (2006). Neoplastic transformation of BALB/3T3 cells and cell cycle of HL-60 cells are inhibited by mango (*Mangifera indica L*) juice and mango juice extract. *Journal of Nutrition*, 136, 1300–1304.
- Riboli, E., & Norat, T. (2003). Epidemiologic evidence of the protective effect of fruit and vegetable on cancer risk. *The American Journal of Clinical Nutrition*, 78(Suppl.), 559S–5569S.
- Rocha-Ribeiro, S. M., Queiroz, J. H., Lopes, M., Campos, F. M., & Pinheiro, H. M. (2007). Antioxidant in mango (*Mangifera indica L*) pulp. *Plant Foods for Human Nutrition*, 62, 13–17.
- Russo, J., & Russo, I. H. (2000). Atlas and histological classification of tumors of the rat mammary gland. *Journal of Mammary Gland Biology and Neoplasia*, 5, 187–200.
- Serafini, M., Maiani, G., & Ferro-Luzzi, A. (1998). Alcohol free red wine enhances plasma antioxidant capacity in humans. *The Journal of Nutrition*, 128, 1003–1007.
- Singletary, K. W., Jackson, S. J., & Milner, J. (2005). A non-nutritive components in foods as modifiers of the cancer process. In A. Bendich & R. J. Deckelbaum (Eds.), *Preventive nutrition: The comprehensive guide for health professionals* (pp. 55–88). Totowa, NY: Humana Press.
- Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152–178.
- Soto-Zamora, G., Yahia, E. M., Brecht, J. K., & Gardea, A. (2005). Effects of postharvest hot air on the quality and antioxidant levels in tomato fruit. *LWT*, 38, 657–663.
- Sun, J., Chu, Y. F., Wu, X., & Liu, R. H. (2002). Antioxidant and antiproliferative activities of common fruits. *Journal of Agricultural Food Chemistry*, 50, 7449–7454.
- Thompson, H. J. (2000). Methods for the induction of mammary carcinogenesis in the rat using either 7,12-dimethylbenz[*a*]anthracene or 1-methyl-1-nitrosourea. In M. Ip & B. B. Asch (Eds.), *Methods in mammary gland biology and breast cancer research* (8th ed., pp. 19–29). New York, NY: Kluwer Academic/Plenum Publishers.
- Verma, A., Johnson, J., Gould, M., & Tanner, M. (1998). Inhibition of 7,12-dimethylbenz[*a*]anthracene and *N*-methylnitrosourea-induced rat mammary cancer by dietary flavonol quercetin. *Cancer Research*, 48, 5754–5758.
- Winston, G. W., Regoli, F., Dugas, A. J., Jr., Fong, J. H., & Blanchard, K. (1998). A rapid gas chromatographic assay for determining oxyradical scavenging capacity of antioxidants and biological fluids. *Free Radical Biology and Medicine*, 24, 480–493.
- Yeum, K.-J., Ahn, S.-H., Rupp de Pavia, S. A., Lee-Kim, Y. C., Krinsky, N. I., & Russell, R. M. (1998). Correlation between carotenoids concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. *The Journal of Nutrition*, 128, 1920–1926.