JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Food Acidulants and Antioxidant Spices on the Bioaccessibility of β-Carotene from Selected Vegetables

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Four common food acidulants—amchur, lime, tamarind, and kokum—and two antioxidant spices—turmeric and onion—were examined for their influence on the bioaccessibility of β-carotene from two fleshy and two leafy vegetables. Amchur and lime generally enhanced the bioaccessibility of β-carotene from these test vegetables in many instances. Such an improved bioaccessibility was evident in both raw and heat-processed vegetables. The effect of lime juice was generally more pronounced than that of amchur. Turmeric significantly enhanced the bioaccessibility of β-carotene from all of the vegetables tested, especially when heat-processed. Onion enhanced the bioaccessibility of β-carotene from pressure-cooked carrot and amaranth leaf and from open-pan-boiled pumpkin and fenugreek leaf. Lime juice and the antioxidant spices turmeric and onion minimized the loss of β-carotene during heat processing of the vegetables. In the case of antioxidant spices, improved bioaccessibility of β-carotene from heat-processed vegetables is attributable to their role in minimizing the loss of this provitamin. Lime juice, which enhanced the bioaccessibility of this provitamin from both raw and heat-processed vegetables, probably exerted this effect by some other mechanism in addition to minimizing the loss of β-carotene. Thus, the presence of food acidulants (lime juice/amchur) and antioxidant spices (turmeric/onion) proved to be advantageous in the context of deriving maximum β-carotene from the vegetable sources.

KEYWORDS: Antioxidant spices; bioaccessibility; β-carotene; food acidulants

INTRODUCTION

Deficiency of vitamin A is a serious public health problem leading to blindness (*1*). A majority of the population in India is dependent on plant foods, which provide carotenoids, especially β-carotene, to meet their requirement of vitamin A. β-Carotene is abundantly found in green leafy and yellow-orange vegetables (*2*). Several factors such as diet composition and methods employed for food processing affect the bioaccessibility of β-carotene from foods (*3*). Studies have shown that absorption of carotenoids from uncooked foods is low and that mild cooking enhances the same (*4*). However, heat treatment, especially in the presence of light and oxygen, causes isomerization of carotene as well as its oxidative destruction, thus decreasing its biological activity. An earlier study in our laboratory revealed that inclusion of food acidulants (tamarind and citric acid) and antioxidant spices (turmeric and onion) during heat processing of vegetables generally improved the retention of β-carotene in the same (*5*). In view of the fact that a majority of the Indian population is dependent on plant foods to meet their requirement of vitamin A, it is desirable to evolve dietary strategies to improve the bioavailability of β-carotene from these sources. Organic acids such as citric, ascorbic, and malic acid are known to promote the bioavailability of iron from plant foods (*6*). We have recently evidenced similar enhancement of the bioaccessibility of zinc from cereals and pulses by these food acidulants/organic acids (*7*). It is possible that these common food ingredients would also have a beneficial stimulating effect on the bioaccessibility of β-carotene from its vegetable sources.

The food acidulants commonly used in Indian cuisine include amchur [dry mango (*Mangifera indica*) powder], lime (*Citrus*) juice, tamarind (*Tamarindus indica*), and kokum (*Garcinia indica*). Turmeric (*Curcuma longa*) and onion (*Allium cepa*) are the spices that are known to possess antioxidant property and are commonly used in Indian households (*8*). Information on the effect of food acidulants and antioxidant spices on the bioaccessibility of β-carotene from vegetables is, at present, lacking. The present investigation was therefore undertaken to examine the effect of these food ingredients on the bioaccessibility of β-carotene from select green leafy and yellow-orange vegetables.

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MATERIALS AND METHODS

Materials. Fresh carrot (*Daucus carota*), pumpkin (*Cucurbita maxima*), amaranth (*Amaranthus gangeticus*) leaves, and fenugreek

10.1021/jf801374d CCC: $40.75
Published on Web 08/27/2008

Table 1. Effect of Food Acidulants on the Bioaccessibility of β-Carotene from Pumpkin[a]

food acidulant	raw		pressure-cooked		open-pan-boiled	
	total	bioaccessible	total	bioaccessible	total	bioaccessible
pumpkin	1707.8 ± 33.4	269.0 ± 5.2 (15.8%)	745.1 ± 30.0	292.1 ± 9.7 (17.1%)	1258.8 ± 33.2	331.6 ± 14.8 (19.4%)
pumpkin + amchur	1696.4 ± 21.8	356.8 ± 22.0* (21.0%)	836.2 ± 36.7	409.0 ± 12.3* (24.0%)	1143.3 ± 61.0	364.6 ± 7.5* (21.4%)
pumpkin + lime	1687.5 ± 19.1	543.8 ± 43.7* (31.8%)	979.1 ± 49.3	386.2 ± 17.1* (22.6%)	1302.0 ± 54.4	386.1 ± 20.1* (22.6%)
pumpkin + tamarind	1697.6 ± 7.6	205.4 ± 5.0** (12.1%)	1131.1 ± 57.4	279.3 ± 19.7 (16.3%)	1060.1 ± 36.4	282.8 ± 12.2** (16.5%)
pumpkin + kokum	1681.3 ± 17.2	292.5 ± 3.1 (17.1%)	903.5 ± 55.0	260.0 ± 10.2 (15.2%)	1037.7 ± 49.2	250.5 ± 28.0** (14.7%)

[a] Values of β-carotene are expressed as μg/100 g of fresh weight and are given as mean ± SEM of pentuplicates. Values in parentheses indicate percent bioaccessibility of β-carotene. *, Significant increase compared to the value in vegetable alone ($p < 0.05$); **, significant decrease compared to the value in vegetable alone ($p < 0.05$).

(*Trigonella foenum-graecum*) leaves were procured from local vendors and cleaned, and the edible portions were used for the study. The other ingredients employed, namely, amchur, lime, tamarind, kokum, turmeric, and onion powder, were locally procured. All chemicals used were of analytical grade. Solvents were distilled before use. Standard β-carotene, porcine pancreatic pepsin, and pancreatin and bile extract (porcine) were procured from Sigma Chemical Co., St. Louis, Mo. Double-distilled water was employed throughout the entire study. All glassware used was acid washed.

Food Sample Preparation. Carrot and pumpkin were cut to a uniform size, whereas the edible portion of the two leafy vegetables was finely chopped. The test vegetables (10 g portions) were used for the study in the following combinations: (1) test vegetable alone; (2) test vegetable + acidulant (amchur/lime/tamarind/kokum); test vegetable + antioxidant spice (turmeric powder/onion powder). The acidulants were added at amounts that reduced the pH of the food by 1 unit, the levels added being 3, 7, 2.5, and 3% for amchur, lime juice, tamarind, and kokum, respectively. Turmeric and onion powder were included at 1% level. The above combinations were also subjected to heat processing by two methods, namely, pressure-cooking and open-pan-boiling.

Determination of Bioaccessibility of β-Carotene in Vitro. The bioaccessibility of β-carotene in vitro was determined according to the method of Garrett et al. (*9*) with suitable modification (*10*). Briefly, the method involved subjecting the sample to simulated gastric digestion at pH 2.0 in the presence of pepsin at 37 °C (16 g in 100 mL of 0.1 M HCl), followed by simulated intestinal digestion in the presence of a pancreatin−bile extract mixture [4 g of porcine pancreatin and 25 g of bile extract (porcine) in 1000 mL of 0.1 M $NaHCO_3$], pH 7.5 at 37 °C for 2 h. At the end of simulated intestinal digestion, the micellar fraction was separated by ultracentrifugation at 70000*g* for 120 min using a Beckman L7-65 ultracentrifuge. The β-carotene present in the micellar fraction represents the portion that is bioaccessible.

Analysis of β-Carotene. β-Carotene was extracted from the samples initially with a mixture of acetone/ethanol (1:1) and subsequently with petroleum ether (*11*). The process was repeated several times to ensure complete extraction of β-carotene. In the case of green leafy vegetables, the extract was saponified (in an additional step to remove chlorophyll) with 30% methanolic potassium hydroxide at room temperature for 3 h. Following saponification, the alkali was removed completely by repeated washing, and the solvent was evaporated to dryness in a rotary evaporator. The residue was redissolved in petroleum ether and stored in the cold pending analysis. Prior to analysis, the petroleum ether was evaporated under nitrogen and the residue was dissolved in the mobile phase used for HPLC determination.

Determination of β-carotene was carried out by reverse-phase HPLC (Shimadzu LC 10 AVP), equipped with a UV−visible detector. β-Carotene was separated on a C_{18} column (S.S. Excil). The mobile phase consisted of a mixture of 65% (v/v) acetonitrile, 15% (v/v) methylene chloride, and 20% (v/v) methanol containing 1.3 mmol/L ammonium acetate. β-Carotene (all-trans isomer) was monitored at a wavelength of 450 nm. The peak identities and λ_{max} were confirmed by their retention time and characteristic spectra of standard chromatograms.

During the steps of simulated gastrointestinal digestion, ultracentrifugation, and extraction of β-carotene, precautions were taken to minimize the exposure of samples to light and air and thus prevent oxidative destruction of β-carotene. Air was replaced by nitrogen before stoppering the flask at all stages of incubation and storage. The experiments were carried out under yellow lighting, and all of the glassware was covered with black cloth to prevent exposure to light.

Statistical Analysis. All determinations were made in pentuplicates, and the average values are reported. Data were analyzed statistically according to the method of Snedecor and Cochran (*12*).

RESULTS

Effect of Food Acidulants on the Bioaccessibility of β-Carotene from Vegetables. The effect of food acidulants—amchur, lime, tamarind, and kokum—on the bioaccessibility of β-carotene from the test vegetables is presented in **Tables 1−4**. **Table 1** presents the influence of food acidulants on the bioaccessibility of β-carotene from pumpkin. Inclusion of amchur significantly increased the bioaccessibility of β-carotene from raw as well as heat-treated pumpkin. The increase brought about in percent bioaccessible fraction was from 15.8 to 21.0 in the case of raw vegetable, from 17.1 to 24.0 in the case of pressure-cooked vegetable, and from 19.4 to 21.4 in the case of open-pan-boiled vegetable. Thus, the extent of increase in bioaccessibility of β-carotene from pumpkin caused by amchur amounted to 32.6, 40.0, and 10.0% from raw, pressure-cooked, and open-pan-boiled pumpkin, respectively. A similar significant increase in the percent bioaccessible fraction of β-carotene from raw as well as heat-treated pumpkin was also evidenced with the addition of lime juice, the extent of increase in the bioaccessibility of β-carotene being 102% (an increase from 15.8 to 31.8%), 32.2% (an increase from 17.1 to 22.6%), and 16.4% (an increase from 19.4 to 22.6%) in raw, pressure-cooked, and open-pan-boiled pumpkin, respectively. Unlike amchur and lime juice, tamarind and kokum generally did not influence the bioaccessibility of β-carotene from pumpkin. On the other hand, these significantly decreased the same from open-pan-boiled pumpkin (14.7 and 24.5%, respectively). Tamarind also negatively influenced the bioaccessibility of β-carotene from raw pumpkin (23.6%).

The effect of acidulants on the bioaccessibility of β-carotene from carrot is presented in **Table 2**. Lime juice brought about an increase in percent bioaccessibility of β-carotene from raw carrot, the extent of increase being 14.2% (from 15.5 to 17.7%). Amchur, on the other hand, had no effect on the bioaccessibility of β-carotene from either raw or heat-processed carrot. As in the case of pumpkin, tamarind negatively influenced the bioaccessibility of β-carotene from open-pan-boiled carrot (36.6% decrease), whereas kokum decreased the same from pressure-cooked carrot by 11.5%.

Table 2. Effect of Food Acidulants on the Bioaccessibility of β-Carotene from Carrot[a]

food acidulant	raw		pressure-cooked		open-pan-boiled	
	total	bioaccessible	total	bioaccessible	total	bioaccessible
carrot	7594.3 ± 81.7	1179.0 ± 30.0 (15.5%)	5075.8 ± 60.0	1546.6 ± 35.5 (20.4%)	6599.1 ± 95.7	1770.0 ± 66.1 (23.3%)
carrot + amchur	7503.2 ± 67.0	1239.0 ± 48.3 (16.3%)	4947.7 ± 57.2	1498.7 ± 41.3 (19.7%)	6091.0 ± 127.4	1774.0 ± 73.7 (23.4%)
carrot + lime	7500.1 ± 207.6	1341.7 ± 52.7* (17.7%)	5677.8 ± 313.0	1602.3 ± 45.3 (21.1%)	6995.0 ± 80.1	1901.0 ± 41.6 (25.0%)
carrot + tamarind	6887.1 ± 33.8	1128.8 ± 9.5 (15.0%)	5600.3 ± 68.8	1535.3 ± 22.6 (20.2%)	6288.6 ± 129.3	1120.9 ± 43.2** (14.7%)
carrot + kokum	6914.8 ± 116.1	1250.1 ± 19.0 (22.6%)	5375.0 ± 112.4	1368.1 ± 51.9** (18.0%)	6263.4 ± 70.6	1702.3 ± 29.8 (22.4%)

[a] Values of β-carotene are expressed as μg/100 g of fresh weight and are given as mean ± SEM of pentuplicates. Values in parentheses indicate percent bioaccessibility of β-carotene. *, significant increase compared to the value in vegetable alone ($p < 0.05$); **, significant decrease compared to the value in vegetable alone ($p < 0.05$).

Table 3. Effect of Food Acidulants on the Bioaccessibility of β-Carotene from Fenugreek Leaves[a]

food acidulant	raw		pressure-cooked		open-pan-boiled	
	total	bioaccessible	total	bioaccessible	total	bioaccessible
fenugreek leaves	9024.5 ± 67.6	703.0 ± 3.8 (7.8%)	6165.1 ± 217.0	1296.8 ± 21.7 (14.4%)	5108.6 ± 61.8	819.7 ± 50.0 (9.1%)
fenugreek leaves + amchur	8922.6 ± 56.2	791.2 ± 2.1 (8.8%)	6458.3 ± 129.0	1585.0 ± 92.2* (17.6%)	5433.5 ± 290.0	859.6 ± 27.4 (9.5%)
fenugreek leaves + lime	9022.7 ± 64.3	790.1 ± 5.8* (8.75%)	6965.0 ± 43.6	1485.7 ± 11.4* (16.5%)	5260.5 ± 39.0	1050.0 ± 43.6* (11.6%)
fenugreek leaves + tamarind	8740.0 ± 107.5	669.4 ± 35.0 (7.41%)	6282.0 ± 143.8	1316.4 ± 30.4 (14.5%)	5496.5 ± 95.0	800.0 ± 7.1 (9.0%)
fenugreek leaves + kokum	8846.8 ± 61.5	683.4 ± 13.4 (7.60%)	6092.0 ± 105.7	1190.0 ± 57.0 (13.3%)	4715.3 ± 55.0	817.5 ± 2.1 (9.10%)

[a] Values of β-carotene are expressed as μg/100 g of fresh weight and are given as mean ± SEM of pentuplicates. Values in parentheses indicate percent bioaccessibility of β-carotene. *, significant increase compared to the value in vegetable alone ($p < 0.05$).

Table 4. Effect of Food Acidulants on the Bioaccessibility of β-Carotene from Amaranth[a]

food acidulant	raw		pressure-cooked		open pan-boiled	
	total	bioaccessible	total	bioaccessible	total	bioaccessible
amaranth	7930.8 ± 65.5	716.2 ± 16.6 (9.0%)	5504.0 ± 59.0	1170.0 ± 38.7 (14.8%)	3965.1 ± 30.0	942.4 ± 30.0 (11.9%)
amaranth + amchur	7836.4 ± 47.1	740.6 ± 28.6 (9.3%)	5566.2 ± 146.7	1399.4 ± 46.1* (17.6%)	3667.8 ± 203.7	813.4 ± 62.4 (10.2%)
amaranth + lime	7825.0 ± 54.0	768.6 ± 29.0 (10.0%)	6067.3 ± 56.0	1124.7 ± 14.2 (14.2%)	5569.2 ± 170.0	1173.1 ± 14.0* (14.7%)
amaranth + tamarind	7781.5 ± 28.1	578.4 ± 15.2** (7.30%)	5360.3 ± 58.0	1295.8 ± 51.4 (16.3%)	5388.1 ± 60.5	930.7 ± 25.5 (11.7%)
amaranth + kokum	7625.6 ± 58.3	691.6 ± 11.1 (8.72%)	5577.0 ± 85.4	1016.1 ± 30.1** (12.8%)	3490.0 ± 251.2	963.3 ± 60.2 (12.1%)

[a] Values of β-carotene are expressed as μg/100 g of fresh weight and are given as mean ± SEM of pentuplicates. Values in parentheses indicate percent bioaccessibility of β-carotene. *, significant increase compared to the value in vegetable alone ($p < 0.05$); **, significant decrease compared to the value in vegetable alone ($p < 0.05$).

Table 3 presents the influence of acidulants on β-carotene bioaccessibility from fenugreek leaf. Amchur brought about a significant increase in the bioaccessibility of β-carotene from pressure-cooked fenugreek leaf, the percent increase being 22.2% (increase from 14.4 to 17.6%). The bioaccessibility of β-carotene from fenugreek leaf was also increased by the presence of lime juice, the percent increase being 12.4, 14.6, and 28 in the raw (increase from 7.8 to 8.75), pressure-cooked (from 14.4 to 16.5%), and open-pan-boiled (from 9.1 to 11.6%) leafy vegetable, respectively. Tamarind and kokum did not have any effect on the bioaccessibility of β-carotene from either raw or heat-processed fenugreek leaf.

The effect of acidulants on the bioaccessibility of β-carotene from amaranth leaf is presented in **Table 4**. Amchur brought about a significant increase in the bioaccessibility of β-carotene from pressure-cooked amaranth leaf (19.6% increase). In the case of lime juice, the positive effect was limited to open-pan-boiled amaranth leaf, where the percent β-carotene bioaccessibility was 14.7, compared to 11.8 in the absence of the food acidulant. Tamarind, on the other hand, brought about a decrease in the bioaccessibility of β-carotene from raw amaranth leaf (23.8% decrease), whereas kokum decreased the same from pressure-cooked amaranth leaf (13.1% decrease).

Effect of Antioxidant Spices on the Bioaccessibility of β-Carotene from Vegetables. The effect of turmeric and onion on the bioaccessibility of β-carotene from carrot and pumpkin is shown in **Table 5**. Whereas turmeric did not have any effect on the bioaccessibility of β-carotene from the raw vegetables when included at 1% level, it had an enhancing influence on the same from pressure-cooked carrot (24.6% increase). In the case of pumpkin, the beneficial influence of turmeric was evident in both open-pan-boiled and pressure-cooked pumpkin, the effect being much greater in the case of the former. The percent increases in β-carotene bioaccessibility were 54.2 and 23.4 for

Table 5. Effect of Antioxidant Spices—Turmeric or Onion—on the Bioaccessibility of β-Carotene from Carrot and Pumpkin[a]

	raw		pressure-cooked		open-pan-boiled	
	total	bioaccessible	total	bioaccessible	total	bioaccessible
carrot	7594.3 ± 81.7	1179.0 ± 30.0 (15.5%)	5075.8 ± 60.0	1546.6 ± 35.5 (20.4%)	6599.1 ± 95.7	1770.3 ± 66.1 (23.3%)
carrot + turmeric	7737.0 ± 68.6	1198.7 ± 11.5 (15.8%)	6183.5 ± 518.9	1927.1 ± 39.0* (25.3%)	7425.2 ± 64.3	1766.7 ± 59.0 (25.0%)
carrot + onion	7259.0 ± 67.6	1507.1 ± 117.4* (19.8%)	5488.3 ± 172.4	1926.3 ± 42.2* (25.4%)	7014.8 ± 66.7	1886.2 ± 102.2 (24.8%)
pumpkin	1707.8 ± 13.4	269.0 ± 5.2 (15.8%)	745.1 ± 30.0	292.1 ± 5.7 (17.1%)	1258.8 ± 33.2	331.6 ± 14.8 (19.4%)
pumpkin + turmeric	1725.3 ± 26.6	286.9 ± 7.7 (16.8%)	1044.7 ± 29.4	360.5 ± 19.4* (21.1%)	1503.2 ± 44.0	511.3 ± 20.0* (30.0%)
pumpkin + onion	1718.7 ± 19.8	250.0 ± 12.0 (14.5%)	772.8 ± 28.4	277.0 ± 6.5 (16.1%)	1300.0 ± 3.20	377.8 ± 13.4* (22.0%)

[a] Values of β-carotene are expressed as μg/100 g of fresh weight and are given as mean ± SEM of pentuplicates. Values in parentheses indicate percent bioaccessibility of β-carotene. *, significant increase compared to the value in vegetable alone ($p < 0.05$).

Table 6. Effect of Antioxidant Spices—Turmeric and Onion—on the Bioaccessibility of β-Carotene from Green Leafy Vegetables[a]

	raw		pressure-cooked		open-pan-boiled	
	total	bioaccessible	total	bioaccessible	total	bioaccessible
amaranth	7930.8 ± 65.5	678.0 ± 39.5 (8.5%)	5504.0 ± 59.0	1170.0 ± 38.7 (14.7%)	3894.7 ± 73.5	942.4 ± 30.0 (11.8%)
amaranth + turmeric	7823.0 ± 53.8	713.3 ± 12.4 (9.00%)	5807.6 ± 58.2	1453.0 ± 27.4* (18.3%)	4213.5 ± 156.2	1590.3 ± 76.6* (20.0%)
amaranth + onion	7868.0 ± 66.6	771.3 ± 27.8 (9.72%)	5859.0 ± 145.0	1427.7 ± 36.5* (18.0%)	4704.7 ± 205.0	1007.3 ± 27.0 (12.7%)
fenugreek leaves	9024.5 ± 67.6	703.0 ± 3.80 (7.80%)	6412.8 ± 277.5	1296.8 ± 21.7 (14.4%)	5075.3 ± 69.0	819.7 ± 50.0 (9.10%)
fenugreek leaves + turmeric	9083.7 ± 89.0	727.2 ± 12.0 (8.10%)	6815.7 ± 52.7	1494.8 ± 53.5* (16.6%)	6052.6 ± 36.6	1117.5 ± 54.5* (12.4%)
fenugreek leaves + onion	9145.5 ± 186.7	718.0 ± 31.3 (8.00%)	6968.0 ± 43.3	1368.8 ± 30.4 (15.2%)	5743.4 ± 89.3	1038.2 ± 67.5* (11.5%)

[a] Values of β-carotene are expressed as μg/100 g of fresh weight and are given as mean ± SEM of pentuplicates. Values in parentheses indicate percent bioaccessibility of β-carotene. *, significant increase compared to the value in vegetable alone ($p < 0.05$).

open-pan-boiled and pressure-cooked pumpkin, respectively. As in the case of turmeric, onion did not have any effect on the bioaccessibility of β-carotene from raw pumpkin, but this antioxidant spice brought about a 27.8% increase in β-carotene bioaccessibility from raw carrot. Onion enhanced the bioaccessibility of β-carotene from pressure-cooked carrot (24.5% increase) and open-pan-boiled pumpkin (13.9%).

The effect of the antioxidant spices turmeric and onion on the bioaccessibility of β-carotene from the two green leafy vegetables is presented in **Table 6**. As in the case of carrot and pumpkin, turmeric significantly increased the bioaccessibility of β-carotene from the heat-processed green leafy vegetables, this effect being more prominent in the case of open-pan-boiled green leafy vegetables. The extents of increase in percent bioaccessibility of β-carotene were 68.7 and 36.3 from open-pan-boiled amaranth and fenugreek leaf, whereas they were 24.2 and 15.3 from pressure-cooked amaranth and fenugreek leaf. Onion enhanced the bioaccessibility of β-carotene from pressure-cooked amaranth leaf (22%) and open-pan-boiled fenugreek leaf (26.6%).

Heat processing of the test vegetables by pressure-cooking or open-pan boiling generally improved the bioaccessibility of β-carotene (**Figure 1**). Whereas the beneficial effect of pressure cooking was higher than that of open pan boiling in the case of the two green leafy vegetables, the reverse was true in the case of carrot and pumpkin.

To verify if the higher bioaccessibility of β-carotene observed in the presence of exogenous food acidulants/antioxidant spices was a result of minimized loss of this provitamin during heat treatment, the concentrations of β-carotene recovered in the two types of heat processing in the presence and absence of food acidulants and antioxidant spices were computed and are presented in **Tables 7** and **8**. Incidentally, higher retention of β-carotene was observed in all four vegetable samples subjected to either pressure cooking or open pan boiling in the presence of lime juice (**Table 7**). Such a prevention of loss of β-carotene by heat processing was restricted to fenugreek leaves in the case of amchur. Tamarind and kokum also had a sparing action on the loss of β-carotene from the test vegetables in a few instances.

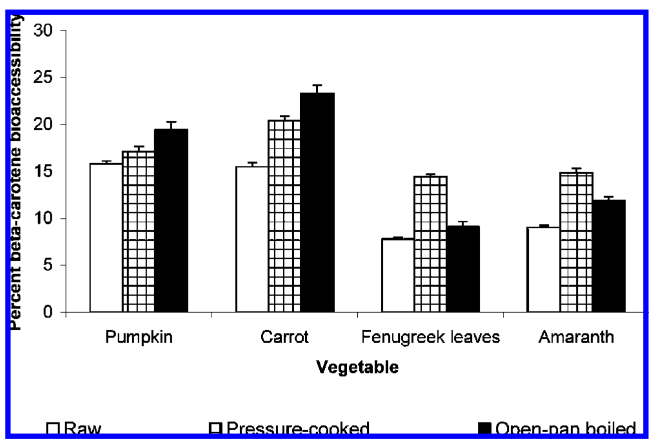

Figure 1. Effect of heat processing on bioaccessibility of β-carotene from vegetables. All the values in pressure-cooked and open-pan-boiled groups were significantly higher than the corresponding value in the raw sample ($p < 0.05$).

Table 7. Effect of Food Acidulants and Antioxidant Spices on the Retention of β-Carotene in Heat-Processed Carrot and Pumpkin[a]

	raw	pressure-cooked	open-pan-boiled
pumpkin	100.0 ± 1.9	43.6 ± 1.7	73.7 ± 1.9
pumpkin + amchur	99.3 ± 1.3	45.8 ± 2.2	66.9 ± 3.6
pumpkin + lime	98.8 ± 1.1	57.3 ± 2.9	76.3 ± 3.2
pumpkin + tamarind	99.4 ± 0.4	66.2 ± 3.4	62.1 ± 2.1
pumpkin + kokum	98.5 ± 1.0	51.9 ± 3.2	60.8 ± 2.9
pumpkin + turmeric	101.0 ± 1.6	61.2 ± 1.7	88.0 ± 2.6
pumpkin + onion	101.0 ± 1.8	45.2 ± 1.1	76.1 ± 1.2
carrot	100.0 ± 1.4	66.8 ± 0.8	86.8 ± 1.3
carrot + amchur	98.8 ± 0.9	65.1 ± 0.8	80.2 ± 1.7
carrot + lime	98.7 ± 2.8	74.7 ± 4.1	92.1 ± 1.1
carrot + tamarind	90.6 ± 1.5	73.7 ± 0.9	82.8 ± 1.7
carrot + kokum	91.0 ± 1.5	77.7 ± 1.5	82.1 ± 0.9
carrot + turmeric	102.0 ± 0.9	81.4 ± 3.8	97.7 ± 0.9
carrot + onion	95.6 ± 0.9	72.2 ± 2.3	92.3 ± 0.9

[a] Values (given as percent) are average of pentuplicates. Values are given as percent relative to β-carotene content of raw vegetables taken as 100%.

Table 8. Effect of Food Acidulants and Antioxidant Spices on the Retention of β-Carotene in Heat-Processed Green Leafy Vegetables[a]

	raw	pressure-cooked	open-pan-boiled
fenugreek	100.0 ± 0.8	68.3 ± 2.4	56.6 ± 0.7
fenugreek + amchur	98.8 ± 0.6	71.6 ± 1.4	60.2 ± 3.2
fenugreek + lime	99.9 ± 0.7	77.1 ± 0.5	58.3 ± 0.4
fenugreek + tamarind	96.8 ± 1.2	69.6 ± 1.6	60.9 ± 1.1
fenugreek + kokum	98.1 ± 0.7	67.5 ± 1.2	52.2 ± 0.6
fenugreek + turmeric	102.0 ± 1.0	75.5 ± 0.6	67.1 ± 0.4
fenugreek + onion	101.0 ± 2.1	77.2 ± 0.5	63.6 ± 1.0
amaranth	100.0 ± 0.8	69.4 ± 0.7	50.0 ± 0.4
amaranth + amchur	98.6 ± 0.6	70.2 ± 1.9	46.2 ± 2.6
amaranth + lime	98.6 ± 0.7	76.5 ± 0.7	70.2 ± 2.1
amaranth + tamarind	98.1 ± 0.4	67.5 ± 0.7	67.9 ± 0.8
amaranth + kokum	96.1 ± 0.7	70.3 ± 1.1	44.0 ± 3.2
amaranth + turmeric	98.6 ± 0.7	73.2 ± 0.7	53.1 ± 2.0
amaranth + onion	99.2 ± 0.8	73.9 ± 1.8	59.3 ± 2.6

[a] Values (given as percent) are average of pentuplicates. Values are given as percent relative to β-carotene content of raw vegetables alone taken as 100%.

The antioxidant spice turmeric had a remarkable protective effect on the loss of β-carotene during heat processing of the test vegetables, the effect being more prominent in the case of yellow-orange vegetables (**Table 8**). Onion, too, offered a similar protection during the heat processing of the four test vegetables, the effect being more prominent in the case of carrot.

DISCUSSION

The present investigation has documented the beneficial bioaccessibility enhancing effect of two common food acidulants—amchur and lime juice—in both raw and cooked vegetables. The beneficial effect of antioxidant spices was observed only during heat processing of these vegetables. We have earlier observed that acidulants (tamarind and citric acid) and antioxidant spices (turmeric and onion) prevented the loss of β-carotene during heat processing of vegetables (*5*). In the present study, the acidulant lime juice, and the antioxidant spices turmeric and onion improved the retention of β-carotene in vegetables during heat processing.

Among the four food acidulants examined, lime juice and amchur enhanced the bioaccessibility of β-carotene from the test vegetables (both yellow-orange and green leafy), whereas tamarind and kokum did not have a similar effect. The enhancing effect of lime juice on the bioaccessibility of β-carotene seems to be higher than that of amchur. These findings on the effect of food acidulants on the bioaccessibility of β-carotene are somewhat in agreement with our earlier observation on their effect on mineral bioaccessibility, where citric acid and amchur generally enhanced the bioaccessibility of zinc and iron, but tamarind and kokum did not have a similar effect (*7*). Whether the lack of an enhancing effect of tamarind and kokum on β-carotene bioaccessibility despite a decrease in the pH similar to amchur or lime and their negative effect in a few instances could be attributable to the high tannin content present in these two acidulants needs to be verified. These two acidulants contain significant amounts of tannin, as we observed earlier (*7*). Thus, food acidulants appear to have an influence on β-carotene bioaccessibility from vegetables very similar to their influence on bioaccessibility of iron and zinc from staple grains. The food acidulants amchur and lime probably exert a favorable influence on β-carotene bioaccessiblity through a loosening of the matrix, thereby rendering β-carotene more bioaccessible.

In a previous study, it was indicated that the bioaccessibility of β-carotene from four specific mango varieties roughly corresponded with the organic acid content of these fruits; that is, the variety with highest organic content also showed the highest β-carotene bioaccessiblity and vice versa (*13*). Organic acids are not the only modifiers of β-carotene bioaccessibility; other factors, especially fiber and carotenoids other than β-carotene (which have not been determined here), may also influence the same. Although it is known that acids promote the isomerization of *all-trans-β*-carotene to cis isomers, which are less absorbed than the all-trans isomer, it is to be noted that the food acidulants exogenously added here did not make the system drastically acidic (lowered the pH by just 1 unit) so as to cause significant extent of isomerization of β-carotene.

Among the two antioxidant spices, the effect of turmeric appeared to be higher in the case of vegetables subjected to open-pan boiling. These antioxidant spices have contributed to higher retention of β-carotene (minimizing the loss due to heat/exposure to air) and hence its bioaccessibility. This is consistent with the observation that the effect of these spices on the bioaccessibility of β-carotene was not apparent in the case of raw vegetables. In the case of antioxidant spices, the improved bioaccessibility of β-carotene from heat-processed vegetables is attributable to their role in minimizing the loss of this provitamin during heat treatment. The food acidulant lime juice, which enhanced the bioaccessibility of this provitamin from both raw and heat-processed vegetables, probably exerted this effect by some other mechanism in addition to minimizing the loss of β-carotene.

Heat processing of the test vegetables by pressure cooking or open pan boiling generally improved the bioaccessibility of β-carotene (**Figure 1**), which is consistent with our earlier observation with pressure-cooked or stir-fried vegetables (*10*). In the present study, the bioaccessibility-enhancing effect of pressure cooking was greater than that of open pan boiling in the case of the two green leafy vegetables, whereas both methods of heat treatment had a similar effect on the yellow-orange vegetables. The bioaccessibility-enhancing effect of either the food acidulants or antioxidant spices discussed above is over and above that brought about by heat processing of the vegetables.

The in vitro method employed here for the estimation of β-carotene availability is based on simulation of gastrointestinal digestion and estimation of the proportion of the nutrient convertible to an absorbable form in the digestive tract, by measuring the fraction that gets into the micellar portion. This method has been well standardized and internationally accepted. The bioaccessibility of β-carotene gives a fair estimate of its availability for absorption in vivo (bioavailability). Such in vitro methods are rapid, simple, and inexpensive. The in vitro method that measures bioaccessibility provides relative rather than absolute estimates of β-carotene absorbability because they are not subjected to the physiological factors that can affect bioavailability.

Reports on the effect of acidulants and antioxidant spices on the bioaccessibility of β-carotene from vegetable sources are lacking, and, to our knowledge, this is the first report of its kind. These food ingredients, especially antioxidant spices, have the double advantage of minimizing loss of β-carotene during heat processing as well as enhancing the bioaccessibility of the thus-retained provitamin from vegetables. The amounts of food acidulants and antioxidant spices observed to bring about such a beneficial influence on β-carotene bioaccessibility are those that are normally encountered in traditional food practices in India. It is a common practice to include turmeric before heat processing of dishes containing vegetables. Onion is also used in such dishes in a majority of households. The common use of onion as a vegetable in many dishes along with green leafy and yellow-orange vegetables probably surpasses the amount evidenced here to produce a beneficial effect on bioaccessibility of β-carotene; therefore, the magnitude of this effect may actually be even higher. Thus, probably a majority of the Indian population is already practicing a food-based strategy to maximize β-carotene bioavailability. Populations in the northern parts of India commonly use amchur as a food acidulant in preference to tamarind or kokum, which are commonly used in the southern parts of India. Lime juice is used in dishes throughout the subcontinent. Thus, the liberal presence of food acidulants such as lime juice and amchur and antioxidant spices—turmeric and onion—proves to be advantageous in the context of deriving maximum β-carotene from the vegetable sources.

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Received for review May 3, 2008. Revised manuscript received July 21, 2008. Accepted July 23, 2008. S.V. thanks the University Grants Commission, New Delhi, for the award of a Senior Research Fellowship.

JF801374D