

# Method for the Rapid Headspace Analysis of Mango (*Mangifera indica* L.) Homogenate Volatile Constituents and Factors Affecting Quantitative Results

Therese M. M. Malundo,<sup>†</sup> Elizabeth A. Baldwin,<sup>\*,‡</sup> Manuel G. Moshonas,<sup>‡</sup> Robert A. Baker,<sup>‡</sup> and Robert L. Shewfelt<sup>§</sup>

United States Distilled Products, 1607 South 12th Street, Princeton, Minnesota 55371, U.S. Citrus and Subtropical Products Laboratory, South Atlantic Area, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 1909, Winter Haven, Florida 33881, and Center for Food Safety and Quality Enhancement, University of Georgia Experiment Station, Griffin, Georgia 30223-1797

A rapid GC method was developed to analyze the headspace composition of cultivar Kent, Keitt, and Tommy Atkins mango homogenates. Factors affecting quantitative results were also studied. Of the 13 volatile compounds identified, 9 were terpene hydrocarbons:  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, myrcene, limonene, *p*-cymene, terpinolene,  $\alpha$ -copaene, and caryophyllene. Volatile concentrations were quantified using peak height calibration curves (peak height vs concentration). Linear relationships were derived for all compounds except caryophyllene. Rate of peak height increase was slower at caryophyllene levels <2 ppm than at those  $\geq$ 2 ppm. Volatile levels increased as homogenate was diluted with up to 50% water. Addition of CaCl<sub>2</sub> during blending, to inhibit degradative enzymes, induced gelation, which resulted in decreased volatile concentrations in the headspace. Sonicating gelled homogenate resulted in partial liquefaction and an increase in headspace volatiles. These results indicate that significant interactions between mango pulp and volatile compounds occur and, depending on sample preparation method, can affect quantitative results.

**Keywords:** Flavor; gelation; hydrocarbons; terpenes

## INTRODUCTION

Mango (*Mangifera indica* L.) and kiwifruit (*Actinidia chinensis* Planch.) are the two most favored specialty fruits in the United States (Anonymous, 1994). With world production totaling 15 million tons in 1989, mango ranks second to banana (*Musa* sp.) in terms of popularity among all tropical fruits cultivated commercially for fresh produce consumption worldwide (Koulibaly *et al.*, 1992).

Mango flavor is critical to consumer acceptability and is an important consideration when the price of the product is determined (Gholap *et al.*, 1986). Mango varieties differ in the amount and type of volatile compounds present, often depending on location. Generally, Old World (Asian) mangoes have more oxygenated volatile compounds such as esters, furanones, and lactones, giving some varieties pineapple- or peach-like aromas, while New World mangoes that are hybrids of Old World stock have higher levels of certain hydrocarbons such as 3-carene (Narain *et al.*, 1997; Wilson *et al.*, 1986; MacLeod and de Troconis, 1982). Several researchers have attempted to identify and quantify volatile compounds contributing significantly to mango flavor. Earlier studies employed the classical flavor isolation procedures of steam distillation and/or solvent extraction (MacLeod and de Troconis, 1982; Engel and Tressl, 1983; MacLeod and Pieris, 1984; MacLeod and Snyder, 1985; Idstein and Schreier, 1985; Gholap *et al.*,

1986), which qualitatively and quantitatively modify the flavor profile of a sample (Schamps and Dirinck, 1982; Ackerman and Torline, 1984; Sakho *et al.*, 1985; Bartley and Schwede, 1987). Furthermore, this technique is not easily applied to large numbers of fruit samples, but volatile compounds present in low concentrations can often be identified by concentrating the sample by vacuum distillation. More recently, investigators have employed purge and trap headspace sampling methods, which involve trapping volatile components over a period of time on a solid support. The trap is later heated so that the volatile compounds can be injected into a GC column (Ackerman and Torline, 1984; Sakho *et al.*, 1985; Bartley and Schwede, 1987; Koulibaly *et al.*, 1992). Bartley and Schwede (1987) suggested that a chromatogram from a headspace sampling procedure will more closely reflect the true flavor profile of a mango sample than one generated by distillation and solvent extraction; however, compounds present at low levels may not be detected using headspace techniques. The purge and trap method for headspace analysis is unsuitable for liquids containing high levels of soluble solids as these may be carried over into the trapping system, where they slowly decompose and affect subsequent GC chromatograms (Moshonas and Shaw, 1992). Therefore, actual volatile concentrations in mango are difficult to quantify using this technique. Ackerman and Torline (1984) considered the results of their purge and trap procedure as qualitative, while Sakho *et al.* (1985) and Koulibaly *et al.* (1992) were able to express volatile concentrations only in terms of percent relative abundance.

There is a need for a quantitative headspace method that involves minimal sample manipulation and time expenditure by the operator. Twenty or more volatile

\* Author to whom correspondence should be addressed [telephone (941) 293-4133; fax (941) 299-8678; e-mail ebaldwin@asrrarsusda.gov].

<sup>†</sup> United States Distilled Products.

<sup>‡</sup> U.S. Citrus and Subtropical Products Laboratory.

<sup>§</sup> Center for Food Safety and Quality Enhancement.

constituents in samples of fresh and processed orange juice were quantified using static headspace GC (Nisperos-Carriedo and Shaw, 1990; Lizotte and Shaw, 1992). A similar technique was also used to quantify volatiles in tomato homogenate (Baldwin *et al.*, 1991a–c). This study was conducted to (1) establish a headspace gas chromatography technique for the rapid identification and quantitative analysis of important volatile compounds in mango and (2) determine how specific factors in sample preparation influence the quantitative determination of volatile mango constituents.

## MATERIALS AND METHODS

**Fruits.** Kent, Keitt, and Tommy Atkins mangoes (*M. indica* L.) were purchased from a commercial mango distributor based in Homestead, FL, on the basis of their availability over the season in tree-ripe condition. Excess fruits were bought, and only fully ripe mangoes, in good condition, and harvested at the morphologically mature stage as described by Medicott *et al.* (1988) were used.

**Identification of Volatile Compounds.** Homogenates of Kent, Keitt, and Tommy Atkins were obtained by blending 300 g of fruit using a Waring blender (Waring Products Division, Dynamics Corp. of America, New Hartford, CT). For each cultivar blended, 2 mL of homogenate was transferred into 6-mL vials. The vials were immediately sealed using crimp-top caps with TFE/silicone septa seals, fast frozen by immersion in liquid nitrogen ( $N_2$ ), and stored at  $-20^\circ C$  until use. Preliminary studies indicated that when mango homogenate was stored in closed containers, headspace composition did not significantly change qualitatively or quantitatively even when held at room temperature for up to 2 h.

Volatile compounds were identified and later quantified by adapting headspace analysis procedures developed previously for citrus (Nisperos-Carriedo and Shaw, 1990) and modified for tomatoes (Baldwin *et al.*, 1991a–c). Analysis was conducted using a Perkin-Elmer Model 8500 gas chromatograph equipped with a Model HS-6 headspace sampler and an FID detector. A 0.53 mm  $\times$  30 m polar Durowax column (1.0  $\mu$ m film thickness; J&W Scientific, Folsom, CA) was used with a 6.0-psi helium head pressure (81 cm/s linear gas velocity). Frozen mango samples were quickly thawed under running tap water and then equilibrated for 15 min at  $80^\circ C$  in the headspace sampler before injection. Injection parameters were a vial pressurization time of 0.5 min and a 0.2-min injection time. Column oven temperature was programmed to initially remain at  $40^\circ C$  for 6 min and then increased to a final temperature of  $180^\circ C$  at a rate of  $6^\circ C/min$ . The FID detector temperature was  $250^\circ C$ , while the amplifier range was set for high sensitivity. Volatile compounds were identified by comparison of retention times with those of standards and by enrichment of "bland" diluted mango homogenate with authentic standards.

Compound identities were also analyzed by gas chromatography/mass spectrometry (GC/MS). Mango homogenate (600 mL) was diluted with 600 mL of deionized water and then centrifuged at 7000 rpm for 15 min. Organic compounds were extracted from the supernatant using GC/MS grade methylene chloride, and the presence of volatile compounds was examined using a Hewlett-Packard Model 5970B, MSD, GC/MS fitted with a 50-m wide bore (0.31–0.32 mm) fused silica column of cross-linked 5% phenylmethyl silicone (1- $\mu$ m film thickness, Hewlett-Packard, Palo Alto, CA). Column oven temperature was programmed to initially remain at  $55^\circ C$  for 9 min, slowly rise at a rate of  $7.5^\circ C/min$ , and maintain a final temperature of  $220^\circ C$  for 30 min. Injector port and ionizing source were maintained at  $275^\circ C$ , and the transfer line was held at  $280^\circ C$ . Mass units were monitored from 25 to 350 at 70 eV. Mass spectral matches were made by comparison of mass spectra and retention time with those of authentic compounds.

**Diluting Mango Homogenate.** In preliminary studies, it was noted that relatively low levels of volatiles were obtained from the headspace of undiluted mango homogenate, which

was very viscous in nature. Therefore, mango pulp (150 g) from a single Kent fruit was homogenized and blended with deionized water, added at levels of either 10, 30, or 50% (45, 35, or 25 mL of homogenate, respectively) to a final volume of 50 mL. Diluted samples (2 mL) were transferred to 6-mL vials, immediately sealed with crimp-top caps, and analyzed for headspace volatile composition. Samples that could not be immediately analyzed were frozen by immersing the vials in liquid  $N_2$  and storing them at  $-20^\circ C$  until needed. Frozen samples were quickly thawed under running tap water before analysis. Two replications were conducted.

**Quantifying Volatile Compounds.** Concentrations of volatile compounds were calculated using regression equations fitted to peak height calibration curves. The calibration curve for a volatile compound was plotted after determination of the peak heights obtained when mango homogenate standards with known volatile concentrations were analyzed for headspace composition. Peak heights resulting from at least five different concentrations were determined. Standard concentrations (parts per million) covered the following ranges: 3-carene, 14.0–140.0; caryophyllene, 0.3–6.0;  $\alpha$ -copaene, 0.12–1.20; *p*-cymene, 0.04–0.40; limonene, 0.15–6.0; myrcene, 0.5–20.0;  $\alpha$ -pinene, 1.5–60.0;  $\beta$ -pinene, 0.1–4.0; terpinolene, 0.32–13.0.

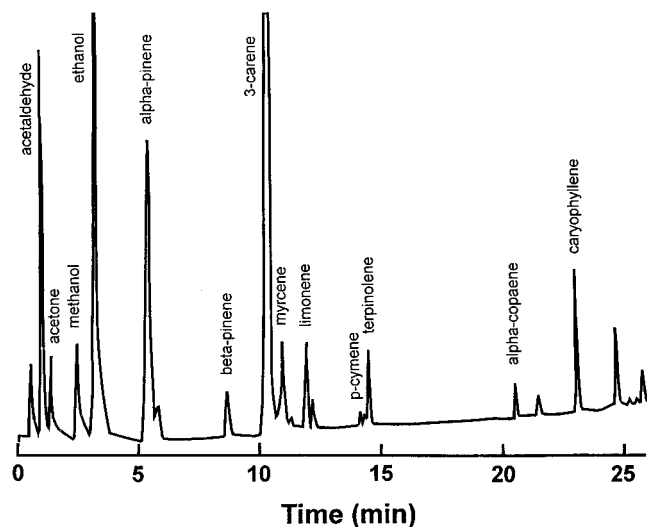
The bland homogenate used for standards was a composite of Kent, Keitt, and Tommy Atkins fruits prepared by blending a total of 600 g of mango (200 g from each cultivar) and diluting with 600 mL of deionized water. The volatile fraction of the blend was volatilized out by evaporation using a Rotavap (Glasapparatefabrik, Buchi, Switzerland) with a water bath temperature set at  $45 \pm 2^\circ C$ . The concentrated mango juice was then reconstituted with deionized water to its original total soluble solids content of 14.5 °Brix, resulting in minimal background (volatile) noise when run on the GC. Authentic volatile compounds were then added back in measured amounts to create the standard curves for GC analysis. This method allows for interaction of volatile standards with fruit pulp tissue in a manner comparable to the fruit homogenate samples for which flavor compounds were to be quantified.

**Gelation of Mango Homogenate.** To study the interaction of fruit volatiles with fruit pulp and the effect of gelation, Tommy Atkins mango flesh (3 kg) from 12 individual fruits were blended in 600-g batches and combined to form a composite sample. The homogenate was then diluted with deionized water or saturated  $CaCl_2$  solutions at levels of either 10, 30, or 50% (180, 140, or 100 mL of homogenate, respectively) in a final volume of 200 mL. Two milliliters or 40 g of the resulting mixture was transferred to a 6-mL vial or a Petri plate, respectively. Vials were sealed using crimp-top caps with TFE/silicone septa seals, and Petri plates were immediately covered. Vials and plates were then cooled using one of three methods: refrigeration, kept at  $10^\circ C$  for 10 h; slow freezing, frozen at  $-20^\circ C$  for 10 h; and fast freezing, frozen by immersion in liquid  $N_2$  and stored at  $-20^\circ C$  until use.

Samples in Petri plates were thawed by leaving them at room temperature for at least 3 h. A thawed sample was then poured over a 1.65-mm mesh sieve covering a circular area with a 7-mm diameter. The extent of gel formation was estimated using a gelation index, which was the total weight (grams) of sample that passed through the sieve 60 s after pouring. Generally, the lower the gelation index, the greater the extent of gelation.

Respective samples in vials were quickly thawed under running tap water. Headspace of the thawed samples was analyzed and volatile concentrations were calculated using methods described above. Three replications of the experiment were performed, and three measurements were taken for each replication. All samples were analyzed within 2 h after thawing.

**Gel Liquefaction.** Pulp (400 g) from four fruits each of Keitt and Tommy Atkins was homogenized. For each cultivar, 70 mL of homogenate was diluted with 30 mL of deionized water or saturated  $CaCl_2$ . Two-milliliter portions of the resulting mixtures were then transferred to 6-mL vials, which were immediately sealed with crimp-top caps, fast frozen by



**Figure 1.** Representative capillary GC chromatogram from the headspace of Tommy Atkins mango.

immersion in liquid  $N_2$ , and stored at  $-20\text{ }^\circ\text{C}$  until use. Some of these samples were later thawed and analyzed for initial headspace composition.

Other frozen samples were quickly thawed under running tap water and mixed with  $20\ \mu\text{L}$  of deionized water or 5% ethylenediaminetetraacetic acid (EDTA; Sigma Chemical Co., St. Louis, MO), injected through the septum with a microsyringe, and sonicated for 30 min. After sonication, the headspace of the sample was analyzed for volatile composition.

**Statistical Analysis.** The data were analyzed using the Statistical Analysis System (SAS, 1988). Analysis of variance (PROC ANOVA) was used to determine the effect of dilution on peak heights generated by volatile compounds from mango homogenate diluted with various levels of water. The regression procedure (PROC REG), which restricted the intercept to 0, was used to fit regression equations to peak height calibration curves. A *t*-test was conducted to check whether the predictive capability of a restricted equation was significantly different from that of the best-fit regression model. ANOVA through the General Linear Model procedure (PROC GLM) was conducted to determine the effects of diluent ( $\text{CaCl}_2$  or water), diluent concentration, and cooling method on gelation index. Correlation analysis (PROC CORR) was used to study the relationships between gelation index and concentration of volatiles quantified. Results of correlation analysis were validated by conducting *t*-tests between volatiles quantified from mango blends with 30% water and 30%  $\text{CaCl}_2$ . Finally, ANOVA and means separation test (Duncan's multiple range test) were performed to determine whether quantitative headspace analyses of mango gel and one treated with additional water or EDTA and sonication were significantly different from each other.

## RESULTS

A representative capillary GC chromatogram from the homogenate headspace of the number one Florida mango variety, Tommy Atkins, is shown in Figure 1. A total of 13 volatile compounds were identified, 9 of which were either mono- or sesquiterpene hydrocarbons. Results from GC headspace analyses of two other popular Florida mango varieties, Kent and Keitt, were qualitatively similar to that illustrated in Figure 1 except that no traces of  $\alpha$ -copaene could be found in Keitt and  $\beta$ -pinene and *p*-cymene were absent from both cultivars. Generally, most volatile levels were lower in Kent mango samples compared to the other two cultivars. Throughout the whole study, 3-carene was quantitatively the major terpene for all three cultivars. Terpenes are considered to be important volatile compounds,

**Table 1.** Effect of Dilution on Headspace Analysis of Kent Homogenate

volatile compound	water level (%)	peak height (ppm)	volatile compound	water level (%)	peak height (ppm)
$\alpha$ -pinene	10	25	terpinolene	10	196
	30	125		30	1395
	50	136 (0.1) <sup>a</sup>		50	1383 (0.3)
3-carene	10	3997	$\alpha$ -copaene	10	NS <sup>b</sup>
	30	18889		30	NS
	50	19454 (10.4)		50	NS
myrcene	10	239	caryophyllene	10	152
	30	1225		30	272
	50	1341 (1.4)		50	792 (1.4)
limonene	10	141			
	30	733			
	50	736 (0.2)			

<sup>a</sup> Values in parentheses are the peak height converted to ppm using standard curves. <sup>b</sup> Not significant ( $\text{Pr} < 0.05$ ).

qualitatively and quantitatively contributing to mango flavor (MacLeod and de Troconis, 1982; Engel and Tressl, 1983; MacLeod and Pieris, 1984; Idstein and Schreier, 1985; MacLeod and Snyder, 1985; Sakho *et al.*, 1985; Bartley and Schwede, 1987; Koulibaly *et al.*, 1992), especially in the case of Florida mangoes. The important Florida variety, Tommy Atkins, for example, has reportedly little or no detectable levels of ester, aldehyde, or ketone flavor compounds reported for Old World mango varieties (Wilson *et al.*, 1990; MacLeod and Snyder, 1988, 1985). Using GC/MS, 98 compounds were tentatively identified with only trace or very low levels of oxygenated compounds in the concentrated composite blend of Tommy Atkins, Keitt, and Kent mangoes. No new compounds were detected that have not already appeared in the literature. Therefore, subsequent quantitative headspace analyses were concentrated only on the hydrocarbon fraction of the headspace volatiles identified.

The effect of homogenate dilution on the peak heights generated by headspace terpene compounds from Kent homogenate is shown in Table 1. Peak heights, not peak areas, were used throughout the study to indicate and calculate volatile concentrations. Generally, quantitative errors due to overlapping peaks were minimized when peak heights are used instead of peak areas (Anonymous, 1988). In addition, Moshonas and Shaw (1992) found that when concentrations of volatiles in orange juice were calculated using peak height and peak area, values obtained from both were comparable with each other but had less variability when using heights instead of areas.

ANOVA indicated that homogenate dilution had a significant effect on the peak height from headspace terpene compounds in Kent except for  $\alpha$ -copaene. Table 1 shows that diluting homogenate with increasing amounts of water generally elevated peak heights, indicating an increase in the amount of volatile released in the headspace. Since volatile levels were much lower in the undiluted homogenate, some of the smaller peaks were difficult to quantify. For this reason, standard curves were prepared in devolatilized mango homogenate diluted 50% with water. Flavor volatile concentrations for Kent mango homogenate diluted 50% with water were calculated from these standard curves and are shown in Table 1. These values are within the range found in the literature for various mango cultivars (MacLeod and de Troconis, 1982; MacLeod and Pieris, 1984; MacLeod and Snyder, 1985; Koulibaly *et al.*, 1992), but are lower in  $\alpha$ -pinene, 3-carene, limonene,

**Table 2. Results of *t*-Tests Comparing the Effects on Quantitative Headspace Analysis of Water and CaCl<sub>2</sub>, Used at 30% Levels, To Dilute Tommy Atkins and Keitt Homogenate**

volatile compound	volatile concn (ppm)					
	Tommy Atkins			Keitt		
	water	CaCl <sub>2</sub>	Pr <   <i>t</i>	water	CaCl <sub>2</sub>	Pr <   <i>t</i>
α-pinene	2.84	0.92	0.0010	0.11	0.04	0.0053
β-pinene	0.33	0.07	0.0002	— <sup>a</sup>	—	—
3-carene	25.34	6.51	0.0009	14.83	3.24	0
myrcene	2.43	0.76	0.0119	1.51	0.44	0.0021
limonene	0.44	0.13	0.0044	0.21	0.05	0.0002
<i>p</i> -cymene	0.07	0.02	0.0728	—	—	—
terpinolene	0.58	0.12	0.0048	0.30	0.08	0.0015
α-copaene	0.44	0.05	0.0137	—	—	—
caryophyllene	0.73	0.12	0.0301	1.10	0.35	0.0377

<sup>a</sup> Not detected.

and terpinolene than values reported for ripe Tommy Atkins or Keitt mango cultivars and higher in myrcene and caryophyllene than reported for Tommy Atkins (MacLeod and Snyder, 1985). In this study, water-diluted mango homogenates for Kent exhibited volatile levels that were also lower compared to those observed for Tommy Atkins, except for caryophyllene, while only 3-carene was lower than values obtained for Keitt (Tables 1 and 2). These homogenates were representative of only one and four fruits for Kent (Table 1) and Tommy Atkins and Keitt (Table 2), respectively, and thus comparison between these three cultivars may not account for possible fruit to fruit variation; however, other studies either do not disclose how many individual fruit were used per sample (Koulibaly *et al.*, 1992; Bartley and Schwede, 1987; Ackerman and Torline, 1984; MacLeod and Pieris, 1984; MacLeod and de Troconis, 1982) or used only two or three mangoes (Wilson *et al.*, 1990; MacLeod and Snyder, 1985). Although these homogenates resulted in chromatograms that were typical of each variety, a larger fruit sampling would be desirable to complement the above studies. The effect of volatile concentration on peak height was observed during the preparation of the peak height calibration curves. A typical calibration curve was easily described by a linear equation with *y*-intercept equal to 0. As indicated by *t*-test, restricting the intercept to 0 did not significantly change the predictive capability of the equation generated through regression analysis. For example, an increase in the concentration of *p*-cymene resulted in a linear increase in the peak height generated ( $R^2 = 0.99$ ). Similar trends were observed for 3-carene, α-copaene, limonene, myrcene, α-pinene, β-pinene, and terpinolene. However, the calibration curve for caryophyllene did not follow a simple linear relationship. The curve was described by a second-order polynomial with a *y*-intercept also equal to 0. Again, a *t*-test indicated that restricting the intercept to 0 did not significantly change the predictive capability of the polynomial. While peak height increased with additional amounts of caryophyllene, the rate of increase was slower when caryophyllene concentration was <2 ppm than when it was ≥2 ppm ( $R^2 = 0.89$ ).

Addition of CaCl<sub>2</sub> to mango homogenate, for insurance against enzymatic changes in the volatile profile (Buttery *et al.*, 1987), with subsequent treatment with liquid N<sub>2</sub> resulted in gelation of the sample, which affected volatile composition in the headspace. Although subsequent studies indicated that the mango homogenate volatile profile did not significantly change over a 2-h

**Table 3. Effect of Diluent and Cooling Method on Gelation Index of Tommy Atkins Homogenate**

diluent	diluent level (%)	cooling method <sup>a</sup>	gelation index <sup>b</sup> (g)
water	10	refrigeration	1.5
		slow freezing	7.4
		fast freezing	2.7
	30	refrigeration	15.0
		slow freezing	23.0
		fast freezing	18.9
	50	refrigeration	31.2
		slow freezing	31.4
		fast freezing	31.2
CaCl <sub>2</sub>	10	refrigeration	1.9
		slow freezing	6.3
		fast freezing	0
	30	refrigeration	15.4
		slow freezing	9.6
		fast freezing	0
	50	refrigeration	21.7
		slow freezing	10.7
		fast freezing	0

<sup>a</sup> Cooling methods were as follows: refrigeration, kept at 10 °C for 10 h; slow freezing, frozen at -20 °C for 10 h; fast frozen, immersed in liquid N<sub>2</sub> until frozen and then stored at -20 °C until analysis. <sup>b</sup> Gelation index was weight (g) of homogenate that passed through a 1.65 mm mesh sieve, covering a circular area with a 7-mm diameter, 60 s after pouring.

period, thus negating the need for CaCl<sub>2</sub>, we decided to investigate the effect of homogenate gelation on volatile release to headspace due to possible relevance for various processed mango products (Lizada, 1993) such as mango juice, jelly, chutney, and puree when the mango matrix is altered.

Different cooling methods in combination with diluents were used in an attempt to obtain a range of gelation in mango homogenate, which is presented as a gelation index. When ANOVA was conducted to study the effects of using different cooling methods (refrigeration, slow freezing, and fast freezing) and diluents (CaCl<sub>2</sub> and H<sub>2</sub>O) at various levels (10, 30, and 50%) on the gelation index of Tommy Atkins homogenate, the results indicated that all main effects, two-way interactions, and three-way interactions were significant at  $P < 0.0001$ . Table 3 shows that gelation index increased (or gel strength decreased) as H<sub>2</sub>O levels increased for all cooling methods employed. A similar trend was observed for homogenates diluted with CaCl<sub>2</sub> when refrigeration and slow freezing were used. However, when CaCl<sub>2</sub>-treated homogenate was fast-frozen, the gelation index was 0 regardless of the amount of diluent added. Thus, the extent of gelation was greatest when homogenate diluted with 50% CaCl<sub>2</sub> was fast-frozen with liquid N<sub>2</sub>.

Results of correlation analysis between gelation index and the concentration of the volatile compounds in the headspace of Tommy Atkins homogenate were not significant for β-pinene, 3-carene, and *p*-cymene. However, α-copaene negatively correlated with gelation index at  $P < 0.10$ , while correlations between gelation index and α-pinene, myrcene, limonene, terpinolene, and caryophyllene were negatively significant at  $P < 0.05$ . These results imply that gelation decreased the amount of volatiles released in the headspace.

This was further investigated using Tommy Atkins and Keitt, from which homogenates were diluted 30% with water or saturated CaCl<sub>2</sub> and fast-frozen with liquid N<sub>2</sub>. Statistical analysis using *t*-tests showed that diluting the homogenate with CaCl<sub>2</sub> instead of water significantly decreased the concentrations of volatiles

**Table 4. Effect of the Addition of 20  $\mu$ L of Deionized Water or EDTA and Sonication on Headspace Analysis of Tommy Atkins and Keitt Homogenate Gelled with  $\text{CaCl}_2$** 

cultivar	volatile compound	volatile concn <sup>a</sup> (ppm)		
		unsonicated	sonicated	
			water	EDTA
Tommy Atkins	$\alpha$ -pinene	0.92b	2.19a	1.66a
	$\beta$ -pinene	0.07b	0.08ab	0.17a
	3-carene	6.51b	11.18a	10.92a
	myrcene	NS <sup>b</sup>	NS	NS
	limonene	NS	NS	NS
	<i>p</i> -cymene	0.02b	0.11a	0.04ab
	terpinolene	NS	NS	NS
	$\alpha$ -copaene	NS	NS	NS
	caryophyllene	NS	NS	NS
Keitt	$\alpha$ -pinene	NS	NS	NS
	3-carene	3.24b	4.78ab	6.14a
	myrcene	NS	NS	NS
	limonene	0.05b	0.06ab	0.08a
	terpinolene	NS	NS	NS
	caryophyllene	NS	NS	NS

<sup>a</sup> Means within a row not followed by the same letter are not significantly different from each other ( $P < 0.10$ ); NS = not significant ( $P < 0.10$ ).

quantified through headspace analysis (Table 2). Since, as indicated previously, gelation would be more extensive in  $\text{CaCl}_2$ -treated homogenate, results in Table 2 validate the conclusions drawn from the correlations of gelation index to volatile concentration described above.

Values for the volatile components measured in Tommy Atkins and Keitt mango diluted homogenate (Table 2) were within ranges reported for other cultivars (MacLeod and de Troconis, 1982; MacLeod and Pieris, 1984; MacLeod and Snyder, 1985). However, levels for  $\alpha$ -pinene, 3-carene, limonene, and terpinolene were somewhat lower than those reported for Tommy Atkins and Keitt extracts (MacLeod and Snyder, 1985), which were in the range of 0.7–16.6, 41–45, 1.3–1.3, and 1.9–1.1 ppm for Keitt and Tommy Atkins, respectively, based on an undisclosed number of ripe fruit.

When the 30%  $\text{CaCl}_2$  homogenate gels were treated with 20  $\mu$ L of deionized water or 5% EDTA and sonicated, partial liquefaction of the gels was observed. Table 4 shows that sonication with water or EDTA significantly increased concentrations of  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, and *p*-cymene quantified for Tommy Atkins and of 3-carene and limonene for Keitt ( $P < 0.10$ ). However, adding EDTA instead of water before sonication did not significantly influence results of headspace analysis.

## DISCUSSION

As with studies on fresh tomato volatiles (Buttery *et al.*, 1987; Baldwin *et al.*, 1991a–c; Buttery, 1993), use of mango homogenate is recommended for headspace flavor studies since it more closely represents masticated fruit pulp experienced when a mango is eaten than whole or cut fruit tissue. When food is eaten, the masticated tissue is also diluted by aqueous mouth saliva, which may aid in the release of volatiles from the fruit pulp as was observed in this study when mango homogenate was diluted with water (Table 1).

Thirteen volatile compounds were concerned in this investigation. Although this number was relatively small when compared to those determined by solvent extraction techniques (Hunter *et al.*, 1974; MacLeod and de Troconis, 1982; Engel and Tressl, 1983; MacLeod and Pieris, 1984; Idstein and Schreier, 1985; MacLeod and

Snyder, 1985; Sakho *et al.*, 1985; Adedeji *et al.*, 1992), identification of all possible flavor compounds in mango was not the aim of this work. Nevertheless, the compounds enumerated using the headspace method described appear to be the most critical to mango aroma and overall flavor in general and especially in the case of Florida mangoes on the basis of previous studies (Bartley and Schwede, 1987; MacLeod and Snyder, 1985; Koulibaly *et al.*, 1992). Although important aroma contribution in other fruits and some Old World mango varieties is usually attributed to compounds containing oxygen, sulfur, or nitrogen, these compounds are not present in detectable levels in the number one U.S. variety, Tommy Atkins, and are found in trace or low amounts in Keitt (MacLeod and Snyder, 1985). Other investigators determined that concentrations of mono- and sesquiterpene hydrocarbons in mango can equal or even exceed 90% (w/w) of total volatile concentration (Idstein and Schreier, 1985; MacLeod and Snyder, 1985; Koulibaly *et al.*, 1992). Levels of the headspace volatile components studied were comparable to other reports in the literature, but were lower in some components for Tommy Atkins and Keitt varieties than values reported by MacLeod and de Troconis (1985) for extracts of these cultivars. In tomato, volatile levels have been shown to vary significantly within the same variety on the basis of harvest maturity, harvest date, and storage conditions (Buttery *et al.*, 1987; Baldwin *et al.*, 1991a, 1992; McDonald *et al.*, 1996), which may have been different in these two studies. In addition, headspace analysis is subject to the interaction of volatile components with the food matrix (in this case, fruit pulp diluted with water), which may result in levels of volatiles released to the headspace that are different than those obtained by solvent extraction/distillation. The method presented here may more closely represent the eating experience, in which volatile flavor components are released from chewed fruit tissue in the mouth.

Among the three cultivars analyzed, 3-carene was consistently the most abundant headspace volatile present. This compound was described as possessing an aroma typical of mango leaves or as mango-like (MacLeod and Snyder, 1985; MacLeod and de Troconis, 1982) and was previously found to be present in very high concentrations in cv. Parrot (MacLeod and Pieris, 1984), Tommy Atkins, Keitt (MacLeod and Snyder, 1985) and in Venezuelan cultivars (MacLeod and de Troconis, 1982). It has been observed that 3-carene was abundant and significant to mango flavor in New World cultivars (*i.e.*, hybrids developed from Old World stock) but contributed very little to flavor of Old World mangoes, examples of which are the Indian varieties (MacLeod and Pieris, 1984; MacLeod and Snyder, 1985). The sesquiterpene  $\alpha$ -copaene was also described as mango-like (MacLeod and Pieris, 1984), and the other monoterpenes are considered useful for citrus and other fruit and "green" flavors, which thus may contribute to the overall mango aroma profile (Narain *et al.*, 1997).

Regarding the other volatile compounds identified, MacLeod and de Troconis (1982) considered  $\alpha$ -pinene to be one of the most important volatile compounds in Venezuelan mango in terms of concentration and/or odor quality. This compound was found to be 28-fold higher in Tommy Atkins homogenate compared to Keitt or Kent in our study (Tables 1 and 2), which is in agreement with MacLeod and Snyder (1985), who reported levels of  $\alpha$ -pinene to be 24-fold higher in

Tommy Atkins samples compared to Keitt. Terpinolene was present in relatively large quantities in Parrot, Willard (MacLeod and Pieris, 1984), and Venezuelan mangoes (MacLeod and de Troconis, 1982), was the major terpene found in cv. Governor (Koulibaly *et al.*, 1992), and was one of the two most abundant volatiles in cv. Bowen (Bartley and Schwede, 1987). Myrcene was quantitatively one of the major compounds in cv. Alphonso, Baladi (Engel and Tressl, 1983), Peach, and Papaya (Koulibaly *et al.*, 1992) and, together with *cis*- $\beta$ -ocimene, was responsible for the typical green aroma in unripe mango (Gholap and Bandyopadhyay, 1977). Limonene was the most abundant volatile compound in Baladi (Engel and Tressl, 1983) and was considered by MacLeod and de Troconis (1982) to be important in the flavor of Venezuelan mango. Koulibaly *et al.* (1992) found caryophyllene to be the major terpene found in cv. Muskat and Amelie, while the aroma of  $\alpha$ -copaene had been described to be typical of mango (MacLeod and Pieris, 1984; MacLeod and Snyder, 1985).  $\beta$ -Pinene was characterized as having an odor reminiscent of polish or varnish (MacLeod and de Troconis, 1982; MacLeod and Pieris, 1984; MacLeod and Snyder, 1985) and may be significant to mango flavor contributing to turpentine notes commonly associated with selected cultivars (Young and Ledin, 1954).

Lactones and furanones, which give peach and pineapple notes to some Old World mango varieties, are not prevalent or sometimes even detectable in New World mango types such as Tommy Atkins or Venezuelan mango and thus are unlikely to contribute to their flavor (Wilson *et al.*, 1990; MacLeod and de Troconis, 1982). Differences between New World mangoes in aroma compounds and flavor are attributed to hydrocarbons such as 3-carene (MacLeod and Snyder, 1985).

The effect of dilution on the quantitative analysis of the volatile compounds was unexpected. Consistent with what has been previously experienced with tomatoes by the authors, one would normally expect that as the mango homogenate was diluted further, volatile concentrations would decrease since there was less fruit material present to generate the volatiles measured in the headspace. However, ripe mango fruit homogenate is much more viscous than ripe tomato homogenate, and the contradicting observations in this study suggest significant physical and/or chemical interactions between the mango pulp and flavor volatiles which were interrupted by additional water. Such interactions have been previously observed between the pulp and terpene hydrocarbons of orange juice (Hernandez *et al.*, 1992; Moshonas and Shaw, 1994; Shaw *et al.*, 1995) and mango puree volatiles (Wu *et al.*, 1993) where, for example, the volatiles remained associated with the pulp portion (retentate) during separation or ultrafiltration of the juice or serum. In a study on the effect of food thickeners on flavor, it was observed that both matrix viscosity and binding of volatiles with hydrocolloids affected release of flavor compounds including  $\alpha$ -pinene (Roberts *et al.*, 1996). In the case of mango homogenate, the viscosity was reduced by the addition of water.

The initial interest on the effect of CaCl<sub>2</sub> on headspace composition was due to results of earlier studies which showed that CaCl<sub>2</sub> reduced changes in volatile concentrations in tomato homogenate, attributed to the inhibiting effect of CaCl<sub>2</sub> on enzymes that catalyze the biogenesis of flavor volatiles (Buttery *et al.*, 1987; Baldwin *et al.*, 1991c; Buttery, 1993). For tomato and

peach, CaCl<sub>2</sub> did not induce gelation in the fresh fruit pulp, even after flash-freezing in liquid N<sub>2</sub>, and thus did not affect volatile concentration other than preventing enzymatic changes.

Gelation of mango homogenate with the addition of CaCl<sub>2</sub> was most likely due to cross-linking among pectinic and pectic acids by Ca<sup>2+</sup> (Whistler and Daniel, 1985). Since only fully ripe fruits were used in this study, the concentrations of these acids in the homogenate were expected to be relatively high as compared to those for protopectin and galacturonic acids. During ripening, pectin methylesterase (EC 3.1.1.11; PME) activity in mangoes increases (Selvaraj and Kumar, 1989; Lizada, 1993) while polygalacturonase (EC 3.2.1.15; PG) activity seems to be relatively low (Lazan *et al.*, 1986; Brinson *et al.*, 1988; Lizada, 1993). The enzyme PME catalyzes the hydrolysis of methyl esters from methylated pectic substances, making them more water-soluble and resulting in carboxyl groups that can bind Ca<sup>2+</sup>. The enzyme PG degrades deesterified pectin chains into smaller molecular weight polymers and component polysaccharides (Fisher and Bennett, 1991).

The extent of gelation was greater when fast freezing was used instead of slow freezing to cool mango homogenate diluted with CaCl<sub>2</sub>. Between the two cooling methods, fast freezing would have induced the formation of smaller ice crystals. The tendency to develop large ice crystals during slow freezing (Reid, 1983) probably interfered with molecular cross-linking among pectinic and pectic acids. The effect of refrigeration on the extent of gelation cannot be readily explained.

Gelation has been previously reported to affect flavor perception and headspace composition of fruit jams (Guichard *et al.*, 1991). It apparently provides a barrier for the escape of volatile compounds into the headspace of mango homogenate. Sonication facilitated the release of some, but not all, volatiles probably because sonication resulted in only partial gel liquefaction. In some gels, mechanical agitation will succeed in disturbing only weak forces of attraction among gel particles but not stronger bonds that are randomly distributed throughout the gel (Matz, 1962; Whistler and Daniel, 1985). Alteration of a food (or fruit) matrix can affect binding, entrapment, and release of flavor compounds (Roberts *et al.*, 1996), which has to be taken into account in quantitative and qualitative studies and in the case of processed fruit products.

#### CONCLUDING REMARKS

A rapid GC method, requiring minimal sample manipulation, for the quantitative analysis of mango flavor volatiles will be useful in flavor research or quality control procedures involving multiple samples or replications. All techniques for flavor volatile analysis have problems, and heating to 80 °C in this headspace technique could create artifacts, although all of the compounds detected have been reported for mango. Current extraction techniques are tedious and time-consuming. Steam distillation and solvent extraction procedures isolate several compounds that might not be necessarily important contributors to mango flavor and may also create artifacts. The technique described here does not require addition of internal standards for quantitative analyses as do techniques involving solvent extraction and steam distillation (MacLeod and de Troconis, 1982).

The GC technique used in this study was adapted from procedures developed previously for citrus (Nis-

peros-Carriedo and Shaw, 1990) and tomatoes (Baldwin *et al.*, 1991a–c). However, results of this investigation demonstrated that modification of the headspace method for citrus and tomatoes is necessary before use in the analysis of mango volatile composition. Because of possible physical or chemical bonding between fruit pulp and flavor compounds, dilution of mango homogenate is necessary for adequate release of volatiles into the headspace. Furthermore, while CaCl<sub>2</sub> is used to inhibit enzymes catalyzing changes in the flavor volatile profile in tomatoes (Buttery *et al.*, 1987; Baldwin *et al.*, 1991c; Buttery, 1993), addition of CaCl<sub>2</sub> to mango homogenate induced gelation, which decreased volatile concentration in the headspace. No enzymatic changes were observed in mango homogenate for up to 2 h at room temperature in any case. Finally, when immediate analysis is not possible, rapid freezing of diluted, homogenized mango samples in liquid N<sub>2</sub>, without the addition of CaCl<sub>2</sub>, and storage at –20 °C was sufficient insurance against possible enzymatic or degradative changes in volatile components and did not affect subsequent quantitative analysis.

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