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DEVELOPMENT OF ELECTRONIC NOSE MEASUREMENTS FOR MANGO (*MANGIFERA INDICA*) HOMOGENATE AND WHOLE FRUIT

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Abstract. **Mango fruit from Latin America (cv. Tommy Atkins), were purchased from a local Florida supermarket, homogenized, and sampled for volatile analysis by static headspace method. Some of the material was analyzed using an electronic nose (e-nose) with metal oxide coated or uncoated sensors (500 µL injection volume) and some by gas chromatography (GC) equipped with a polar Carbowax column and a flame ionization detector. Dilution of homogenate and homogenate volume were analyzed to determine effect on e-nose and GC headspace measurements. Mango homogenate (1.0, 1.5, and 2.0 mL) was diluted with DI water to 50, 25, and 12.5% of original concentration. The resulting e-nose signal intensities (changes in resistance across the metal oxide sensor due to non-selective interactions with volatile compounds in the headspace) were analyzed by discriminant factor analysis (DFA), which resulted in grouping by dilution factor, regardless of sample size. A combination of 2.0 mL and 25% dilution of mango homogenate was determined to be optimal. These results were compared to analysis of 13 characteristic mango volatiles by gas chromatography (GC) headspace analysis of the mango homogenate for the same volume/dilution combinations. Concentration of volatiles in the headspace generally increased with volume and decreased with dilution, but there were some exceptions and inconsistencies. The increase in headspace concentration was not directly proportional to the homogenate volume, indicating matrix effects on aroma partitioning into the headspace, which varied for different compounds. Whole mangoes (cv. Keitt and Kent) harvested in Homestead, Fla., were put in sealed containers for 3 hours to accumulate enough volatiles for headspace analysis. A large injection volume injected into the e-nose (2000 µL) was necessary to get ample signal and reproducible results, and separated the two varieties based on their volatile emission to the headspace.**

Mango fruit, *Manguifera indica* L., originated in Thailand and Burma, and are currently grown in tropical regions around the world. There are 49 species of mango and thousands of cultivars (Narain et al., 1997). New World or Asian mangoes have different flavor characteristics than Old World or Western hemisphere mangoes (Malundo et al., 1997). Many mono-embryonic mango cultivars were selected in Florida and are grown throughout south and Central America, the Caribbean, and Florida. Mango fruit are climacteric and the fruit matures between the eleventh and fourteenth week after fruit set. Postharvest disorders are observed when the fruit are harvested too early (immature) (Lizada, 1993; Mitra and Baldwin, 1997; Narain et al., 1997).

Terpene hydrocarbon is the major class of compounds in New World mangoes, with contents from 16% to 90%. 3-Carene is the major compound in most New World mango cultivars, with limonene, β-ocimene, myrcene and α-terpinolene having some importance in some cultivars (MacLeod and de Troconis, 1982; Narain et al., 1997; Wilson et al., 1986). Sesquiterpene hydrocarbons may also be present in amounts as high as 10% in some cultivars. There is a large variation in the quality and quantity of alcohols, ketones, and esters in mangoes, especially those of the Old World varieties. Those compounds, together with esters, are responsible for much of the characteristic aroma of Old World mangoes (Narain et al., 1997). Important biochemical changes occur during the respiratory climacteric, just before ripening. Most of the volatile compounds, such as terpenic alcohols, norisoprenoids, and aromatic alcohols are glycosidically bound (Adedeji et al., 1992) prior to ripening, and become part of the volatile profile when they are released as part of the ripening process (Mitra and Baldwin, 1997).

Aside from GC and GC-MS methods, which identify and quantify individual volatile compounds, there are new sensors available that have a broad range of selectivity. These sensor

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arrays (called electronic noses) are useful to discriminate among samples based on the interaction of volatile components with the various sensors. The resulting response pattern allows a particular sample or flavor component(s) to be detected by pattern recognition. However, these instruments do not give information that leads to identification/quantification of individual compounds. Four basic sensor technologies have been commercialized to date. Metal oxide semiconductors (MOS), metal oxide semiconductor field effect transistors (MOSFET), conducting organic polymers (CP), piezoelectric crystals (bulk acoustic wave, BAW) or quartz crystal microbalance (Schaller et al., 1998). Bai et al. (2003, 2004) used this technology to separate whole and cut apples based on flavor differences due to postharvest treatments.

It would be of interest to industry to determine the stage of maturity for harvest that resulted in optimal flavor quality upon ripening (Mitra and Baldwin, 1997). One way to possibly determine this would be to use an electronic nose or gas chromatography (GC). In this study we explore different methodologies to best analyze mango volatiles by electronic nose and GC.

Materials and Methods

Plant material. Preliminary tests for the electronic nose optimization were done by using mangoes (cv Tommy Atkins) from Latin America purchased in a local supermarket (Winter Haven, Fla.). Mangoes (cv Keitt and Kent) were harvested in Homestead, Fla., and used to evaluate the feasibility of whole fruit analysis with the electronic nose. In both cases, the fruit were at the turning stage (starting to color, but still firm) and were allowed to complete ripening at ambient temperatures before analysis.

Gas Chromatography. Static headspace: The homogenate (2 mL) was placed in a 6 mL vial sealed with a TFE/silicone septum. The vial was equilibrated at 80 °C for 15 min in a static headspace sampler Perkin Elmer HS6 coupled to a Perkin Elmer 8500 GC equipped with an FID detector. The column used was a polar Durowax (J&W Scientific, Folsum, Calif.) (30 m, 0.53 mm i.d., 1 µm film thickness); carrier gas was He at 56 cm s^{-1}. Injections were performed in triplicate for each sample and under the following conditions: 30 s pressurization followed by injection, where the injection port temperature was 250 °C. The temperature of the run was 40 °C held for 6 min, then to 180 °C at 6 °C min^{-1}. Compound identification was by retention time comparison with known standards, as well as by spiking the homogenate with specific compounds. Quantification of known compounds was done by performing calibration curves at 5 dilutions spiked into deodorized homogenate (Malundo et al., 1977; Shaw et al., 1991). Compound identities were also analyzed by gas chromatograpy/mass spectrometry (GC/MS). Mango homogenate (600 mL was diluted with 600 mL DI water and then centrifuged at 7000 rpm for 15 min. Organic compounds were extracted from the supernatant using methylene chloride and examined using a Hewlett-Packard Model 597OB, MSD, GC/MS fitted with a 50 m long wide bore (0.31-0.32 mm) fused silica column of cross-linked 5% phenylmethyl silicone (Malundo et al., 1997).

Electronic nose. The electronic nose (e-nose) FOX 4000 (Alpha MOS, Toulouse, France) was equipped with an automatic headspace sampler HS100 and with 18 metallic oxide sensors (coated and uncoated). Mangoes were analyzed as homogenates, or whole fruits. Peeled mango pieces were homogenized in a Waring blender (Waring Products Corp, New York, N.Y.) at 15,000 rpm for 40 s. Two-mL of homogenate were placed in a 10-mL vial and allowed to equilibrate for one hour at 10 °C on the HS100 headspace autosampler (Alpha MOS, Toulouse, France). The samples were heated to 50 °C and shaken for 3 min just before headspace sampling. Mango homogenate was diluted with deionized (DI) water to 50, 25 and 12.5% of original homogenate (w/w) (homogenate/water). Headspace (500 µL) was injected at 2000 µL s^{-1}, and signal acquisition lasted 2 min, followed by 8 min. for baseline recovery. Each injection was repeated six times per sample.

Whole mangoes (8-10), of similar maturity, were sorted by weight and placed in sealed plastic containers (18.9 L). Container lids, fitted with a rubber gasket, were equipped with septa for headspace sampling, and with a flexible bladder to equilibrate the internal pressure during headspace sampling. Fruit were held at 28 °C for 3 hr, then 30 mL of headspace were withdrawn from the container. The headspace sample was injected into a 10-mL sampling vial equipped with a venting tube for flushing several times the vial volume with sample (venting tube was removed after flushing). The sampling vials were equilibrated for one hour at room temperature on the HS100 autosampler. The vials were then heated to 40 °C for 60 s, and 2 mL of vial atmosphere were injected into the electronic nose.

Statistics. Data were analyzed using Discriminant Factor Analysis (DFA). The Prometheus software (Alpha MOS., Toulouse, France) was used for data analysis, as well as for sensor optimization when appropriate (i.e., when sensors were duplicating each other, or not sensing, their data were deleted from the analysis).

Results and Discussion

Electronic nose optimization, homogenate samples. Headspace was generated by heating and shaking vials containing homogenate. Samples were first equilibrated at 10 °C for one hour on the autosampler before transfer to the incubator. Incubator temperature was 30 °C, 40 °C, 50 °C, or 60 °C for one to 5 min, and shaking speed was 200 to 800 rpm. After testing these different methods, the following parameters were retained for an optimum signal: samples heated for 3 min at 50 °C and shaken at 500 rpm.

Injection volume and speed were optimized at 500 µL (volume of headspace injected) at 2000 µL s^{-1}. With those parameters, the maximum signal was obtained in the first minute for all the sensors, which then returned to the baseline after 10 min.

Sample quantity and dilution are of foremost importance to obtain an adequate signal in response to headspace volatiles, without saturating the sensors, and to get reproducible and sensitive measurements (Malundo et al., 1997). Since Malundo et al. (1997) had shown that 50% dilution of mango homogenate increased volatiles in the headspace using GC analysis, due to the viscosity of the pure homogenate trapping volatiles, it was decided to explore the dilution and volume effect using the e-nose starting with 50% dilution. To this end, homogenate (1.0, 1.5 or 2 mL in quantity, or Q1, Q1.5, and Q2, respectively) were diluted to 50%, 25% or 12.5% of pulp (dilution by half, to one quarter or one eighth of full strength or D2, D4 and D8, respectively) in distilled water, and analyzed by the e-nose with DFA. The first axis (56.5% of variabil-

ity) showed separation between all dilutions, and the second axis (35.1% of variability) separated 50% dilution from the other two (Fig. 1). The volumes of homogenate were not separated within each dilution. Although one would intuitively expect to have a signal of similar intensity for 1 mL of pulp diluted to 50% (Q1D2) and 2 mL pulp diluted to 25% (Q2D4), or for 1 mL diluted to 25% (Q1D4) and 2 mL diluted to 12.5% (Q2D8), the resulting signal intensities were grouped by dilution factor regardless of sample quantity when performing a DFA analysis of the e-nose signal (Fig. 1). This means that the volatiles in the headspace differed more based on dilution rather than by volume or quantity of homogenate. Further testing of the quantity of homogenate from 0.5 to 3.0 mL at 50% dilution showed that the sensors were not saturated within this range of sample volume, yet there was enough homogenate to obtain a signal (data not shown). There was some separation, based on quantity of homogenate, with the lower volumes (0.5-1.5 mL) generally separating from the higher homogenate volumes (2.0-3.0 mL) (Fig. 2). Therefore, 2 mL of homogenate diluted to 25% was chosen for the study. The higher volume allows more precise measurements, and the dilution reduces sample viscosity, releasing more volatiles to the headspace in agreement with Malundo et al. (1997).

Validation of e-nose results with GC analysis of static headspace. It was apparent that the volatiles in the headspace of the homogenate were different based on dilution, with some volume effect at 50% dilution according to the e-nose analysis. However, that analysis did not give information on whether the total volatile concentration was higher or lower due to dilution, or whether it was simply a different profile. To determine this, GC analysis was conducted. One, 1.5, and 2 mL of homogenate were diluted with distilled water to one half (D2), one quarter (D4) and one eighth (D8) or to 50, 25 and 12.5% of full strength, respectively, and static headspace sampled and injected into a GC. Thirteen compounds characteristic of mango

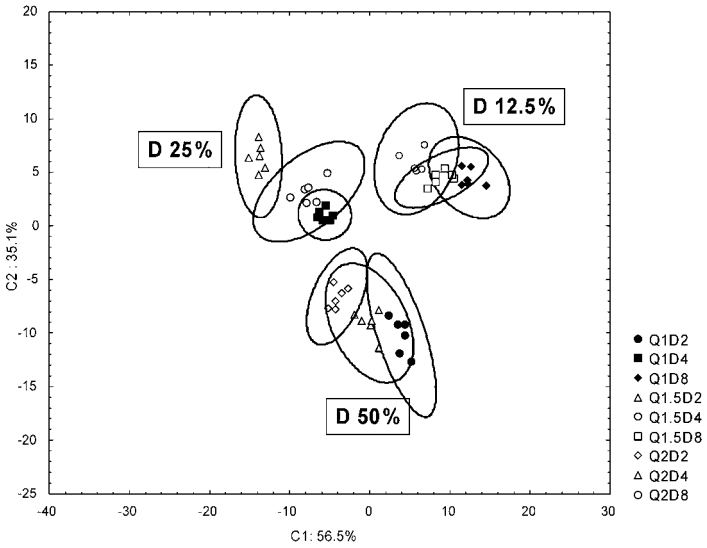

Fig. 1. DFA analysis of electronic nose signals for mango homogenate sampled at different volumes (Q1 to Q2) and dilutions (D2 to D8): Q1D2 = 1.0 ml, dilution to 50%; Q1D4 = 1 ml, dilution to 25%; Q1D8 = dilution to 12.5%; Q1.5D2 = 1.5 ml, dilution to 50%; Q1.5D4 = 1.5 ml, dilution to 25%; Q1.5D8 = 1.5 ml, dilution to 12.5%; Q2.D2 = 2 ml, dilution to 50%; Q2D4 = 2 ml, dilution to 25%; Q2D8 = 2 ml, dilution to 12.5%. Ellipses represent 95% confidence level.

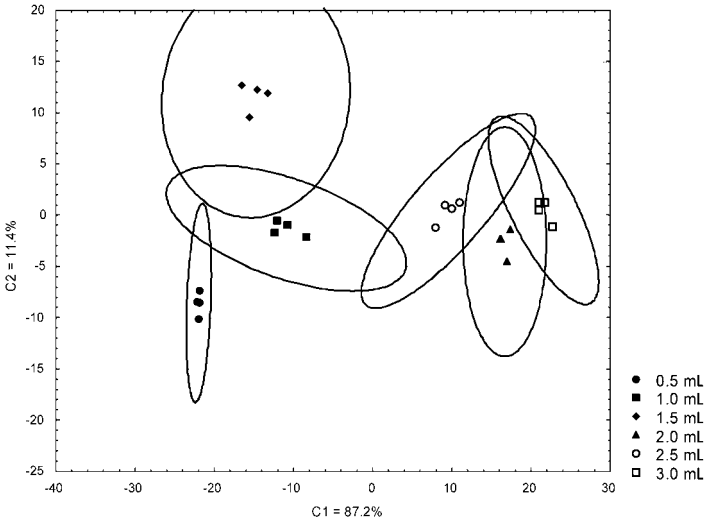

Fig. 2. DFA analysis of electronic nose signals for mango homogenate sampled at different volumes (Q0.5 to Q 3): Q0.5 = 0.5 ml, Q1.0 = 1.0 ml, Q1.5 = 1.5 ml, Q2.0 = 2.0 ml, Q2.5 = 2.0 ml, Q3.0 = 3.0 ml in a 20 ml vial. Ellipses represent 95% confidence level.

aroma (Malundo et al., 1997) were analyzed quantitatively for each sample volume (Table 1). Generally, headspace concentration increased with homogenate volume and decreased with dilution. Methanol, α-copaene and α-caryophyllene did not change with either dilution or sample volume. Acetone and p-cymene did not consistently change with dilution or volume either, but p-cymene increased slightly with increasing volume. Ethanol decreased with increasing dilution, but was not affected by sample volume. The remaining compounds (acetaldehyde, α-pinene, β-pinene, 3-carene, myrcene, limonene, and α-terpinolene) increased with increasing sample volume, and decreased with increasing dilution.

Overall, the increase in headspace concentration was not directly proportional to the homogenate quantity, indicating the matrix effect of aroma retention varies for each compound. Also, there was little variation with sesquiterpenes, due to the fact that they were near the detection threshold.

Whole fruit sampling optimization. It would be useful to determine volatiles non-destructively in whole fruit, to determine degree of fruit maturity or ripening, or to distinguish varieties. The parameters chosen for homogenate headspace appeared to give a weak signal when whole fruit headspace was sampled using the e-nose or GC. This is due to the fact that whole fruit headspace is usually less concentrated in volatiles (Malundo et al., 1997). Attempts to concentrate the headspace with liquid nitrogen were not successful. Whole fruit were placed in sealed containers for one to four hours. An adequate signal was obtained after 3 hr incubation. To determine that anaerobic conditions did not arise over the 3 hr incubation, internal O_2 was measured and found to be adequate for aerobic respiration and ranging between 14 to 18% O_2. Under those conditions, an injection volume of 2000 µL of headspace preheated for 60 sec at 40 °C appeared to be satisfactory. Fig. 3 shows separation of whole 'Keitt' and 'Kent' mango fruit based on the volatiles released by the fruit into the headspace.

The variability of each sensor when measuring whole fruits was estimated by taking 10 headspace measurements from 'Keitt' and 'Kent', and five blank measurements from an

Table 1. Headspace volatiles from mango homogenate, identified and quantified by gas chromatography +/- Std. Error (acetald = acetaldehde, α-terp = α-terpinolene, α-cop= α-copaene, α-cary = α-caryophyllene).

		Mango volatiles (ppm)												
Sample		Acetald	acetone	methanol	ethanol	α-pinene	β-pinene	3-carene	myrcene	limonene	p-cymene	α-terp	α-cop	α-cary
1QD2	mean	27.97	0.33	107.70	123.06	3.99	0.24	19.43	1.15	0.53	0.04	1.09	0.05	0.51
1QD2	stderr	0.08	0.00	0.99	6.24	0.20	0.08	1.28	0.10	0.02	0.00	0.03	0.00	0.00
1QD4	mean	7.12	0.44	106.01	92.85	3.24	0.25	15.17	0.99	0.45	0.03	1.01	0.03	0.51
1QD4	stderr	3.03	0.13	0.57	1.71	0.25	0.03	1.48	0.05	0.03	0.00	0.03	0.02	0.00
1QD8	mean	3.63	0.35	105.36	45.39	2.80	0.20	11.88	0.73	0.40	0.02	0.95	0.03	0.50
1QD8	stderr	0.11	0.01	0.85	1.19	0.15	0.01	0.68	0.03	0.00	0.00	0.01	0.03	0.00
1.5QD2	mean	30.26	0.34	106.86	153.11	5.09	0.44	24.65	1.48	0.61	0.05	1.18	0.04	0.53
1.5QD2	stderr	2.11	0.05	1.95	31.56	0.78	0.08	5.24	0.37	0.09	0.01	0.10	0.02	0.01
1.5QD4	mean	12.20	0.23	104.99	66.25	3.42	0.27	16.11	0.96	0.43	0.03	1.01	0.05	0.51
1.5QD4	stderr	1.23	0.01	1.21	5.22	0.09	0.01	0.32	0.02	0.04	0.00	0.02	0.00	0.01
1.5QD8	mean	6.97	0.21	103.92	37.26	2.96	0.21	12.39	0.74	0.40	0.02	0.95	0.03	0.50
1.5QD8	stderr	0.47	0.00	0.83	11.89	0.29	0.02	1.33	0.09	0.02	0.00	0.02	0.02	0.00
2QD2	mean	30.46	0.30	105.59	93.54	6.68	0.59	33.69	2.15	0.73	0.06	1.31	0.02	0.56
2QD4	mean	15.60	0.26	105.49	92.16	4.46	0.36	21.20	1.35	0.52	0.04	1.07	0.06	0.51
2QD4	stderr	2.02	0.03	0.91	15.14	1.58	0.15	8.71	0.58	0.14	0.02	0.10	0.01	0.02
2QD8	mean	7.65	0.22	104.78	50.63	3.10	0.24	14.15	0.90	0.44	0.03	0.98	0.02	0.50
2QD8	stderr	0.18	0.00	0.34	7.09	0.02	0.00	0.25	0.01	0.01	0.00	0.01	0.02	0.00

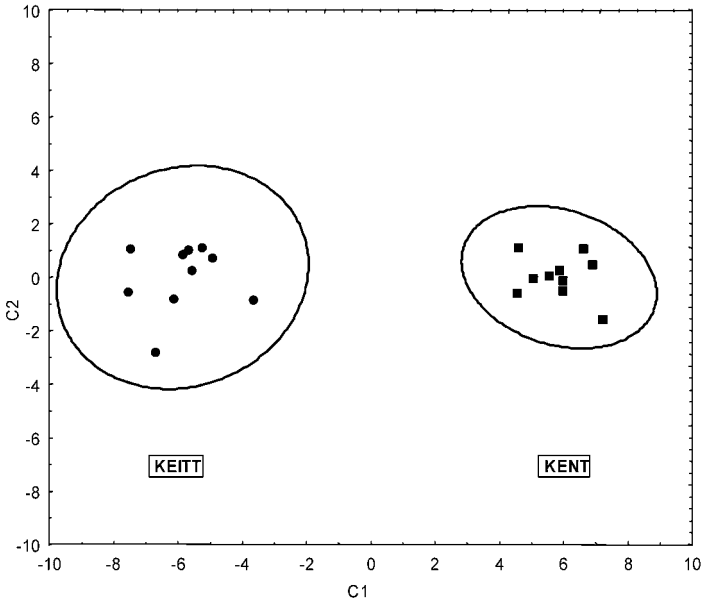

Fig. 3. Separation of intact mango fruit, variety 'Keitt' and 'Kent', by electronic nose based on headspace volatiles of 8 fruit/variety.

empty container. QCM sensors had variations from 3.5 to 11.6%, which still shows high reproducibility, but these specific sensors may not be the most appropriate sensors for mango headspace. Other sensors had an acceptable reproducibility with coefficients of variation varying from 1.9 to 5.8% (Table 2). These other sensors were most likely metallic oxide sensors, but the exact material is proprietary information of the manufacturer. The variation was higher with the blank samples, due to signal near the threshold of detection. Overall, the coefficients of variation for all sensors (average of 10 measurements of all sensors) appear to be within the same order of magnitude than those obtained by gas chromatography (Table 2).

Table 2. Coefficient of variation of the 18 electronic nose sensors used for mango analysis.

	Keitt	Kent	Control
QCM1	3.5	2.8	4.2
QCM1	8.3	8.3	10.5
QCM1	7.0	7.8	10.0
QCM1	11.6	8.6	12.0
QCM1	11.1	8.8	7.9
QCM1	9.5	5.8	4.7
SY/LG	5.9	4.7	9.2
SY/G	3.0	1.9	4.3
SY/AA	3.3	2.0	4.8
SY/Gh	3.0	1.9	4.1
SY/gCTI	5.6	4.8	9.9
Sy/gCT	3.6	2.7	6.4
T30/1	5.8	4.7	9.7
P10/1	3.0	2.3	6.8
P10/2	3.4	2.5	5.6
P40/1	3.1	2.0	5.9
T70/2	4.0	2.5	5.0
PA2	3.7	2.6	4.8
Overall Average Performance	0.113	0.119	0.109
Overall Coefficient of Variation	2.8	1.6	6.2

Conclusion

The optimal method for analyzing mango homogenate using electronic nose is to dilute the homogenate to 25% with DI water and place 2 mL in a 10 mL vial, and heat to 50 °C, while shaking at 500 rpms. Inject 500 µL volume of headspace, at 2000 µL s^{-1}, into the e-nose. For whole fruit, 8-10 fruit in a 18.9 L container sealed for 3 hr will give enough volatiles to the headspace if 2000 µL of headspace, preheated for 60 sec at 40 °C, is injected at 2000 µL s^{-1} into the e-nose.

The use of the electronic nose coupled on a sorting line is not a commercially available technique yet. But the current application of the electronic nose appeared to be useful, and data was validated with gas chromatography.

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