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# Use of an Electronic Nose to Classify Avocado Pulp by Maturity Stage

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**Mature-green 'Booth 7' avocados were stored at 20 °C and assessed for electronic nose (Cyranose® 320) analysis at three maturity stages: mature-green, mid-ripe, and ripe. The training session consisted of six replicates of one fruit for each maturity stage. The pulp (100 g of cubes; 1 $cm^3$) was immediately placed in a 1.7-L glass jar, and sealed for 5 min prior to headspace sampling. The sequence of conditions for each sample reading was: 60 s baseline purge, 60 s sample draw, 30 s sample gas purge, 150 s air intake purge. The identification session was performed using seven ripe fruit under the same set of conditions. The results showed that the electronic nose was successfully trained. Cross-validation of the model was 100%, suitable for class discrimination. Canonical discriminant analysis separated the pulp into three clusters according to the maturity stage. Interclass M-distance was lower between mid-ripe and ripe fruit (15.32), and much higher between mature-green and mid-ripe (55.74) or mature-green and ripe (68.84). However, the electronic nose had poor performance on the identification of test samples (43%). Changes in methodology to improve sample identification are discussed.**

The characteristic smell of a food is a result of the interaction of volatile compounds with the human olfactory system. When sniffed by the human nose, volatiles interact with the olfactory epithelium, which generates a signal that will be interpreted by the brain as a smell. Although thousands of volatiles have been identified, humans only detect the aromatic compounds (odorants). Human sensitivity to odorants varies from person to person and is influenced by factors such as age and fatigue (Reineccius, 2006).

The electronic nose (EN) is an instrument that attempts to mimic the human nose as an artificial olfactory system. Several commercial models are available, and they are composed of an array of sensors that are stimulated when exposed to the volatile compounds of a sample, including the non-aromatic. The sensors generate a signal which is captured and interpreted by software as a smell, called "smellprint." The smellprint is the pattern of volatiles produced by the sample. As the human nose, the EN cannot separate individual volatiles. Additionally, the EN needs to be trained first to create a library of smellprints of the samples to be later analyzed. Then, the identification can take place by pattern comparison (Röck et al., 2008).

There are several ENs commercially available featuring different technologies (Röck et al., 2008). The applications of the EN are diverse and include uses in the food industry (Ampuero and Bosset, 2003), environment analyses (Littarru, 2007), and in medicine for disease diagnostics (Turner and Magan, 2004). The use of ENs in food control has been increasing over the years because they are cost-effective and provide a short-time analysis (Peris and Escuder-Gilabert, 2009). Several studies demonstrate the ability of the EN to discriminate fruit maturity stages and assess fruit ripeness, in the field or after harvest, for intact or processed products (Athamneh et al., 2008; Benedetti et al., 2008; Brezmes et al., 2005; Lebrun et al., 2008; Maul et al., 2000; Pathange et al., 2006). The purpose of this study was to develop a methodology to use an EN to classify avocado pulp by maturity stage.

## Materials and Methods

**Plant material.** Avocado (*Persea american* Mill.) cv. Booth 7 fruit were used for this study. This cultivar is a Guatemalan–West Indian hybrid and a major commercial variety in Florida harvested in mid-season (Crane et al., 2007). Fruit were harvested from experimental plots at the Tropical Research Education Center, Homestead, FL, on date A (Sept. 2008), as established in the official shipping schedule for avocado by the Florida Avocado Administrative Committee (GPO, 2009). Fruit were harvested at the mature-green stage early in the morning and immediately transported to the Postharvest Horticulture Laboratory in Gainesville, FL. Upon arrival fruit were held overnight at 20 °C. The next day the fruit were sorted for absence of major defects and diseases and stored at 20 °C prior to testing with the EN.

The avocados were sorted into three maturity stages: mature-green, mid-ripe, and ripe, based upon fruit firmness (Fig. 1).

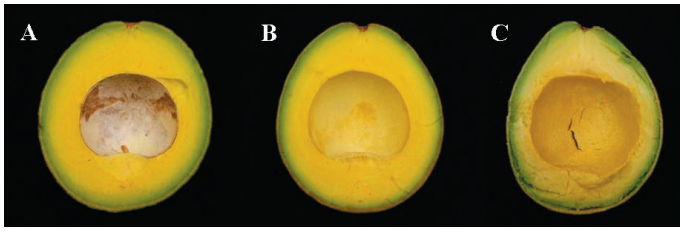


Fig. 1. Maturity stages assessed for electronic nose analysis: (**A**) mature-green; (**B**) mid-ripe; (**C**) ripe.

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Firmness of each maturity stage was determined by a non-destructive compression test on whole, unpeeled fruit using an Instron Universal Testing Instrument (Model 4411, Canton, MA) fitted with a flat-plate probe [5-cm (1.968 inch) diameter] and 50-kg (110.2-lb) load cell. After establishing zero force contact between the probe and the equatorial region of the fruit, the probe was driven with a crosshead speed of 10 mm·min$^{-1}$ (0.394 inch/min). The maximum force was recorded at 2.5 mm (0.098 inch) deformation from two equidistant points on the equatorial region of each fruit and averaged. Ten fruit were measured every other day until they reached the full-ripe stage (10–15 N firmness). The average firmness was 193 N for mature-green, 98 N for mid-ripe, and 14 N for ripe fruit.

**Sample preparation and sampling conditions for EN training and sample identification.** The EN (Cyranose® 320; Smith Detections, Pasadena, Inc., Pasadena, CA) is a portable instrument composed of 32 individual thin-film carbon-black polymer composite chemiresistors configured into an array (Cyrano Sciences, 2001) (Fig. 2). An accessory apparatus was constructed to improve accuracy of the EN based on the apparatus developed by M.O. Balaban and Luis Martinez (Martinez, 2007) (Fig. 3A). Compressed air was used during the analyses, purified by activated carbon and two moisture traps. An initial purge of the electronic nose was performed for 6 min prior to the first reading to avoid the influence of water desorbed from sensors due to long inactive time. The sequence of conditions for each reading was determined according to the time required for most sensors to have a constant signal level for the baseline, namely: 60 s baseline purge, 60 s sample draw, 30 s sample gas purge, 150 s air intake purge. Pump speed was set to medium (approximately 120 mL/min) during baseline purge and sample draw, then was set to high (approximately 180 ml/min) during sample gas and air intake purge. The apparatus was purged with air for 1 min between samples. All 32 sensors were active for the analyses.

Each fruit was peeled, halved, and the pulp sliced into cubes of approximately 1 cm$^3$ (0.061 inch$^3$). A sample of 100 g (0.220 lb) of pulp cubes was immediately placed in a 1.7-L (0.449-gal) glass jar, which was immediately sealed for 5 min. Headspace samples were drawn inserting the 10.2-cm (4-inch) needle on one of the two vents on the lid of the jar, keeping the other vent open to avoid vacuum (Fig. 3B). Room conditions during analysis were 22 °C and 56% R.H. The training session allowed the EN to establish a "smellprint" for each class, which is the pattern of the headspace in the jar containing the volatile compounds. The training session utilized six individual fruit for each maturity stage.

In the identification session the EN was challenged to identify the maturity stage of seven test samples (100 g of ripe avocado pulp sliced into 1-cm$^3$ cubes) by comparing their smellprints with the known smellprints generated during the training session. The same set of conditions was used for both sessions.

**Experimental design and statistical analysis.** The experiment was conducted in a completely randomized design with three maturity stages and six replicates. A cross-validation of the model was performed by the EN to validate the model. Canonical discriminant analysis was performed and the interclass M-distances were generated by the software PCNose (Fig. 4) (Cyranose® 320; Smith Detections, Pasadena, Inc., Pasadena, CA).

## Results and Discussion

The training session was considered successful as the EN accurately separated the three maturity stages targeted in this study. The model with three maturity stages was suitable for clear class discrimination. Cross-validation of the model was 100%, meaning that all samples trained as one particular maturity stage were classified as being of that maturity stage (Table 1). The Interclass Mahalanobis distance (M-distance) is the distance between two classes, and the ability of the model to successfully discriminate between classes increased as the M-distance increased. A minimum M-distance of 5 is required for a good class separation and it was 15.32 between mid-ripe and ripe fruit. The M-distance was much higher between mature-green and mid-ripe (55.74) or mature-green and ripe (68.84) (Table 2). Therefore, mature-green fruit were

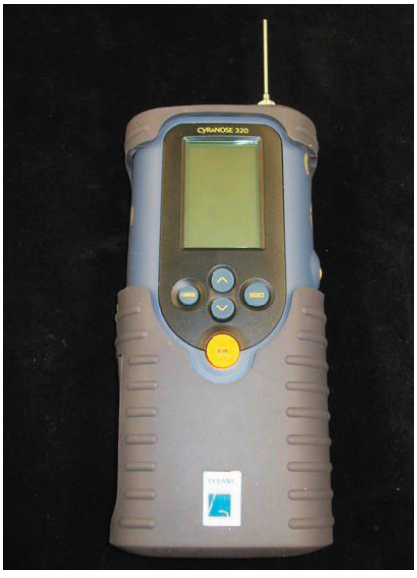


Fig. 2. The electronic nose Cyranose® 320.



Fig. 3. (**A**) Apparatus constructed to improve the accuracy of the electronic nose, based on the apparatus developed by M.O. Balaban and Luis Martinez (Martinez, 2007). Design used with permission. (**B**) Detail of headspace sample draw.

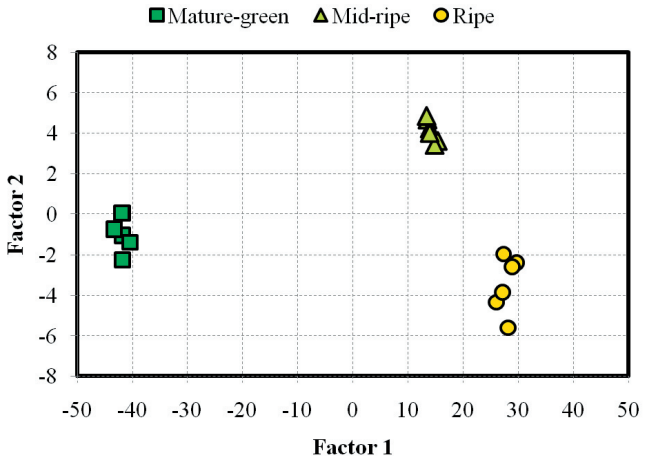


Fig. 4. Canonical projection plot of electronic nose readings (n=6) of mature-green, mid-ripe, and ripe 'Booth 7' avocado pulp.

Table 1. Cross-validation of Cyranose® 320 readings of electronic nose signals for mature-green, mid-ripe, and ripe 'Booth 7' avocado pulp (training session).

| | Classified as: | | |
|---|---|---|---|
| | Mature-green | Mid-ripe | Ripe |
| Trained as | | | |
| Mature-green | 6 | | |
| Mid-ripe | | 6 | |
| Ripe | | | 6 |

Table 2. Interclass M-distances between readings of electronic nose signals for mature-green, mid-ripe and ripe 'Booth 7' avocado pulp (training session).

| | Mature-green | Mid-ripe | Ripe |
|---|---|---|---|
| Mature-green | --- | 55.74 | 68.84 |
| Mid-ripe | --- | --- | 15.32 |

more distinctively separated from other stages than mid-ripe from ripe fruit. This was confirmed by canonical discriminant analysis plot, which shows three distinct clusters representing the three maturity stages considered in this study.

Informal analysis of fruit aroma revealed that mature-green fruit had a characteristic green, woody aroma, which is mostly lost during ripening. Ripe fruit had weak greenish, nutty aroma. Terpenoids are the main volatile compounds in avocado (Pino et al., 2000, 2004; Sinyinda and Gramshaw, 1998) and they may contribute to class distinction in avocado using the EN. Changes in volatiles were significantly correlated with EN signals in tomato (Maul et al., 2000) and mango (Lebrun et al., 2008). In 'Castlebrite' apricots, the capacity of an EN to distinguish between stages of ripening may have been associated with levels of hexanal and less with ethylene production (Defilippi et al., 2009). Avocado produces ethylene at very low rates in mature-green fruit but large amounts during ripening (Eaks, 1978). It is possible that ethylene influenced the separation of maturity stages since the EN also detects non-aromatic compounds.

The use of a portable EN could be an advantage over benchtop instruments for maturity determination, particularly for field studies. Fruit quality parameters (firmness, acidity and starch index) correlated significantly with EN signals in 'Pinklady' apples. The values were predicted non-destructively as the apples ripened (Brezmes et al., 2001). The same approach was proven to be valid for pears, peaches and nectarines (Brezmes et al., 2005), but not always for tomato (Gómez et al., 2006a, 2008; Maul et al., 2000). Although different maturity stages of grapes could not be distinguished solely based on physicochemical analysis, they were successfully separated by the Cyranose® 320 in the field in two consecutive years (Athamneh et al., 2008).

In this test the EN had poor performance when challenged to classify the maturity stage of test samples (ripe fruit) in the sample identification session (Table 3). It correctly classified only 43% of the samples as ripe fruit. It did not misclassify the other 57% of the samples as mature-green or mid-ripe fruit, but rather as "unknown," meaning that some samples were out of the range of training. Therefore, although the EN was successfully trained to generate one distinct smellprint for each maturity stage, adjustments in the methodology are needed to increase the efficacy of sample identification.

The first corrective action would be to increase the number of fruit samples per maturity stage during the training session. Use of a larger sample size would expose the EN to more variability within each maturity stage and therefore the patterns would be more representative of this variation, increasing the chances of correct identification. Second, headspace volume could be reduced and surface area increased to accelerate the accumulation of volatile compounds in the headspace. A third action would be to reduce identification quality in the EN software setup, since there is a very low possibility that a test sample will fall outside of the three maturity stages established during the training session.

Lastly, all 32 sensors were used in the present study for the method development; however, sensor responses vary. As supported by other studies, a selection of the most sensitive sensors to changes in avocado maturity stage should improve the specificity of the analysis. Few specific Cyranose® 320 sensors responded more favorably to apple aroma than others (Pathange et al., 2006). Significant correlations were also found for 'BHN-189' tomatoes between specific aroma compounds and individual sensors of the e-Nose 4000 (Neotronics Scientific, Flowery Branch, Ga.) (Maul et al., 2000). The electronic nose, PEN2 (WMA Analytics Inc., Schwerin, Germany), is composed of an array of 10 metal-oxide semiconductor type sensors, yet a single sensor was able to discriminate peaches according to their ripeness stage (Benedetti et al., 2008). A subset of sensors explained nearly all the variance during ripening of tomato (Gómez et al., 2006a) and mandarin (Gómez et al., 2006b).

In conclusion, the Cyranose® 320 was successfully trained

Table 3. Results from the identification session using ripe avocado pulp as test samples.

| Tested as: | Identified as: | | | |
|---|---|---|---|---|
| Ripe fruit (no.) | Mature-green | Mid-ripe | Ripe | Unknown |
| 1 | --- | --- | --- | × |
| 2 | --- | --- | --- | × |
| 3 | --- | --- | --- | × |
| 4 | --- | --- | × | --- |
| 5 | --- | --- | --- | × |
| 6 | --- | --- | × | --- |
| 7 | --- | --- | × | --- |
| Results (%) | 0 | 0 | 43 | 57 |

to classify avocado pulp by maturity stage. However, changes in methodology are required to improve sample identification.

## Literature Cited


Ampuero, S. and J.O. Bosset. 2003. The electronic nose applied to dairy products: a review. Sensors and Actuat. B-Chem 94:1–12.

Athamneh, A.I., B.W. Zoecklein, and K. Mallikarjunan. 2008. Electronic nose evaluation of cabernet sauvignon fruit maturity. J. Wine Res. 19:69–80.

Benedetti, S., S. Buratti, A. Spinardi, S. Mannino, and I. Mignani. 2008. Electronic nose as a non-destructive tool to characterize peach cultivars and to monitor their ripening stage during shelf-life. Postharvest Biol. Technol. 47:181–188.

Brezmes, J., E. Llobet, X. Vilanova, J. Orts, G. Saiz, and X. Correig. 2001. Correlation between electronic nose signals and fruit quality indicators on shelf-life measurements with Pinklady apples. Sensors and Actuat. B-Chem 80:41–50.

Brezmes, J., M..L.L. Fructuoso, E. Llobet, X. Vilanova, I. Recasens, J. Orts, G. Saiz, and X. Correig. 2005. Evaluation of an electronic nose to assess fruit ripeness. IEEE Sensors J. 5:97–108.

Crane, J., E. Evans, and C. Balerdi. 2007. A review of the Florida avocado industry. Proc. VI World Avocado Congr., Viña Del Mar, Chile.

Cyrano Sciences. 2001. Cyranose 320 user's manual. 4th ed. Cyrano Sciences, Pasadena, CA.

Defilippi, B.G., W. San Juan, H. Valdés, M.A. Moya-León, R. Infante, and R. Campos-Vargas. 2009. The aroma development during storage of 'Castlebrite' apricots as evaluated by gas chromatography, electronic nose, and sensory analysis. Postharvest Biol. Technol. 51:212–219.

Eaks, I.L. 1978. Ripening, respiration, and ethylene production of 'Hass' avocado fruits at 20° to 40° C. J. Amer. Soc. Hortic. Sci. 103:576–578.

Gómez, A.H., G. Hu, J. Wang, and A.G. Pereira. 2006a. Evaluation of tomato maturity by electronic nose. Comput. Electron. Agr. 54:44–52.

Gómez, A.H., J. Wang, G. Hu, and A.G. Pereira. 2006b. Electronic nose technique potential monitoring mandarin maturity. Sensors and Actuat. B-Chem 113:347–353.

Gómez, A.H., J. Wang, G. Hu, and A.G. Pereira. 2008. Monitoring storage shelf-life of tomato using electronic nose technique. J. Food Eng. 85:625–631.

Government Printing Office (GPO). 2009. Code of federal regulations. Title 7, Part 915. Avocados grown in South Florida. Available at: <http://www.gpoaccess.gov/ecfr/>.

Lebrun, M., A. Plotto, K.L. Goodner, M. Ducamp, and E.A. Baldwin. 2008. Discrimination of mango fruit maturity by volatiles using the electronic nose and gas chromatography. Postharvest Biol. Technol. 48:122–131.

Littarru, P. 2007. Environmental odours assessment from waste treatment plants: dynamic olfactometry in combination with sensorial analysers "electronic noses." Waste Mgt. 27:302–309.

Martinez, L.F. 2007. Quality enhancement of coffee with acid and enzyme treatment. M.Sc. Thesis, Univ. of Fla., Gainesville.

Maul, F., S.A. Sargent, C.A. Sims, E.A. Baldwin, M.O. Balaban, and D.J. Huber. 2000. Tomato flavor and aroma quality as affected by storage temperature. J. Food Sci. 65:1228–1237.

Pathange L.P., P. Mallikarjunan, R.P. Marini, S. O'Keefe, and D. Vaughan. 2006. Non-destructive evaluation of apple maturity using an electronic nose system. J. Food Eng. 77:1018–1023.

Peris, M. and L. Escuder-Gilabert. 2009. A 21st century technique for food control: Electronic noses. Anal. Chim. Acta 638:1–15.

Pino, J.A., A. Rosado, and J. Aguero. 2000. Volatile components of avocado (*Persea americana* Mill.) fruits. J. Essent. Oil Res. 12:377–378.

Pino, J.A., R. Marbot, A. Rosado, and V. Fuentes. 2004. Volatile components of avocado (*Persea americana* Mill.) cv. Moro grown in Cuba. J. Essent. Oil Res. 16:139–140.

Reineccius, G. 2006. Flavor chemistry and technology. 2nd ed. Taylor & Francis Publ., London, UK.

Röck, F., N. Barsan, and U. Weimar. 2008. Electronic nose: Current status and future trends. Chem. Rev. 108:705–725.

Sinyinda, S. and J.W. Gramshaw. 1998. Volatiles of avocado fruit. Food Chem. 62:483–487.

Turner, A.P.F. and N. Magan. 2004. Electronic noses and disease diagnostics. Nature Rev. 2:161–166.