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**Electronic Sensing of Fruit Ripeness Based
on Volatile Gas Emissions**

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הספריה המרכזית
למדעי החקלאות
בית דגן

**ELECTRONIC SENSING OF FRUIT RIPENESS BASED ON VOLATILE
GAS EMISSIONS**

BARD Project No. US-2234-92R

Final Scientific Report

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ELECTRONIC SENSING OF FRUIT RIPENESS BASED ON VOLATILE GAS EMISSIONS

BARD Project No. US-2234-92R

Final Annual Scientific Report: Summary

An electronic sensory system for the evaluation of headspace volatiles was developed to determine fruit ripeness and quality. Two prototype systems were designed, constructed, and later modified. The first is an improved version of our original prototype electronic sniffer using a single head sensing unit for use as a single or paired unit placed on an individual fruit surface for applications in the field, lab, or industry. The second electronic sniffer utilizes a matrix of gas sensors, each selected for differential sensitivity to a range of volatile compounds. This system is more sophisticated as it uses multiple gas sensors, but was found to enhance the ability of the sniffer to classify fruit ripeness and quality relative to a single gas sensor. This second sniffer was designed and constructed for the sampling of fresh-cut or whole packs of fruits such as packaged strawberries and blueberries, and can serve as a prototype for research or commercial applications. Results demonstrate that electronic sensing of fruit ripeness based on aromatic volatile gas emissions can be used successfully with fresh fruits. Aroma sensing was successful for classifying ripeness in muskmelons, including different cultivars, apples, blueberries, strawberries, and in a complimentary BARD project on tomatoes. This system compared favorably to the physicochemical measurements traditionally employed to assess fruit maturity. This nondestructive sensory system can detect the presence of physically damaged fruits and shows excellent application for use in quality assessment.

Electronic sensors of the tin oxide type were evaluated for specificity toward a wide range of volatiles associated with fruit ripeness. Sensors were identified that detected a broad range of alcohols, aldehydes, esters, hydrocarbons, and volatile sulfur compounds, as well as individual volatiles associated with fruit ripening across a wide concentration range. Sensors are not compound specific, thus, the matrix of sensors coupled with discrimination analysis provides a fingerprint to identify the presence of compounds and to assess alterations in fresh products due to alterations in volatile emissions. Engineering developments led to the development of a system to compensate for temperature and relative humidity relative to on-line aroma sensing with melons for ripeness determination and to reduce response time, thus permitting the electronic sniffer to be used for monitoring both fresh and processed food products. The sniffer provides a fast, reliable and nondestructive tool to assess fruit ripeness and quality. We hope that our work will foster the introduction and utilization of this emerging technology into the agricultural and horticultural fields.

ELECTRONIC SENSING OF FRUIT RIPENESS BASED ON VOLATILE GAS EMISSIONS

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Introduction and Objectives

Commercial gas sensors for the detection of volatile gases appeared around 1923 and by the 70's, research concentrated mainly on finding the best materials for these sensors. By the 1980's, commercial gas sensors were available to cover a wide range of applications. The sensor technology covers a wide range of physical phenomena that led to the development of the sensors. Among these are the tin oxide technology which is commercially available (Ihokura and Watson, 1994), the conducting polymer sensors (Gardner and Bartlett, 1991), and the piezoelectric sensors (Ema et al. 1989; Nakamoto et al. 1990). These technologies are undergoing major developments and advances in the field of solid-state sensors opened an opportunity to electronically sense odors and discriminate products by their aroma (Persaud and Pelosi, 1985; Shurmer and Gardner, 1992; Corcoran, 1993; Gardner and Bartlett, 1994). In general, sensors change their electrical resistance when exposed to certain volatile chemicals. The detection process of each sensor element appears to be relatively non-specific, yet there is an overlap in sensitivity among sensors allowing for the possibility of discrimination for a class of compounds and targeted compounds. Due to the complex relationship, response to wide range of gases, and low correlation between odor and the response of an individual sensor, multivariate analysis or neural networks can be applied to discriminate the objects sensed to predetermined classes. The developments in this field included the improvement in the sensing elements and the supporting mathematical algorithms that are required to extract discriminating features from the raw data. Existing statistical analysis methods such as partial least square, discriminate function, principle component, cluster analysis and specific algorithms such as neural networks simulators have been developed (Nakamoto et al. 1990; Nakamoto et al. 1992; Corcoran, 1993; Davide et al. 1994; Hines and Gardner, 1994; Pisanelli et al. 1994). This technology which permitted the opportunity to analyze, recognize and identify odors, aromas and other volatile chemicals at low ppb levels can be far reaching, and as such, there are now several bench-top large scale commercially available units based upon odor or aroma sensing technology. Electronic sensing of volatiles have been used to detect alcohols and tobacco smoke (Shurmer et al. 1989), coffee (Gardner et al. 1990), perfume and flavor (Nakamoto et al. 1993), fish (Olafsson et al. 1992), brewery (Tylor et al. 1994), fruit ripeness (Benady, 1993; Benady et al. 1995; Hetzroni et al. 1994), and in fire alarms (Nakamoto et al. 1994). To use such an electronic sensing technology effectively, each of these studies first have to define quality and acceptability, and then use traditional parameters of quality (i.e. physicochemical, human sensory response) to define the sensing response. This technology can enable us to electronically recognize differences in aroma, or alteration in

aroma over time, and hence the quality, of incoming raw food materials, such as fresh fruits, or potentially for use with processed materials. The speed, sensitivity and accuracy of the technology translates into improved productivity and reduced costs. Odor sensing technology has potential application in the determination of fruit ripeness and fruit quality and to detect alterations in quality of fresh fruit, and it is in this area where we focused our research efforts. Statistical pattern recognition techniques can be successfully used with such multi-sensors. While the interest and use in this emerging technology continues, very little basic research has been conducted to provide a comprehensive understanding in characterizing the actual volatiles that are being indirectly measured, nor on the differential sensitivity of individual volatiles to the sensing surfaces. The direct linkage from specific aromatic volatiles to odor sensing responses has not been achieved. While none of the commercial units have been designed for continuous sampling, for industrial use under on-line conditions, under industrial contaminated air conditions, or for compensating for environmental changes, their availability and use does illustrate the use of this technology as a tool for quality control.

An electronic sensory system for the evaluation of headspace volatiles was developed at the Center for New Crops and Plant Products and the Department of Agricultural and Biological Engineering, Purdue University, to determine quality and ripeness stages of agricultural products (Benady et al. 1995; Simon et al. 1996). This system employs a matrix of stannic oxide sensors (Ihokura and Watson, 1994) that are sensitive to a wide range of volatiles. In the course of this research project, we have demonstrated that this system and modifications to this system can be successfully used as a research tool for ripeness determination of fresh fruits such as different melon cultivars (Benady, 1993; Benady et al. 1995), strawberries (Hetzroni et al. 1994) apples (Hetzroni et al. 1995), blueberries (Benady et al. 1994, Simon et al. 1996), raspberries (Simon et al. unpublished data), and tomatoes (Edan et al. 1994). The sensory system was also successful in detecting presence of small quantities (5%) of physically damaged (soft and leaky) blueberry fruits in packages (Simon et al. 1996) and shows excellent application for use with fresh fruit. Engineering developments in the final year of this study also led to the development of a system to compensate for temperature and relative humidity relative to on-line aroma sensing with melons (Grinshpun et al. 1997) for ripeness determination and to reduce response time, thus permitting the electronic sniffer to be used for monitoring both fresh and processed food products.

In the original objectives set forth in the BARD proposal we sought to:

1. Determine the correlation between the response of the 'electronic sniffer' to aromatic volatiles, ethylene, and other physiological indices of fruit development including stem detachment force, flesh

firmness, and total soluble solids during the ripening process of different cultivars of melons, watermelons and apples.

2. Develop the sensor fusion algorithm for accurately determining the degree of fruit ripeness based upon the electronic sniffer response;
3. Design the sensory system as a self-contained apparatus including the detection sensors, relevant environmental sensors, and the signal processing system;
4. Examine the impact of ambient and microenvironment on the capture of fruit volatiles, and modify the electronic sensory system and the data acquisition hardware to adjust for laboratory, field, processing plant and storage conditions.

The reduced budget awarded to the project from BARD mandated a reduction in the scope of these objectives. During the course of the study, preliminary studies using the sniffer cup on the surface of the watermelon rind resulted in very low to nearly undetectable volatile gas emissions. Seeking to keep the system nondestructive, we substituted a number of other highly perishable high value fruit (blueberries and strawberries) instead of further work with watermelons.

Electronic Sensing of Volatile Compounds Related to Fruit Ripening and Food Quality

Abstract: Electronic sensors of the tin oxide type were calibrated with selected volatiles associated with fruit ripeness. The results show that electronic sensing provides an effective tool to detect the presence of aromatic volatiles across a broad concentration range. The sensors are not compound specific, thus requiring a matrix of sensors coupled with discrimination analysis to provide a fingerprint to identify the presence of a class of compounds and to quantify alterations in the tested atmospheres. Electronic sensing of volatile compounds are best achieved using such a matrix, though for specific applications a single gas sensor may provide adequate information. This controlled study comparatively evaluated the response of gas sensors to single compounds associated with fruit ripeness in order to identify those gas sensors that would be more useful in monitoring fruit ripeness and food quality. Preliminary studies also evaluated the ability of the electronic sniffer to detect air borne contaminants, such as those found associated with pesticides. Although not the central focus of this project, it was noteworthy that the sniffer was able to detect the presence of selected insecticides, hydrocarbons, and herbicides, also across a wide concentration range, and that individual fingerprints for each compound were developed. While the applicability of detecting such contaminants on ripening fruit is still unknown, the ability of an aroma sensing system to detect air quality contaminants such as those found in agricultural environments should be pursued.

Introduction: Gas sensing is becoming increasingly important as an emerging technology (Gardner and Bartlett, 1994) for the remote sensing of industrial and agricultural environments (Mizei and Lantto, 1992; Reichert et al., 1993) and food products (Benady et al., 1995; Shurmer et al., 1990; Gardner et al., 1992; Nakamoto et al., 1993). Applications cover a broad range, going from single gas monitoring in industrial plants or mines, to complex gas mixtures as in beer making, coffee aromas and essential oils. The advantage of using electronic sensors to monitor gases resides in the rapidity and precision of the response, the convenience of the sensing device which can easily be used outdoors, in a field, as well as indoors, and a relatively low price. Also, electronic sensing can be adapted to monitor many chemicals. The use of an array or matrix of sensors allows the fingerprinting of a sample, be it a single component or a complex mixture of gases, such as the aroma of a ripe fruit. However, as most sensors are not compound specific, there is a need to select the appropriate sensors and to evaluate their threshold limit. In this study, related to our work on the monitoring of fruit ripeness (apples, blueberries, melons and

strawberries) with an electronic sensor system (electronic sniffer), we have calibrated different tin dioxide semiconductor gas sensors with volatiles associated with ripening fruits, as well as a range of commonly used insecticides and herbicides, in concentrations varying from below 1 ppm up to 300 ppm.

Semiconductor Gas Sensors: Semiconductor gas sensors have a decreasing electrical resistance as concentration of gas or vapor in the ambient atmosphere increases. More specifically, there are two types of metal oxide semiconductor sensors, classified according to the predominance of the electrons, n-type, or holes, p-type, in a crystal that are responsible for the semiconducting properties. Tin dioxide (SnO₂) semiconductors are of the n-type, and their conductivity increases in the presence of reducing compounds such as hydrogen, carbon monoxide, methane, alcohols, ketones, and esters. In this article, the term semiconductor gas sensor refers specifically to tin-dioxide semiconductor gas sensors. The chemical reactions across membrane surfaces and the electrical resistance changes associated with adsorption and desorption have been previously described (Kubokawa, 1965; Streetman, 1980; and Tarama, 1958).

Objectives: The specific objectives of this study were (1) to identify the specificity and sensitivity of an array of semiconductor gas sensors that can rapidly monitor fruit ripeness, and quality factors influenced by ripeness time for use in quality fruit sorting; and (2) to detect the presence of common farm pesticides in agricultural environments.

Materials and Methods: In this study, all chemicals were obtained from commercial sources and used as received: ethanol (McCormick, Weston, Missouri); 1-octanol, *cis*-3-hexen-1-ol, hexanal, butyl acetate, ethyl hexanoate, ethyl 2-methylbutyrate, ethyl propionate, ethanethiol and dipropyldisulfide (Aldrich, Milwaukee, Wisconsin); ethyl acetate, toluene and xylene (Mallinckrodt, Paris, Kentucky); pentyl acetate (Ladd Research, Burlington, Vermont); benzaldehyde (Fisher Scientific, Fair Lawn, New Jersey); isobutane (Ronson, Woodbridge, New Jersey); carbon dioxide (Pain Entreprises, Marion, Indiana); ethylene (Matheson, Montgomeryville, Pennsylvania); 2,2,5-trimethylbutane (Chemical Samples, Columbus, Ohio).

Purity levels of all standard volatiles, except carbon dioxide, were determined by GC analysis, and were found to be at least 98% pure. These analyses were conducted using a Varian (Walnut Creek, CA) model 3700 gas chromatograph, fitted with a flame ionization detector and a Hewlett-Packard model

3396 series II integrator (Palo Alto, CA). A capillary column, Supelco (Bellefonte, PA) SPB-5 (30m X 0.25 mm i.d., 0.25 μ m film thickness), was used. The injector and detector temperatures were 220C and 240C, respectively, while the program temperature was 2 min at 40C, up to 220C, at a rate of 10C/min, and held 10 min at the final temperature. For liquid samples, volumes of 0.1 μ l, with the same split ratio of 100:1, were injected, while for gas samples (ethylene), volumes of 1.0 μ l, with the same split ratio, were injected. the carrier gas was helium, at a linear velocity of 20.0 cm/s.

The Electronic Sniffer: The electronic sniffer developed consisted of a power supply, a sampling chamber, and data acquisition hardware. The sampling chamber, which had a volume of 5.40 l, was made of plexiglass, and six semiconductor gas sensors were mounted on a top lid (Simon et al. 1996). A miniature electrical fan, used to effect efficient gas mixing and vaporization, was also fixed inside the box. The semiconductor gas sensors were of tin dioxide type and were selected to cover the broadest range of chemical compounds. The sensors were selected from commercially available gas sensors to include those that may be sensitive to volatiles produced from food products. The gas sensors came from Figaro (Osaka, Japan) and included TGS800, TGS822, TGS824, TGS825, TGS842, and TGS880. To record the data, the LabView software (National Instruments, Austin, Texas) was used on a Toshiba (Japan) model T1950CT laptop computer. This output was continuously recorded into computer files using our data acquisition system. The output value after the system stabilizes was recorded and was used for the analysis.

Ripeness and Classes of Natural Volatiles: Fruit aroma and flavors consist of complex mixtures of volatiles whose composition and concentration change, dependent on the fruit and stage of ripeness. This process is also accompanied by a rapid and significant rise in ethylene production (Bliss and Pratt, 1979). It has been reported that for apples, mostly esters were synthesized during the climacteric phase (Dimick and Hoskin, 1983) while 6-carbon aldehydes would be related to damaged tissues (Flath et al, 1967). For blueberries, alcohols and some aldehydes comprise most of the reported volatiles constituents (Hivi and Honkanen, 1983; Howatt and Senter, 1985). In muskmelons (Yabuto and Jennings, 1977; Kemp et al., 1972), well as in strawberries (Schreier, 1980) esters were the most abundant volatiles in headspace analysis. Minor compounds included alcohols, aldehydes and disulfides. As our objectives were to monitor fruit ripeness based on the load of volatiles emitted by the fruits as they mature, and as a secondary objective to detect the presence of residual chemical contaminants, the following classes of volatiles related to fruit ripeness and respiration, along with aromatic hydrocarbons used in herbicide and

pesticide formulations, were selected for the present study: alcohols, aldehydes, esters, hydrocarbons, sulfur compounds and carbon dioxide.

Gas Sensor Calibration: To perform the calibration of the sensors, an additional element was needed: an estimate of the volatile load that would be produced by a real sample present in the sampling chamber, and to which the concentration of the chemicals used for the calibration would be adjusted.

Natural Volatile Concentration: While many studies report volatile emission rates by plants, and ethylene emission rates, most studies focusing on aromatic volatiles have described terpene emissions by trees. The units used to report those results relating to atmospheric emissions were microgram of volatile emitted per gram of dry plant material per hour ($\text{mgg}^{-1}\text{h}^{-1}$), with most values being in the range of 0.1 to $3.0 \text{ mgg}^{-1}\text{h}^{-1}$. From these data, and to serve as a guideline for the calibration, we tentatively estimated that a reasonably sized sample ($\pm 1 \text{ kg}$) having an averaged emission rate of $1 \text{ mgg}^{-1}\text{h}^{-1}$ could release more than $33 \mu\text{g}$ of a single component, over a two minute period. Considering that the sample is contained in a 4.0 l sealed chamber and with an average molecular weight of 140, the figure gives a concentration that approaches 4 ppm. Based upon these assumptions, and to obtain the best possible view of the behavior of each sensor in different atmospheres, a broad range of concentrations was considered for most volatiles, extending, in general, from below 1 ppm up to 300 ppm as the limit values (Table 1).

Concentration of Sample Chemicals: All samples were injected inside the box either as gases or as liquids. For the latter, vaporization was performed with the assistance of the miniature electrical fan. For these samples, the amounts required to obtain a given concentration were calculated with Antoine's law, when the data were available. Otherwise, the perfect gas law was used. Antoine's law is: $\log P_n = A - B/(C-t)$, with P_n being the vapor pressure (Atm) of compound "n" at a given temperature "t" ($^{\circ}\text{C}$). A, B and C are constants experimentally determined and available from the appropriate literature sources (Nelson, 1991). Comparison of the two methods showed no or very slight differences. For example, at 24°C , the volume of liquid ethanol required to obtain a 300 ppm concentration inside the chamber was 3.83 ml with both methods. For toluene, and for the same concentration, the calculated volumes were 7.06 ml with Antoine's law and 7.07 ml with the perfect gas law. The similarity of the results obtained with both methods were expected since the concentrations and pressure are low. All calculations were

performed with relative values: the numbers obtained for a 300 ppm concentration of ethanol being used as references for each sensor.

Results and discussion: All the tested sensors responded to low concentrations of all screened compounds with the exception of carbon dioxide. The gas sensors did respond to ethylene, but only poorly. Significant differences were observed in the sensors sensitivity to changes in the concentration of most chemicals. Sensor sensitivities to concentration changes were evaluated with plots of normalized sensor responses versus the volatile concentrations plotted on log-log scales. Thus, the higher the absolute value of the slope, the more sensitive the sensor is to a change in concentration. Plots of the slope values for the different chemicals are presented as radar graphs, or star-like graphs, with each line pointing to the center corresponding to a different sensor (Fig. 1). In all cases but isobutane, the TGS842 sensor was the least sensitive to concentration variations: the values obtained for the slopes ranging from 0.00 with carbon dioxide to -0.39 with ethanethiol. Among the other sensors, none seemed to be more sensitive, in general, to concentration variations, though this was not true for specific cases. None of the plots could be superimposed upon another, as can be seen from the graphs, demonstrating the capability of the sensor matrix to fingerprint a compound across of concentration range. Both sulfur compounds (dipropyl disulfide and ethanethiol) exhibited the steepest slopes among the volatiles tested. This is one of the advantages in the use of a multi-sensor (or matrix) system, compared to the use of a single sensor, in the monitoring of a mixture of gases. With such an array of sensors, it is unlikely that different gas mixtures will have exactly the same fingerprint, which is not so with a single sensor.

Specificity of gas sensors: This study screened selected alcohols, aldehydes, carbon dioxide, esters, hydrocarbons, and sulfur compounds, to assess whether each could be detected by one or more gas sensors. Each compound was individually injected inside the sniffer chamber. Responses of the sniffer reported as R_s/R_o , where R_s is the resistance of the sensor and R_o is that for a 300 ppm concentration of ethanol, to concentration of the volatiles, when plotted on $\log(R_s/R_o)$ vs $\log(\text{concentration})$ scales, exhibit somewhat linear relationships (Figs. 1 and 2). The slopes of those lines vary from one sensor to the other, for a given volatile, and from one compound to another, relative to a specific sensor. The later indicates that variation in sensor response to changes of volatile concentration constitute a good indication on the ability of a specific sensor to detect such changes, but must be completed with a knowledge of the sensitivity of the sensors to the different volatiles, that is the ability to “smell” a compound, as it varies from one volatile to another for a given sensor. Another way to present the sensitivity of a single gas sensor to a concentration range of selected volatiles is illustrated in Fig. 2,

where the steeper the slope and the lower the slope the more sensitive the sensor is to that given concentration range of the volatile. These results also clearly illustrate that, as predicted, carbon dioxide does not contribute to sniffer response, and could actually be considered as a control check for false positive signals within a sniffer system (Figs. 1 and 2). As an ethylene probe, such sensors would have found tremendous applicability, but unfortunately as predicted, the gas sensors tested were simply not very sensitive to ethylene response (Figs. 1-2).

Canonical distribution of fruit volatiles and selected organic solvents monitored by a matrix of six gas sensors at a single concentration shows that this system has potential to distinguish classes of compounds (Fig 3). This data supports the concept that sensors can be selected for use based upon their differential specificity and predictable response range, that the sensory system is able to discriminate between volatiles common to fruits and vegetables, and to detect variation in their concentration (Figs. 1-3). While we recognize that the controlled calibration of a single compound is far simpler than the complex array of the many volatiles that are naturally emitted by fruit and foodstuffs, these results strongly support the concept that pattern recognition techniques can be used with this technology to develop fingerprints for products and for single and multiple volatile constituents. With the new development in the electronic sniffers within this project to compensate for temperature and relative humidity (see the later section on: Development of On-Line Measurement Methods of Gas Sensors for Melon Ripeness Determination), improved results are anticipated. The significance in knowing the specific aromatic volatiles emitted by the fruit of interest is apparent from these studies, as specific gas sensors can be placed into a sniffer as needed to achieve the best matrix combination for the specific application and the specific fruit. These studies demonstrated the sensitivity of individual gas sensors to a wide range of specific volatiles associated with fruit ripening.

As a preliminary study, we also evaluated the sniffers response to a selected number of pesticides, and observed that across concentration ranges of 0.5-100 ppm, the sniffer was able to fingerprint all of the herbicides (clomazone, dichlorprop, oxyfluorfen, and trifluralin) and insecticides (endosulfan and phosnet) evaluated (Figs. 4-5). While such initial studies do not suggest that this odor sensing system can detect and discriminate pesticide residues on food products, it does suggest that such technology may be applied to detect adverse air quality in agricultural environments.

In conclusion, this study illustrated that aroma sensing technology via an electronic sniffer succeeded in the detection of alcohols, aldehydes, esters, hydrocarbons, and volatile sulfur compounds. In addition, this study demonstrated the feasibility of detecting individual compounds, and most importantly demonstrated the specificity of a range of gas sensors to volatiles associated with fruit ripening. The elegance of this technology and approach is that by using multiple gas sensors, with each varied in their relative sensitivity to a given compound, rapid qualitative assessments of fruit ripening can be achieved. The sniffer does not provide interpretive values, instead, it is a corresponding informational base generated by the sniffer that is used to give meaning to the sniffer signals. Detection and fingerprinting of known aromas by the use of a matrix of sensors can be best utilized to identify unripe to overripe fruits, and to potentially detect damaged fruit products. The sniffer detected volatiles related to fruit quality and the stage of ripeness of fruits, and provides a fast, reliable and objective tool to assess those fruit attributes.

Table 1. Selected volatile chemicals associated with fruit ripening and food quality that were screened against the matrix of gas sensors

List of chemicals	concentration range (ppm)	
Alcohols	min.	max.
ethanol	1.6	600
<i>cis</i> -3-hexen-1-ol	0.76	300
1-octanol	0.55	300
Aldehydes		
benzaldehyde	1.0	300
hexanal	0.75	300
Carbon dioxide	50	500
Esters		
butyl acetate	0.69	300
ethyl acetate	0.90	300
ethyl hexanoate	0.55	300
ethyl 2-methyl butyrate	0.50	300
ethyl propionate	0.79	300
pentyl acetate	0.60	300
Hydrocarbons		
ethylene	0.05	15.4
isobutane	300	3500
toluene	0.11	300
2,2,5-trimethylbutane	0.16	300
xylene	0.74	300
Sulfur compounds		
dipropyldisulfide	0.58	300
ethanethiol	1.2	300

Figure 1. Slopes of the plots of relative responses vs concentrations, for the selected chemicals. The scales are as follows: a-TGS800, b-TGS822, c-TGS824, d-TGS825, e-TGS842 and f-TGS880 sensors.

ethyl 2-methylbutyrate

ethyl propionate

pentyl acetate

ethylene

isobutane

toluene

trimethylbutane

xylene

dipropyl disulfide

ethanethiol

Fig.2. Relative response (R_s/R_o) of the electronic sniffer using a single gas sensing head TGS822) to concentrations of selected volatiles common to fruits and vegetables. The higher the relative response, the less sensitive the sensor is to a given compound. A steep slope, reflects a high sensitivity to variation in concentration.

Figure 3. Canonical distribution of volatile compounds by chemical classes (A: alcohols; B: aldehydes; C: short-chain hydrocarbons; D: sulfur-containing volatiles; E: esters; and F: across chemical classes).

Figure 4. Slopes of the plots of relative responses vs concentrations (0.5-100 ppm, on average), for the selected herbicides (H) and insecticides (I). The axes reflect individual tin dioxide gas sensors (TGS) and include: a-TGS800, b-TGS822, c-TGS824, d-TGS825, e-TGS842 and f-TGS880 sensors.

Figure 5. Relative response (R_s/R_o) of the electronic sniffer using a single gas sensing head (TGS824) to concentrations of selected herbicides (H) and insecticides (I). The higher the relative response, the less sensitive the sensor is to a given compound. A steep slope reflects a high sensitivity to variation in concentration.

Classification of Ripening Muskmelon Fruit Using Aromatic Volatiles

Progressive Changes of Volatile Gas Emissions from ripening Muskmelon Fruit

Abstract: Production of aromatic volatiles, C_2H_4 and CO_2 , were measured both in the internal cavity and in the headspace of enclosed harvested muskmelons (*Cucumis melo* L. var *reticulatis* Ser. cv. Mission) using gas chromatography over four stages of ripeness: unripe, half-ripe, full ripe, and over-ripe. Ripeness stages were determined visually, and ripeness parameters were measured including stem detachment force, flesh firmness, soluble solid contents, and electronic sensing of total volatile emissions. There was a significant increase in the production of aromatic volatiles and CO_2 during fruit ripening, but C_2H_4 typically peaked at the half-ripe stage. Similar results were obtained with two other melon cvs. Superstar and Mediterranean Delight. The same eleven volatile compounds were present in all three cultivars. Changes in total production of aromatic volatiles and CO_2 were correlated highly with changes in flesh firmness ($r=-0.92$ volatiles; $r=-0.80$ CO_2), and stem detachment force ($r=-0.86$ volatiles; $r=-0.77$ CO_2). Bayesian classification of Mission fruit into three ripeness classes (unripe, half-ripe; and the combination of full and over-ripe) using total aromatic volatiles in the fruit cavity and in the fruit headspace results in 88% and 81% classification accuracy, respectively. Electronic sensing of total aromatic volatiles in the fruit headspace resulted in 82% success in classification of fruit into the three ripeness stages. Classification accuracy was lower using C_2H_4 and CO_2 measured in the headspace and cavity of the fruit (69% and 63% for C_2H_4 ; 75% and 65% for CO_2 , respectively).

Introduction: For many fruits, ripening is associated with significant changes in respiratory activity: a lowering of respiration in the mature green fruit, a large increased in respiration during the period of ripening, and then, after reaching a *climacteric peak*, a decrease as the fruit enters a senescent decline (Leopold and Kriedemann, 1975). During melon ripening evolution of both ethylene (C_2H_4) and aromatic volatiles increase. Ethylene, a natural hormone associated with ripening has been extensively studied (Abeles, 1973; Reid, 1985). Muskmelon, a climacteric fruit (Pratt, 1971), undergoes increased rates of respiration along with ethylene production during fruit ripening. Bliss and Pratt (1979) reported a close association between internal ethylene production and muskmelon ripening. When the critical concentration of ethylene is reached, physiological changes associated with fruit ripening are induced.

Pratt (1971) reviewed the role of ethylene and abscission in melons, and suggested that abscission of melon from the vine (known as the *slip*) occurs during increased rates of endogenous ethylene. Rates of

ethylene production and internal ethylene concentrations in muskmelon appear to be under genetic control. (Kendall and Ng, 1988). Formation of the abscission zone can vary by cultivar (Reid, 1985). Development of slip is an important index of fruit maturity, and the consequent decrease in force required to detach fruit from the plant is strongly correlated with ripeness.

Aromatic volatiles are partly responsible for the flavor, aroma, and taste of muskmelons. Volatiles accumulate during fruit ripening for most climacteric and nonclimacteric fruits (Kitamura, 1976). More than forty aromatic volatiles have been identified in muskmelons and significant changes in the composition and relative concentration of aroma compounds occur during fruit ripening (Horvat and Senter, 1987; Kemp et al., 1972; Kemp et al., 1973; Yabumoto et al., 1977). With continued ripening fruit may develop an undesirable aroma and flavor apparently associated with over-production of certain volatiles. Yabumoto et al. (1978) reported that regulation of volatile production by ripening melons is not independent for each aromatic compound, but two groups of compounds appear to be regulated simultaneously. Some showed a continuously accelerating rate of production (*e.g.*, ethyl esters, acetaldehyde, ethanol), whereas others increased rapidly up to the full-ripe stage and then remained constant (*e.g.* acetate esters). Miyazaki and Ookubo (1989) reported that aromatic volatiles and ethylene were much better indicators of fruit ripeness than was visual appearance. Chachin and Iwata (1988) investigated the relationship between production of high boiling point volatiles and quality and concluded that volatiles are a better quality index than total soluble solids (TSS).

Objectives: The objectives of this research were to characterize volatile constituents emitted by three different cultivars of muskmelons during ripening and to determine correlations between the production of aromatic volatiles and ripeness stage of fruits.

Materials and Methods: Plant Material. The cultivars evaluated included 'Mission', a popular Western shipped (Asgrow Seed Co.); 'Superstar', the leading Midwestern and Eastern melon (Harris Moran Seed Co.), which is deeply sutured, and the Israeli green-fleshed muskmelon 'Makdimon', also known as 'Mediterranean Delight' (Hazera Seed Co., Haifa, Israel). The main cultivar tested was 'Mission'; the other cultivars were included to determine whether results could be generalizable across different types of melons. Fields of melons were grown at the Purdue University O'Neill Vegetable Research Center, Lafayette, IN, 15 km south of Purdue University. Melon seeds were sown in the greenhouse and transplanted to the field 17 days later. The melons were grown on black plastic mulch using overhead irrigation with fertility and pest management practices as described by Latin et al. (1991).

Visual Classification. Fruit of each cultivar were harvested and manually classified into one of four ripeness stages: unripe-late green stage prior to slip; half-ripe as associated with half-slip; full ripe at full slip; and over-ripe as associated with post-full slip exhibiting external fruit color changes and cracking along stem detachment section, yet prior to external physical breakdown. Visual classification was based upon a variety of assessments that would be performed by a melon grader and included: color, the degree of slip, formation of net, cracking of fruit, and overall firmness and rind integrity.

Stem Detachment Force. Stem detachment force was measured using a Chatillon DFGHS-50 strain gauge (Chatillon Inc., Greensboro, NC). A cotton cord was attached from the instrument to the fruit stem, at the location of attachment of the fruit stem to the runner. The stem was then pulled manually along the direction of growth until it detached, and the peak force recorded.

Production of Volatile Gases. Fruit were harvested from the field, brought into the laboratory, and left to equilibrate overnight. The day after harvest, single fruit were placed in glass containers (Ca. 9.4 l), sealed for a period of 1 hour and then two gas samples were withdrawn from the headspace, the first to determine emission of ethylene, and the second for emission of CO₂ and aromatic volatiles, via GC analysis. Two additional gas samples were withdrawn from the seed cavity of each fruit using a 5-cm-long stainless-steel needle to determine internal levels of ethylene, as was as CO₂, and aromatic volatiles.

The internal cavity gas samples were diluted to a 1:5 ratio with ambient air. One 2-ml sample from the headspace, and a 1-ml sample from the cavity were directly injected into a Varian 3700 GC for determination of aromatic volatiles and CO₂ (Charles and Simon, 1990). Two 1-ml samples, one from the headspace and one from the cavity, were injected into a Shimadzu GC for ethylene determination as previously described (Simon et al. 1983). Compound identification was verified by using standards and by Mass Spectrometry (MS) with a Finnigan 4000 CG/MS (Finnigan, San Jose, CA) using electron impact analysis (Charles and Simon, 1990). Headspace emission rates were normalized by individual fruit weight and net volume.

Electronic Sampling of Aromatic Volatiles: Production of aromatic volatiles were measured using the first version of the electronic sniffer (see Benday et al. 1995). Each fruit was sampled for 30 seconds on

four different fruit surface locations; adjacent to the stem end, adjacent to the blossom end; and at two points along the equatorial line (from the top and side of the fruit), and the average response for each fruit calculated. There were 4-5 fruit per ripeness stage. The output of the sniffer is a change in the electrical resistance of the semi-conductor. An increased concentration of aromatic volatiles and ethylene induces a decrease in the sensors resistance, which is expressed as the ratio of resistance at a specified time relative to its initial resistance (R_s/R_o).

Internal Characteristics. Internal flesh firmness was measured with an Ametek CADET (Ametek Inc., Hatfield, PA) strain gauge mounted vertically to a manual drill press using a 1.1-cm diameter cylindrical plunger (Mohsenin, 1970). Fruit were halved along the equatorial line, and a 3 cm-thick slice of the entire diameter was placed on a wooden board and three readings per slice were recorded and averaged. The readings were evenly distributed around the slice with each location equidistant from the external rind and the seed cavity. Total soluble solids (TSS) were measured from three locations on the same slice, also equidistant from the rind and the seed cavity. Liquid was manually squeezed from tissue (1 cm diameter), then sieved through sharkskin paper. The percentage of soluble solids was read directly with an ATAGO ATC-1 hand-held temperature compensated refractometer (Atago Inc., Japan).

Statistical Classification of Fruit by Ripeness Stage. Mean separation is a general indicator of classification ability, but the actual rate of classification success depends on the specific classifying method used. Fruit were classified into four ripeness classes based on Bayesian maximum likelihood classification supplied by the SAS DISCRIM procedure (SAS Institute Inc., Cary, NC) using the natural gases produced by the fruit as the classification variable. To evaluate the classification error for an unknown population, the CROSS-VALIDATE option was used, in which each observation being classified is excluded when constructing the discriminating function, from which the expected error rate is calculated. Fruit ripeness classes also were merged to evaluate classification performance into three ripeness classes: unripe, half-ripe and full-ripe.

Results and Discussion: Analytical Characterization of Ripeness. Physical indices of ripeness (stem detachment force, flesh firmness and TSS) were measured to verify the accuracy of the visual classification. Stem detachment force and flesh firmness typically decreased with increased ripening, whereas TSS reached peak value at the half-ripe stage for 'Mission' and 'Superstar', but at the full-ripe stage for 'Mediterranean Delight' (Fig. 1). There were significant differences in each physical index between the ripeness classes based on LSD 5%, yet differences between full-ripe and over-ripe fruit

appeared least separable. We presume this to be largely due to the fruit in the over-ripe class being only slightly riper than those in the full-ripe class, and thus, not significantly differing from the fruit in the full-ripe class. That is, the purposeful selection of fruit that were very over-ripe should have presented a clearer separation.

Measurement of Volatile Compounds. In this initial and preliminary study, eleven different gases and volatiles were detected in the headspace and in the cavity of ripening melon cv. Mission fruit including C_2H_4 , CO_2 , and nine aromatic volatile (AV) compounds (Table 1). Most aromatic volatile compounds detected under our experimental conditions included ethyl-esters (ethyl acetate, ethyl propanoate, ethyl-2-methylbutanoate, ethyl hexanoate), various substituted acetates, and one unidentified compound (mol. weight 144). These volatiles were also reported in other melon cultivars (Yabumoto et al., 1978; Wyllie and Leach, 1990). Many additional volatile compounds are known to be in muskmelon fruit, and perhaps could have been detected using another column or different extraction technique (Kemp et al., 1971). The retention time of compound No. 9 did not match that of any known compound from our standards or from our MS libraries and is therefore noted as unidentified. Identical peaks were found in cvs. Superstar and Mediterranean Delight, except for compounds No. 7, 9, and 11, which did not appear in the gas samples from fruit of 'Mediterranean Delight'.

Ripening and AV Emissions. An increase both in the number of compounds detected and the emission rates of aromatic volatiles with ripening was detected both in the headspace and cavity of 'Mission' melons (Fig. 2). Buyl acetate, isobuyl acetate, and 2-methylbutyl acetate were the predominant volatiles detected in full-ripe melons both in the headspace and internally. Ethyl acetate was the volatile of highest internal concentration at the full-ripe stage but appeared only in the headspace with over-ripe fruit. The rapid acceleration of ethyl acetate also was observed with ethyl-esters, acetaldehyde and ethanol by Yabumoto et al. (1978). For unripe fruit, there were only trace amounts of aromatic volatiles in the headspace, yet sampling of the internal cavities revealed the presence of isobutyl-acetate and 2-methylbutyl acetate. Both compounds later appeared in the headspace of half-ripe and mature melons. The extremely low levels of aromatic volatiles emitted by the unripe fruit are particularly significant (Fig. 3). Total aromatic volatiles from both the headspace and fruit cavity increased with increasing ripeness stage. Measurement of volatile gases captured in the headspace and by internal sampling confirms reports by others (Horvat and Senter, 1987; Kemp et al., 1973; Yabumoto et al., 1978) that the ripeness stage of muskmelons is correlates with the production of aromatic volatiles.

Measurement of total headspace gas emissions using the electronic sniffer indicated a significant decrease in the ratio of resistance of the sensor, which corresponds to an increase of headspace gas emissions as fruit ripen (Fig. 4). Benady et al. (1995) reported successful use of the electronic sniffer as a method to nondestructively assess fruit ripeness.

A strong negative correlation was found between the log transformation of total aromatic volatile emissions and physical indices of ripeness measured; headspace production rates were significantly correlated with stem detachment force ($p=0.0001$) and flesh firmness ($p=0.0002$) at $r=-0.92$ and $r=-0.86$, respectively. Internal cavity measurements were significantly correlated with stem detachment force ($p=0.0001$) at $r=-0.86$, and flesh firmness ($p=0.0002$) at $r=-0.85$.

The respiration rate of fruit, as measured by changes in concentration of CO_2 in the fruit headspace and cavity, increased over the ripeness stages (Fig. 3). The increase in CO_2 emissions in the headspace gases and internal cavity of muskmelons was anticipated, as this type of melon exhibits a climacteric increase. Correlations between the log-transformed date for CO_2 production in the cavity and headspace with stem detachment force and flesh firmness ranged between $r=-0.77$ and $r=-0.80$, at $p \leq 0.0005$. An eventual decrease in CO_2 emissions would have been expected if the fruit were kept in storage. Pratt et al., (1977) reported that in 'Honey Dew' melons, decreases in CO_2 emission were observed following several days in storage. The 'Honey Dew' melon (*C. melo* L.) also are reported to show less increase in CO_2 and C_2H_4 during ripening (Miccolis and Saltveit Jr., 1991).

Internal C_2H_4 production reached a peak at the half-ripe stage for 'Mission' and 'Mediterranean Delight', however, no peak was detected for 'Superstar' (Fig. 5). In the headspace gas, C_2H_4 emission reached a peak at the half-ripe stage for 'Mission' and 'Superstar', while no peak was observed for 'Mediterranean Delight'. Presumably, less mature and younger fruit would have emitted lower amounts of C_2H_4 . Pratt (1971) reported that a critical concentration of C_2H_4 (about 3 ppm) occurs when the fruit is mature, a level of C_2H_4 already exceeded in these unripe fruit.

Ripeness Classification from Production of Volatiles. The success of placing 'Mission' fruit into discrete ripeness classes according to natural gas emissions profiles was compared with that of other quantitative indicators (Table 2). Classification accuracy into four ripeness stages as 65% for both flesh firmness and stem detachment force, whereas classification accuracy based on AV in the headspace, internal cavity, or

the electronic sniffer were 63%, 59% and 64.0%, respectively. Classification accuracy was lower when based on C_2H_4 or CO_2 . Merging the full-ripe and over-ripe classes, which were not significantly different from each other in most cases (Fig. 1), improved the classification accuracy. For the 3-way classification, firmness or stem detachment force reached 85% accuracy; AV increased to 81% and 88% for headspace and cavity measurements, respectively, and to 82% using the electronic sniffer.

Summary. This study confirms that emissions of aromatic volatiles are excellent indicators of fruit ripeness, and that there are characteristic quantitative and qualitative changes in volatiles during fruit ripening. The demonstrated ability to non-destructively measure these changes with an electronic sniffer that measures aromatic volatile emissions could lead to the development of on-line, continuous monitoring of fruit ripeness and quality during harvesting, storage and marketing, or a system that could be used off-line for inspection and grading.

References:

- Abeles, F.B. 1973. *Ethylene in plant biology*. Academic Press, N.Y.
- Benady, M., J.E. Simon, D.J. Charles, and G.E. Miles. 1995. Fruit ripeness determination by electronic sensing of aromatic volatiles. *Trans. of the ASAE* 38:251-257.
- Bliss, M.L. and H.K. Pratt. 1979. Effect of ethylene, maturity, and attachment to the parent plant on production of volatile compounds by muskmelons. *J. Amer. Soc. Hort. Sci.*, 104:273-277.
- Chachin, K. and T. Iwata. 1988. Physiological and compositional changes in 'Prince Melon' fruit during development and ripening. *Bull. Univ. Osaka Pref. Series B*, 40:27-35.
- Charles, D.J. and J.E. Simon. 1990. Comparison of extraction methods for the rapid determination of essential oil content and composition of basil (*Ocimum* spp.). *J. Amer. Soc. Hort. Sci.*, 115:458-462.
- Horvat, R.J. and S.D. Senter. 1987. Identification of additional volatile compounds from cantaloupe. *J. Food Sci.*, 52:1097-1098.
- Kemp, T.R., D.E. Knavel, and L.P. Stoltz. 1971. Characterization of some volatile components of muskmelon fruit. *Phytochemistry*, (pp. 1925-1928).
- Kemp, T.R., D.E. Knavel, and L.P. Stoltz. 1973. Volatile *Cucumis melo* components: Identification of additional compounds and effects of storage conditions. *Phytochemistry*, 12:2921-2924.
- Kemp, T.R., L.P. Stoltz, and D.E. Knavel. 1972. Volatile components of muskmelon fruit. *J. Agric. Food Chem.*, 20:196-198.
- Kendall, S.A. and T.J. Ng. 1988. Genetic variation of ethylene production in harvested muskmelon fruits. *HortScience*, 23:759-761.
- Kitamura, T., T. Umenoto, T. Iwata, and T. Akazawa. 1976. Studies on the storage of melons, III: Varietal differences in the fluctuation of the amount of volatile constituents produced during ripening. *J. Jap. Soc. Hort. Sci.*, 44:417-421.
- Latin, R.X., R.E. Foster, and J.E. Simon. 1991. *Vegetable production guide for commercial growers*. ID-56. Purdue Univ. Coop. Ext. Serv.
- Leopold, A.C. and P.E. Kriedemann. 1975. *Plant Growth and Development*. McGraw Hill Book Company.
- Miccolis, V. and M.E. Saltweit Jr., 1991. Morphological and physiological changes during fruit growth and maturation of seven (*Cucumis melo* L.) cultivars. *J. Amer. Soc. Hort. Sci.*, 116:1025-1029.
- Miyazaki, T. and M. Ookubo. 1989. Effects of maturity and postharvest techniques on keeping quality of melons. *J. Jap. Soc. Hort. Sci.*, 58:361-368.
- Mohsenin, N.N. 1970. *Physical Properties of Plant and Animal Materials*. Gordon and Breach Science Publishers.

- Pratt, H.K. 1971. Melons. In A.C. Hulme (Ed.), *The biochemistry of fruits and their products*, volume 2 (pp. 207-232). Academic Press, London.
- Pratt, H.K., J.D. Goeschl, and F.W. Martin. 1977. Fruit growth and development, ripening and the role of ethylene in the Hone Dew muskmelon. *J. Amer. Soc. Hort. Sci.*, 95:814-815.
- Reid, M.S. 1985. Ethylene and abscission. *HortScience*, 20:45-50.
- Simon, J.E., D.R. Decoteau, and L.E. Craker. 1983. Rapid detection of phytotoxic compounds using stress-induced ethylene and ethane. *Environ. Tech. Letters*, 4:157-162.
- Wyllie, S.G. and D. Leach. 1990. Aroma volatiles of *Cucumis melo* cv. Golden Crispy. *J. Agric. Food Chem.*, 38:2042-2044.
- Yabumoto, K., W.G. Jennings, and M. Yamaguchi. 1977. Volatile constituents of cantaloupe, *Cucumis melo*, and their biogenesis. *J. Food Sci.*, 42:32-37.
- Yabumoto, K., M. Yamaguchi, and W.G. Jennings. 1978. Production of volatile compounds by muskmelon, *Cucumis melo*. *Food Chemistry*, 3:7-16.

Table 1: Initial studies on the volatile compounds identified by gas chromatography in muskmelons, cvs. Mission, Superstar, and Mediterranean Delight.

Peak No. ^a	Volatile Constituent ^b	Compound	Molecular weight
1	Ethylene	C ₂ H ₄	24
2	Carbon dioxide	CO ₂	44
3	Ethyl acetate	C ₄ H ₈ O ₂	88
4	Ethyl propanoate	C ₅ H ₁₀ O ₂	102
5	Isobutyl acetate	C ₆ H ₁₂ O ₂	116
6	Butyl acetate	C ₆ H ₁₂ O ₂	116
7	Ethyl-2-methylbutanoate	C ₇ H ₁₄ O ₂	130
8	2-methylbutyl acetate	C ₇ H ₁₄ O ₂	130
9	Unidentified	C ₈ H ₁₆ O ₂	144
10	Ethyl hexanoate	C ₈ H ₁₆ O ₂	144
11	Hexyl acetate	C ₈ H ₁₆ O ₂	144

^aListed in order of elution except for C₂H₄, which was detected using a different column and GC as described in text.

^bAll compounds were verified by GC/MS, except for C₂H₄, which was verified only by a standard.

Table 2: Classification accuracy (%) of muskmelon cv. Mission fruit into ripeness classes using different volatile measurements and physical ripeness indicators. Data based on 16-20 fruit per measurement.

No. of Ripeness Classes	Headspace			Cavity			Electronic	Flesh	Detachment	TSS
	AV ^a	CO ₂	C ₂ H ₄	AV	CO ₂	C ₂ H ₄	Sniffer	Firmness	Force	
4 ^b	62.5	43.8	50.0	58.8	52.9	57.9	64.0	65.0	65.0	50.0
3 ^c	81.3	75.0	68.8	88.2	64.7	63.7	81.7	85.0	85.0	55.0

^aAV=aromatic volatiles.

^b4=Unripe, Half-ripe, Full-ripe, Over-ripe.

^c3=Unripe, Half-ripe, Full-ripe and Over-ripe.

Figure 1. Changes in stem detachment force, flesh firmness, and percent soluble solids of muskmelon cvs. Mission, Superstar and Mediterranean Delight, over 4 ripeness stages. Data are for 83 'Mission' fruit, 16 'Superstar' fruit and 16 'Mediterranean Delight' fruit. Vertical bars represent 5% LSD for each measurement.

Figure 2. Changes in production of aromatic volatile constituents from (A) the headspace of ripening melons, and (B) the cavity of ripening 'Mission' melons. Aromatic volatile constituents 3-11 are identified in Table 1. Data are based on 4 fruit per ripeness class.

Figure 3. Changes in production of total aromatic volatiles (AV) and CO₂ by Mission melons over 4 ripeness stages from (A) the headspace of ripening fruit and (B) the internal cavity. Data are based on 4 fruit per ripeness stage. Vertical bars represents the 5% LSD for each measurement.

Figure 4. Changes in the ratio of resistance of the electronic sniffer response across four ripeness stages of 'Mission', 'Superstar' and 'Mediterranean Delight' melons. R_s = sensor resistance following sampling; R_0 = sensor resistance immediately prior to sampling. Data are for 83 'Mission' fruit, 16 'Superstar' fruit and 16 'Mediterranean Delight' fruit. Vertical bars represent the overall 5% LSD.

Figure 5. Changes in production of C_2H_4 by melons cvs. Mission, Superstar and Mediterranean Delight over 4 ripeness stages, as measured in (A) the fruit headspace, and (B) the fruit cavity. Data are for 83 Mission fruit, 16 Superstar and 16 Mediterranean Delight. Vertical bars represent the overall 5% LSD.

Chemical Characterization of Melon Volatiles

Volatile Constituents of muskmelon, cv. Mission: The volatiles of muskmelon were studied by dichloromethane extraction and dynamic headspace sampling methods and gas chromatography and gas chromatography-mass spectroscopy. Of the 34 identified volatile compounds, four have been detected in muskmelon for the first time: ethyl 3-hydroxybutyrate, hexyl formate, ethyl 3-methylpentanoate, and 1-methylbutyl acetate. Muskmelon fruits were also separated into five layers including the exocarp, outer mesocarp, middle mesocarp, inner mesocarp and seed cavity. The volatile constituents differed significantly in both content and composition by fruit tissue.

Background on Melons: Muskmelon (*Cucumis melo* L.var. *reticulatus* Ser.), a polymorphic species (Whitaker and Davis, 1962) is an important fresh food crop in the world. Several species of *C. melo* are used as fresh fruits or in processed foods, but muskmelon (syn. netted melon, cantaloupe) probably is the most economically important melon. Aroma volatiles are a major determinant of muskmelon fruit quality as perceived by the consumer, and as such have been examined by several investigators (Kemp et al., 1971, 1972a, b, 1973, 1974; Yamaguchi et al., 1977; Yabumoto et al., 1977; Yabumoto et al., 1978; Buttery et al., 1982; Horvat and Senter, 1987; Wyllie and Leach, 1990, 1992; Homatidou et al., 1992). These reports show that the aroma volatiles of melon consist of a complex mixture of esters along with other constituents such as nine carbon unsaturated aldehydes, alcohols, and sulfur-containing compounds (Kemp et al, 1971, 1972a,b, 1974; Yabumoto et al., 1977; Buttery et al., 1982). The aroma volatiles of 'Mission', a major commercial cultivar produced in the western U.S. states and shipped nationwide, has not been investigated. This study reports the isolation and characterization of the aroma volatiles obtained from *Cucumis melo* cv. Mission grown in Indiana, and the influence of distinct fruit tissue regions, including the exocarp, outer mesocarp, middle mesocarp, inner mesocarp, and seed cavity lining, on the emissions of aromatic compounds.

Methods: Plant material. Ripe fresh muskmelons, cv. Mission were manually harvested from experimental fields at the O'Neill Vegetable Research Farm, Department of Horticulture, Purdue University, West Lafayette and analyzed immediately after harvesting. Ripeness was judged visually by color, size of fruit, and full-slip stage.

Volatile composition. Volatile constituents were extracted either by solvent extraction or by dynamic headspace methods and analyzed by gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS) as described below.

Solvent extraction. Small pieces (ca.4×2×0.5 cm) of melon tissue, free of the exocarp, seeds and seed cavity, were immersed in 30ml dichloromethane and stored at 5°C for 5 days. After 5 days the tissue was blended at 25°C in a blender for 2min. After filtering and removing the aqueous layer, the dichloromethane solution was dried for 1 hr. with anhydrous sodium sulfate. The solution was then concentrated by rotary evaporator at 20 °C to 100 µl.

Dynamic headspace sampling. Fruits were cut in half and placed in a modified 4-L sealed wide-mouth desiccator. Air was purified by passing through activated charcoal into the desiccator's headspace at 35 ml/min and subsequently exited through an adsorbent trap. A photograph of the Dynamic Headspace Volatile Gas Capture System is in the **Appendix**. Volatile compounds were removed from the headspace of the desiccator by gas purging and trapped onto a porous adsorbent (ORBO 32, Supelco, Bellefonte, PA). After 2 hr, vials were removed and the adsorbent placed in a 5 ml vial with 2 ml dichloromethane. The vials were placed on a sample agitator (Supelco, Bellefonte, PA) for 60 min, the dichloromethane was removed and concentrated to 100 µl with a gentle stream of air at room temperature.

Contribution of different fruit tissues to volatile constituents. Fruits were divided into five distinct sections (layer 1-5 from exocarp to core). The five fruit sections involved: Layer 1: exocarp or rind; Layer 2: outer mesocarp, including the green colored area adjacent to the rind; Layer 3: middle mesocarp, including the orange flesh; Layer 4: the inner mesocarp, of orange flesh adjacent to the seed cavity and between Layers 3 and 5; and Layer 5: the seed cavity or core, but without seeds. Each section was cut into small slices (ca.5×5×0.5 cm) and extracted with dichloromethane at room temperature as described. The solution containing fruit residues and water was discarded, and the organic phase dried by anhydrous sodium sulfate and concentrated by rotary evaporator at 20 °C to 100 µl.

Gas chromatography analysis. The concentrated solution was analyzed using a Varian 3700 gas chromatography equipped with FID and a Varian electronic 4270 integrator (Varian, Walnut Creek, CA.). A fused silica capillary column (30 m × 0.25 mm i.d.) with the stationary phase RSL-200 (Alltech) was used. Chromatographic conditions were as follows: initial temperature, 40 °C for 5 min; program rate, 5 °C / min.; final temperature, 160 °C for 2 min.; injector temperature, 160 °C; detector temperature, 300 °C; carrier gas, helium (100: 1 split-vent ratio) (Charles and Simon, 1995).

Gas Chromatograph- Mass Spectroscopy. The MS conditions were: ionization voltage of 70 eV; emission current of 40 µA; scan rate and range of one scan per second and 40 to 500 Da, respectively;

and a source temperature of 160 °C. The identification of volatile constituents were accomplished by comparing the mass spectra of each compound with different MS compound libraries for best fit (Stenhagen, 1974; Heller and Milne, 1978; Adams, 1989), and confirmed by notation MS based upon interpretation of mass spectral data.

Results and Discussion: Two different methods were used to isolate the volatile components of Mission melon. The concentrates obtained by each method were subjected to GC and GC-MS analysis of volatile constituents. Thirty-four volatile compounds were identified in these fruits (Table 1). Twenty-three of the 34 constituents were detected in concentrates of both extraction methods but their relative amounts differed. One compound, hexanal, was only detected by dynamic headspace sampling. In contrast, ten compounds were detected only by solvent extraction. While most compounds have been reported in *Cucumis melo* volatiles by previous researchers (Table 1), we identified four compounds that have not been reported in this plant species. Of these, ethyl 3-hydroxybutyrate was only detected by solvent extraction, hexyl formate was only detected by headspace sampling, while 1-methylbutyl acetate and ethyl 3-methylpentanoate were present by both sampling methods.

Aliphatic hydroxy-esters have been seldom reported as aroma volatiles of muskmelon. The only hydroxy-ester previously reported was ethyl 3-hydroxyhexanoate (Homatidou et al., 1992). It has been suggested that the low molecular weight esters (C_3 - C_{12}), a major class of volatiles, are enzymically synthesized by combining acid and alcohol moieties. The acids and the alcohols are originated by metabolism of amino acids and fatty acids (Yabumoto et al., 1977). The 3-hydroxy-carboxylic acids can be produced from the β -oxidation intermediate (White et al., 1978). The discovery of *Cucumis melo* volatile 3-hydroxy-esters, the ethyl 3-hydroxybutyrate and ethyl 3-hydroxyhexanoate, strongly supports this suggestion.

Vanillin (3-methoxy-4-hydroxybenzaldehyde) has been found to be a volatile component of many fruits including the Hami melon (*Cucumis melo*) by Moshonas et al. (1993), and our study supports this finding as vanillin was also present in 'Mission' melon. Vanillin occurs naturally in vanilla and potato parings and is used as a flavoring because of pleasant aromatic vanilla odor and taste (Stecher, 1968). Vanillin, contains a phenyl-hydroxy group and an aldehyde group which is not stable, and its relatively high boiling point (285°C) makes it difficult to trap by steam distillation, headspace sampling, and other evaporating methods. The low concentration of vanillin in the fruit (< 1% of total volatiles) as found in our study and by Moshonas et al. (1993) also suggests that this compound is difficult to detect by GC. These factors may explain the lack of reports showing that melons synthesize this volatile. Vanillin is

widely used as a 'sweetener' in fruit flavors (Arctander, 1969), and recently considered as an important component in orange flavor (Marin et al., 1992). The occurrence of vanillin in muskmelons is an interesting observation though its contribution to the fruit's aroma is unknown.

The related potent odorant (Z)-6-nonenal, known as a characteristic melon aroma and previously detected in *Cucumis melo* by Kemp et al (1972) and Homatidou et al. (1992), was not detected in this study. Whether this is due to sampling technique or simply the use of a different cultivar is unknown. Sulfur-containing compounds, including thioether-acid ester, thioether-alcohol ester, and thioether alcohol, have been reported (Wyllie and Leach, 1992; Homatidou et al., 1992). The importance of these sulfur compounds in the fruit aroma volatiles were suggested and reviewed by Wyllie and Leach (1992). The presence of the five sulfur-containing compounds in other muskmelon cultivars is summarized in Table 2. Wyllie and Leach (1992) reported that there are cultivar differences in sulfur-containing compounds in melons (Table 2). In our study, we detected two sulfur-containing compounds, ethyl (methylthio)acetate and methyl (methylthio)acetate. The distribution of sulfur-containing compounds, methyl (methylthio)acetate and ethyl (methylthio)acetate, can be affected by fruit location difference as shown in this study (Table 3). Exocarp and outer mesocarp contained no methyl (methylthio)acetate while middle mesocarp contained 0.57 %; exocarp and seed cavity contained more ethyl (methylthio)acetate (0.36 % in exocarp, 0.42 % in seed cavity), while middle mesocarp had 0.21 %.

Formic esters have been reported as aroma volatiles of tomato and tomato products (Petro-Turza, 1987), but have not been previously detected in melon volatiles. Hexyl formate is the first formic ester found in muskmelon (Table 1). In addition, 1-methylbutyl acetate and ethyl 3-methylpentanoate were also detected in muskmelon for the first time (Table 1). Esters were the major components found in Mission melon volatiles in this study. Although the (Z)-6-nonenal and sulfur-containing compounds have been reported as having important roles in the overall aroma profiles of melon fruit (Kemp et al., 1972; Wyllie and Leach, 1992), it is reasonable to suggest that the large quantities of highly volatile and aromatic esters also contribute to the strong and characteristic muskmelon aroma.

The relationship between melon fruit volatiles and ripeness or storage conditions have been studied previously (Kemp et al., 1973; Benady et al., 1995), but such work had not attempted to isolate volatile constituents by fruit tissue section. This study examined volatile emissions by fruit sections and significant differences of aroma volatiles between fruit section were observed (Table 3, Fig. 1). Samples from each of the different layers had a distinct contribution to the total volatiles (based on weight). The levels of the total volatiles contained in Layer 4 (inner mesocarp) and Layer 3 (middle mesocarp) were

higher than all other layers (exocarp, outer mesocarp, and seed cavity). The exocarp (Layer 1) contained the lowest level of total aromatic volatiles. The composition of aroma compounds in each layer was also generally different. The relative amount of some compounds was greater in the fruit tissue section closest to the rind, such as methyl hexanoate and methyl butyrate; ethyl acetate was recovered as a major component from the middle mesocarp tissue section (Layers 3 and 4). In contrast, the relative amount of butyl acetate increased from the exterior or exocarp to the interior or core. Vanillin and methyl (methylthio)acetate were absent in the exocarp. The relative amount of ethyl (methylthio)acetate or benzyl alcohol was greater in the middle mesocarp relative to both the exocarp and the core. Other compounds did not appear to be strongly linked with specific tissue regions. These results suggest that volatile constituents differed significantly in both content and composition by fruit tissue sections.

Table 1. Volatile Constituents Identified in Muskmelon, cv. Mission

Chemical constituents	Order of elution	Characteristic MS ions ^a , m/e	Previously reported ^d
benzaldehyde	23	106, 105, 77, 74, 51	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
benzyl acetate	30	150, 108, 91, 79, 65, 43	Buttery, 1982; Homatidou, 1992;
benzyl alcohol	25	108, 107, 91, 79, 77, 65, 51	Buttery, 1982; Homatidou, 1992;
butyl acetate	5	116, 73, 61, 56, 55, 54, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
butyl propionate ^c	16	87, 75, 71, 57, 56, 55	Yabumoto, 1977
ethyl acetate	1	88, 73, 70, 61, 45, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
ethyl propionate	2	102, 75, 74, 73, 57, 43	Homatidou, 1992; Wyllie, 1990
ethyl butyrate	8	116, 101, 88, 73, 71, 60, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
ethyl 2-methylbutyrate ^c	11	130, 115, 102, 85, 74, 57, 45	Buttery, 1982; Homatidou, 1992;
ethyl 3-hydroxybutyrate ^c	19	117, 114, 88, 87, 60, 45, 43	^d
ethyl 3-hydroxyhexanoate	27	117, 97, 88, 73, 71, 60, 57, 55, 43	Homatidou, 1992
ethyl hexanoate	22	144, 99, 88, 73, 60, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
ethyl (methylthio)acetate	21	134, 119, 105, 88, 61, 45	Wyllie, 1990; Homatidou, 1992
ethyl 2-methylpropionate	4	116, 89, 88, 73, 71, 45, 43	Wyllie, 1990
ethyl 3-methylpentanoate		144, 99, 88, 73, 60, 45	^d
hexanal ^b	7	99, 82, 72, 67, 57, 56, 44, 43	Homatidou, 1992; Moshonas, 1993
hexyl formate ^c	12	84, 71, 69, 56, 55, 43	^d
hexyl acetate	24	101, 84, 73, 69, 61, 56, 55, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
hexyl hexanoate ^c	28	117, 99, 84, 71, 69, 67, 55, 43	Kemp, 1972
hexadecanoic acid ^c	33	256, 213, 185, 157, 129, 115, 73, 43	Homatidou, 1992; Moshonas, 1993
1-methylbutyl acetate	10	130, 87, 72, 70, 45, 43	^d
3-methylbutyl acetate	13	130, 87, 73, 70, 61, 55, 43	Buttery, 1982; Homatidou, 1992;
methyl hexanoate	18	130, 99, 87, 74, 59, 55, 45, 43	Homatidou, 1992
methyl 2-methylbutyrate	6	116, 101, 88, 85, 69, 59, 57	Wyllie, 1990; Homatidou, 1992
1-methylpropyl acetate	9	117, 87, 73, 61, 56, 43	Wyllie, 1990; Yabumoto, 1977
1-methylpropyl butyrate	20	101, 89, 73, 71, 60, 56, 43	Homatidou, 1992
methyl butyrate	3	102, 87, 74, 71, 59, 55, 43	Buttery, 1982; Homatidou, 1992;

methyl (methylthio)acetate ^c	14	120 , 105, 74, <i>61</i> , 46, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
1-octanol	26	84, 83, 70, 69, 56, 55, 43	Homatidou, 1992
propyl butyrate	15	101, 89, 73, <i>71</i> , 61, 60, 43	Homatidou, 1992
phenylpropyl alcohol ^c	29	136 , <i>117</i> , 105, 91, 77, 65, 51	Homatidou, 1992
phenylpropyl acetate	31	118, <i>117</i> , 105, 91, 77, 43	Horva, 1987
pentyl acetate	17	73, 70, 61, 58, 55, 43	Buttery, 1982; Wyllie, 1990; Homatidou, 1992
vanillin ^c	32	152 , <i>151</i> , 123, 109, 81, 65	Moshonas, 1993

^a Ions are listed in descending order of m/e value. Not necessarily the most intense ions but considered characteristic for that compound. The molecular ions are presented in bold if they are detected. The base ions are presented in italic.

^b Compound detected only in dynamic headspace method.

^c Compound detected only by solvent extraction method.

^d Where no reference is provided, the compound is detected in muskmelon for the first time.

Table 2. Presence of Thioether Esters in *Cucumis melo* Cultivars

Cultivar	ethyl (methylthio) acetate	ethyl 3-(methylthio) propanoate	methyl (methylthio) acetate	2-(methylthio) ethyl acetate	3-(methylthio) propyl acetate	Previously reported ^a
Crenshaw	-	-	-	-	-	Yabumoto, 1978
Cantaloupe 'PRM-45'	-	-	-	-	-	Yabumoto, 1978
Cantaloupe 'Top Mark'	-	-	-	-	-	Yabumoto, 1978
Golden Cispy	+	+	+	+	+	Wyllie, 1990
Hami	-	-	-	-	-	Moshonas, 1993
Honeydew	-	-	-	-	-	Yabumoto, 1978
Mission	+	-	+	-	-	^a
Penn Sweet	-	-	-	-	-	Kemp, 1972
Saticoy	+	-	-	-	-	Horva, 1987

^a Where no reference is provided, the cultivar is studied for the first time.

Table 3. Differences in Total Volatiles and Individual Volatile Compounds by Different Fruit Tissue

Layer ID ^b	Relative total volatiles ^a	Volatile Constituents (As area % of total volatiles)													
		ethyl acetate	ethyl propionate	methyl butyrate	butyl acetate	2-methyl butyrate	ethyl butyrate	ethyl 2-methyl butyrate	3-methyl butyl acetate	methyl (methylthio) acetate	methyl hexanoate	ethyl (ethylthio) acetate	ethyl hexanoate	benzyl alcohol	
1	25.4 %	4.81	1.01	30.04	3.17	0.53	0.53	0.26	1.29	0	0.19	0.36	0.49	10.09	
2	44.2 %	30.56	6.58	17.88	12.14	1.84	1.05	0.87	5.0	0	0.61	0.34	2.64	4.88	
3	74.5 %	47.07	4.06	8.39	14.2	2.59	0.89	3.02	3.72	0.57	0.03	0.21	0.89	3.35	
4	100.0 %	38.28	2.64	9.38	18.38	1.33	1.56	0.83	7.46	0.44	0.03	0.27	0.93	4.55	
5	49.49 %	24.81	5.01	14.46	21.56	1.38	1.75	2.85	7.61	0.35	0.04	0.42	0.76	7.25	

^a Relative total volatiles = total volatiles contributed by fresh fruit (g)/ total volatiles contributed by layer No.4 (g fresh fruit tissue)

^b 1 = exocarp, 2 = outer mesocarp, 3 = middle mesocarp, 4 = inner mesocarp, 5 = seed cavity.

Figure 1. The Differences of Relative Total volatile and Relative Percentages in Different Layers
 A = relative percentage of ethyl acetate in the total volatiles; B = relative total volatiles; C = relative percentage of butyl acetate in the total volatiles; D = relative percentage of benzyl alcohol in total volatiles.

Classification of Ripening Apples Using Aromatic Volatiles and an Electronic Sniffer

Abstract: The determination of apple ripeness was evaluated using aroma sensing technology. Golden Delicious and GoldRush apples were harvested weekly (19 Sept. - 17 Oct.; and 27 Sept. - 2 Dec., respectively). Initial harvest for Golden Delicious occurred after fruits began to ripen while GoldRush fruits were first harvested when fruit were visually unripe. Harvest continued until fruit of each cultivar were fully ripe. Apples from each cultivar were classified into three ripeness stages. The fruits were tested by an electronic sniffer which was mounted in a sealed chamber. The electronic sniffer was composed of an array of four tin-oxide gas sensors, each sensitive to a different range of reductive gases, which include the aromatic volatiles naturally emitted by ripening apples. Fruits were also subjected to a wide range of physio-chemical tests including ethylene, starch value, firmness, total soluble solids and acidity, currently used to assess apple ripeness. Multivariate analysis of the changes in the normalized sensor's resistance indicated that Golden Delicious apples can be classified into the three ripeness stages with a 13% classification error, while classification of GoldRush apples resulted in only a 1% error.

Introduction: Respiration rate of climacteric fruits such as apples (Kingston, 1991) increase as fruits reach maturity and ripening is initiated (Dirink et al. 1989; Knee et al. 1989). As fruits ripen, CO₂, ethylene and aromatic volatile emission also increases. While ethylene concentration both rises and falls, there appears to be a continuous increase in total aromatic volatile emissions in ripening apple fruit. Alternatively, increased aromatic volatiles might be used to determine ripeness stages of climacteric fruits. The burst of ethylene production is one of the criteria used to determine optimal harvesting date of apples, but determination of ethylene concentration (as well as other volatiles) requires gas chromatography (Chu, 1988) which is time consuming. Blankenship and Unrath (1988) also reported that ethylene alone cannot be used as a single criteria to determine ripeness. In contrast, Watkins et al. (1989) reported that ethylene production is an effective maturity index for apples, but the results are cultivar specific. Aroma volatiles associated with apple fruit maturation have been characterized in apples (Yahia, 1994), but have not yet been used to monitor fruit ripeness. While real-time analysis is available for ethylene and aromatic volatiles from apples, the use of gas chromatography is often inappropriate and

difficult due to the time required for results for use on farms or during the sorting and grading process of apples.

The emerging technology of gas sensors coupled with the use of multivariate statistics has demonstrated increasing potential to detect the presence of natural volatile gases from fruits and food products and as quality control detectors in food and fruit quality. The principle of using a matrix of gas sensors for odor discrimination was originally demonstrated by Persaud and Dodd (1982) who incorporated three sensors to discriminate a variety of odors. An array of eight sensors was able to classify thirty substances into four families of odors (Abe et al. 1987). An array of six metal oxide sensors were used to identify vapors for liquors (Aishima, 1991a; Cao et al. 1995) and coffee (Aishima, 1991b). An electronic nose was also used to monitor flavors of beer, thus reducing the time to obtain the results from 2-3 days in traditional methods to 40 min (Pearce et al. 1993; Bailey et al. 1995). The advantage of using electronic sensors to monitor fruit volatiles is in the rapidity and precision of response, and the convenience of using the sensing device under industrial or outdoor conditions.

A prototype of an “electronic sniffer” for fruit ripeness was developed in the Indiana Center for New Crops and Plant Products and the Department of Agricultural Engineering, Purdue University (Benady et al. 1995). This system can consist of either a single or multiple stannic oxide gas sensors (Ihokura and Watson, 1994) that are sensitive to a wide variety of volatile gas compounds by changing the electrical resistance of the sensor when exposed to these volatiles. The sensors are mounted on a lid which seals a chamber with an effective volume of 5400cc which can accommodate moderate size fruit or bulk of small fruits. This unit is equipped with a fan to mix the gas inside the chamber. The voltage output from the sensors is continuously digitized and recorded into a portable laptop computer. The voltage output of each sensor is translated into sensor resistance and normalized with respect to the sensor resistance when exposed to a reference gas concentration (R_s/R_0). The electronic sniffer was successfully employed to monitor ripeness of melons (Benady et al. 1992; Benady et al. 1995), strawberries (Hetzroni et al. 1994), and ripeness and quality of blueberries (Benady et al. 1994, Simon et al., 1995), and compared favorably to the traditional physio-chemical indices of fruit maturity.

Individual gas sensors are generally not compound specific. As such, an array of sensors may be required to develop a specific sample fingerprint, whether the sample consists of a single

volatile or complex mixture of gases. In the case of a ripening fruit, the selection to use a single or a matrix of sensors can only be determined by the evaluation of a series of sensors with the specific fruit. The electronic sniffer using an array of sensors has shown compound specificity toward a wide range of volatiles commonly associated with ripening fruit (Angers et al., 1996a) and associated malodors (Angers et al. 1996b). In this study, we examined the potential of using aroma sensing technology to determine the fruit maturity of apples. A matrix of four gas sensors were employed in the electronic sniffer.

Methods: Fruit Samples. Apples were manually harvested from an orchard at the Purdue Horticulture Orchard Farm in West Lafayette, IN during the fall of 1994. Two cultivars were included in this study: "Golden Delicious" (GD) and "GoldRush" (GR). Fruits were manually harvested once a week, starting prior to the expected time of ripeness based on previous years experience, and ending two weeks after the fruit were expected to be fully ripened. For Golden Delicious, apples were sampled from 19 Sept. through 17 Oct.; for GoldRush, 27 Sept. through 2 Dec., 1994. At each harvest, nine apples of each cultivar were subjected to a series of destructive and nondestructive measurements to ascertain fruit ripeness.

Measurements of Ripeness. Skin color was measured in three locations around the equatorial axis (using Minolta CR-200, C illuminant). Color is reported in the a, b, and L range. Fruit weight and diameter were recorded. Headspace volatiles of individual apples were captured inside the test chamber (effective volume was reduced to 3470cc) by the electronic sniffer equipped with four tin oxide gas sensors (TGS: 800, 822, 842, and 880 from Figaro Engineering INC. Osaka, Japan). Headspace gases were directly sampled from inside the chamber with a 1cc syringe and concentration was determined by GC analysis. Concentration of headspace ethylene (sampled in the test chamber) and core ethylene (sampled near the core of the whole apple) were measured. Fruits were then subjected to several physio-chemical measurements (starting about 5h after picking). These tests included: core ethylene (Sfakiotakis and Dilley, 1973); Total Soluble Solids (TSS) (Refractometer, Brix scale); firmness (rupture force) using a Chatillon force gauge (Greensboro, NC) with 11mm diam Plunger (ASAE, 1988); pH (using a Corning pH meter); total acidity (10 ml of apple juice is diluted with 50 ml dist. water and the solution is titrated with 1N NaOH to an end point of 8.2 pH (units are g malic acid/100 mls); and Starch Iodine test (Smith et al. 1979). Starch values, as a starch value index (Smith et al. 1979) was determined using an iodine test (iodine scale 1 to 6). Correlation analysis was conducted on all measured properties for both cultivars.

Ripenes Determination. Fresh apples were classified into three ripeness stages termed I, II and III. To judge the ripeness stage of individual fruit, a method for ripeness classification had to be determined. However, there is no single measurement that can be used to determine ripeness of apples and the measures that are commonly used vary among cultivars, seasons, orchards and individual trees (Kingston, 1991).

We found that the starch index value (SIV) in “GoldRush” apples harvested for this study, monotonously increased from 1.0 to 5.4 over time (from day 270 to day 336). Therefore, the starch index level was used to classify the ripeness stage of individual apples as follows:

stage I $SIV \leq 2$

stage II SIV is between 2 to 5

stage III $SIV \geq 5$

In contrast, the “Golden Delicious” apples did not exhibit any major changes in the same physical properties measured during the time period evaluated, therefore calendar date was used to determine ripeness stage as follows:

stage I harvested before 20 Sept.

stage II harvested between 20 Sept. 1 Oct.

3. stage III harvested after 1 Oct.

The Streif Index of apple ripeness (SI) which is based on the relationship between firmness, TSS and starch was also calculated for both cultivars of apples and also used to determine fruit ripeness as follows:

1. stage I if $SI > 0.2$

2. stage II if $SI > 0.17$ and < 0.2

3. stage III if $SI < 0.17$

Data Acquisition and Signal Analysis. The gas sensors were exposed to the volatiles emitted by whole apples after the apples were placed in the test chamber. The testing chamber was flushed with compressed nitrogen gas prior to placing the samples inside. Data acquisition was initiated as soon as the lid was sealed onto the chamber. Voltage output from the four sensors was recorded in a rate of 0.5Hz for 12min. A typical response of the gas sensor when exposed to a volatile emitting fruit can be described by an exponential function in the form of :

$$V = a + be^{ct}$$

where V is the voltage output of the sensor, t is time, and a , b , and c are coefficients which are governed by the rate of emission through the rind and the volume of the testing chamber. The coefficients were evaluated by regression. The constraints for the regression coefficients were as follows: $a \in \{0,10\}$, the range of values determined by the electronic hardware; $b < 0$; and $c < 0$. According to this model and constraints, the sensor response will monotonously increase during a transitional period and will converge to a .

Each data set was preprocessed to remove the first section of the signal that marks a transitional period of closing the chamber lid and initiation of volatiles emission from the apple. For that, the data file was scanned from the beginning until the data were monotonously not decreasing for at least five consecutive data points (10s). All data points prior to that sequence were rejected. All remaining data were used to evaluate the model parameters to extrapolate sensor output at infinity. The model parameters for each sample were determined using Gauss-Newton method of non-linear regression (SAS, Institute Inc. 1988).

Results: Physio-Chemical Measurements of Ripeness. Few changes in the physicochemical parameters of Golden Delicious apples were found between day 262 and day 290 suggesting that the initial harvest began too late and that the fruit were already mature. There was however, a continual decrease in firmness over time (Table 1). The data on ‘Golden Delicious’ suggests that the unripe stage was passed by the first harvest. In contrast, the harveting time frame for ‘GoldRush’ resulted in the expected changes in the traditional indices of fruit maturity (Table 1). In GoldRush apples, the SIV increased over time, while firmness decreased. No clear trends in apple color during this time frame were observed, though the B color range appeared to minimally increase in value with harvest date. Both pH and TSS appeared to exhibit a weakly nonmonotonous inverse relationship. Core ethylene levels were significantly greater than headspace ethylene concentrations.

The electronic sniffer. Each of the gas sensors in the electronic sniffer was evaluated to determine whether any single sensor could classify the apples into three ripeness groups. Analysis of the means of GoldRush apples (Table 2) suggests that only TGS-822 could be used to discriminate these apples into three classes. The TGS-800 and TGS-842 could classify the GoldRush apples into two groups only, while the change of means between ripeness stages was not monotonous in TGS-880, despite the means separation into three ripeness classes. Discrimination analysis using only a single sensor results with the following classification (cross-

validation) errors: TGS-800 – 51%; TGS-822 – 47%; TGS-842 – 52%; and TGS-880 – 31%, suggesting an unacceptably high error rate.

Analysis of the means of Golden Delicious apples suggests that no single sensor could classify Golden delicious apples into the three predetermined ripeness stages in a monotonous manner (Table 3). Discrimination analysis using only a single sensor resulted in the following classification (cross-validation) error: TGS-800 – 37%; TGS-822 – 37%; TGS-842 – 16%; and TGS-880 – 37%, also indicating an unacceptably high error rate for this second cultivar, though this may be partially explained by an earlier than normal ripening of this cultivar in 1994, thus, missing the unripe stage even at the first harvest.

Ripeness Classification. Multivariate techniques were then applied to evaluate the prospect of ripeness classification using multiple gas sensors. Canonical discrimination is a method in which data points in a multivariate space are linearly mapped to a new coordinate system of reduced dimensionality in which differences between predetermined classes are enhanced (Johnson and Wichern, 1992). Canonical discrimination for apples, and plotting the transformed data onto the first two canonical axis showed that when all four gas sensors are used, individual apples can be classified into three distinct groups (Fig. 1). This observation was supported by quadratic discriminant procedure which classified the “Golden Delicious” apples into three ripeness stages with 7% error (Table 4). Similarly, classification of GoldRush apples to the three predetermined ripeness stages resulted in only 1% error (Table 4). The higher error rate with “Golden Delicious” presumably in part reflects that this cultivar was not initially harvested at an early unripe stage, and this supports both the lack of physio-chemical changes as well as the lack of sniffer response differences across the harvest window used this year for this cultivar.

Discussion: A major problem encountered in this study was the determination and independent classification of the ripeness stage of an individual apple. There is no generally accepted (industrial or scientific) categorization rule, and ripeness determination, though based on fruit physiology, is governed by local environmental and horticultural conditions which may vary within an orchard or even within a tree and by cultivar (Kingston, 1991).

The emission of ethylene is expected to increase for a short period of time indicating the initiation of the climacteric. Significant change in ethylene (either from headspace or from the

core) generally coincides with initial rapid increase in SIV in some apples. Yet, in this study, no major change could be detected in ethylene emission. Firmness, as expected, decreased monotonously as the fruit softens while SIV increased (in 'GoldRush') as starch is converted to sugars. The change in SIV was not significant in the 'Golden Delicious' apples because it was already high (>4) on the first week of harvest indicating the fruit were more mature than visually judged and estimated. Fruit acidity and pH did not show any significant change over time in either of the two cultivars. Total soluble solids in 'Golden Delicious' did not show any significant change with time, while a substantial increase was observed in 'GoldRush'. However, 'Golden Delicious', although edible at the earliest stages became unacceptably soft at the later stages, while 'GoldRush' was never considered organoleptically over-mature, rather it continued to improve in quality.

No single sensor was able to correctly classify the apples into the three ripeness groups as suggested by the analysis of means (Tables and 3). Moreover, the sensor response over time was not always monotonous. This implies that even if analysis of the means indicated that the ripeness classes were significantly different, ambiguity might still occur for measurements that do not clearly belong to any of the predetermined classes.

Improved determination of ripeness stage of the apples was achieved by applying multivariate quadratic classification using the four gas sensors in the electronic sniffer. The prospect of correct discrimination was indicated by the clustering of apples, that belong to the same ripeness stage, in the canonical space (Fig. 1). In this figure, 'GoldRush' apples form three very distinct clusters in the parametric space of canonical variables generated from the electronic sniffer response. While 'Golden Delicious' apples also formed distinct groups, the distinction was less clear than with 'GoldRush'. Improved separation would be expected if 'Golden Delicious' was actually harvested at an earlier initial ripeness stage. Canonical discrimination is a method in which data points in a multivariate space are linearly mapped to a new coordinate system of reduced dimensionality in which differences between predetermined classes are enhanced (Johnson and Wichern, 1992). Canonical discrimination for apples, and plotting the transformed data onto the first two canonical axis show that when all four gas sensors are used, individual apples can be classified into three distinct ripeness groups (Fig. 1). This conclusion is supported by quadratic discriminant analysis using a cross-validation technique procedure which classified

the 'Golden Delicious' and 'goldRush' apples into three ripeness stages based upon harvest/calendar date with 13% classification error for 'Golden Delicious' and based upon a starch index value with a 1% classification error for 'GoldRush'. These results show that electronic sniffer has the potential to separate apple ripeness, but only when multiple gas sensors rather than single sensors are used and when coupled by quadratic classification techniques. We also conclude from this data, that the results will be cultivar specific.

Ripeness determination of apples by analysis of volatile emissions is a nondestructive technique that can be used on individual fruit as well as on bulked and packaged fruit. Although we cannot yet determine whether the classification parameters acquired during this year's study are applicable to classify apples from other sites, the results of this work indicate that such technique may be promising and should be pursued to evaluate whether there are general rules that govern ripeness discrimination by electronic sniffers.

Bibliography:

- Abe, H., T. Yoshimura, S. Kanaya, Y. Takahashi, Y. Miyashita, and S. Sasaki. 1987. Automated odour-sensing system based on plural semiconductor gas sensors and computerized pattern recognition techniques. *Analytica Chimica Acta* 194:1-9.
- Aishima, T. 1991a. Discrimination of liquor aromas by pattern recognition analysis of responses from gas sensor array. *Analytica Chimica Acta* 243:293-300.
- Aishima, T. 1991b. Aroma discrimination by pattern recognition analysis of responses from semiconductor gas sensor array. *J.Agric.Food Chem.* 39(4):752-756.
- Angers, P., A. Hetzroni, D.J. Charles and J.E. Simon. 1996. Electronic sensing of volatile compounds related to fruit ripening and food quality. (unpublished data).
- Angers, P., A. Hetzroni, D.J. Charles and J.E. Simon. 1996. Monitoring air quality in agricultural environments by electronic aroma sensing technology. (unpublished data).
- ASAE. 1988. ASAE Standard: Compression test of food materials of convex shape. S368.1 ASAE. :356-359.
- Bailey, T.P., R.V. Hammond, and K.C. Persaud. 1995. Applications for an electronic aroma detector in the analysis of beer and raw materials. *J.Amer.Soc.Brew.Chemist.* 53(1):39-42.
- Benady, M., A. Hetzroni, J.E. Simon, and B. Bordelon. 1994 Electronic sensing of aromatic volatiles for quality sorting in blueberries. Presented at the annual meeting of the Amer. Soc. for Hort. Sci. *Hortscience* 29:463(Abstract)
- Benady, M., J.E. Simon, D.J. Charles, and G.E. Miles. 1992. Determining melon ripeness by analyzing headspace gas emissions. ASAE paper 92-6055. St. Joseph MI. ASAE.
- Benady, M., J.E. Simon, G.E. Miles, and D.J. Charles. (1995) Fruit ripeness determination by electronic sensing of aromatic volatiles. *Trans. of the ASAE* 38(1):251-257.
- Blankenship, S.M. and C.R. Unrath. 1988. Internal ethylene levels and maturity of 'Delicious' and 'Golden Delicious' apples destined for prompt consumption. *J.Amer.Soc.Hort.Sci.* 113(1):88-91.
- Cao, Z., H.G. Lin, B.F. Wang, Z.Z. Chen, F.L. Ma, K.M. Wang, and R.Q. Yu. 1995. Discrimination of vapours of alcohols and beverage samples using piezoelectric crystal sensor array. *Anal.Lett.* 28:451-466.
- Chu, C.L. 1988. Internal ethylene concentration of 'McIntosh', 'Northern Spy', 'Empire', 'Mutsu', and 'Idared' apples during the harvest season. *J.Amer.Soc.Hort.Sci.* 113(2):226-229.

Dirink, P., H. De Pooter, and N. Schamp. 1989. Aroma development in ripening fruits. In: ed. Teranishi, R., R.G. Buttery, and F. Shahidi. *Flavor chemistry*. ACS symposium series. Washington: American Chemical Society. 23-34.

Hetzroni, A., J.E. Simon, M. Benady, and B. Bordelon. 1994. Electronic Sensing of Aromatic Volatiles for Quality Sorting in Strawberries. Presented at the ASAE summer meeting. ASAE paper No. 94-6029. St. Joseph, MI: ASAE.

Ihokura, K. and J. Watson. 1994. *The Stannic Oxide Gas Sensor*. CRC Press, Inc.

Johnson, R.A. and D.W. Wichern. 1992. *Applied multivariate statistical analysis*. Englewood, NJ: Prentice Hall,

Kingston, C.M. 1991. Maturity indices for apple and pear. In: ed. Janick, J. *Horticultural Reviews*. Vol.13, John Wiley & Sons, Inc. 407-432.

Knee, M., S.G.S. Hatfield, and S.M. Smith. 1989. Evaluation of various indicators of maturity for harvest of apple fruit intended for long-term storage. *Journal of Horticultural Science* 64(4):403-411.

Pearce, T.C., J.W. Gardner, and S. Friel. 1993 Machine olfaction: intelligent sensing of odours. (Abstract)

Persaud, K. and G. Dodd. 1982. Analysis of discrimination mechanisms in the mammalian olfactory system using a model nose. *Nature (London)* 299:352-355.

SAS Institute Inc. 1988. *SAS/STAT User's Guide*. Cary, NC: SAS Institute Inc.

Sfakiotakis, E.M. and D.R. Dilley. 1973. Internal ethylene concentrations in apple fruits attached to or detached from the tree. *J.Amer.Soc.Hort.Sci.* 98(5):501-503.

Simon, J.E., A. Hetzroni, B. Bordelon, G.E. Miles and D.J. Charles. 1995. Electronic sensing of aromatic volatiles for quality sorting of blueberry fruit. *J. Food Sci.* 61(5):967-972.

Smith, R.B., E.C. Loughheed, E.W. Franklin, and I. McMillan. 1979. The starch iodine test for determining stage of maturation in apples to determine first acceptable harvest date. *Canadian journal of plant science* 59(3):725-735.

Watkins, C.B., J. H. Bowen and V.J. Walker. 1989. Assessment of ethylene production by apple cultivars in relation to commercial harvest dates. *New Zealand J. of Crops and Hort. Sci.* 17:327-331.

Yahia, E. M. 1994. Apple Flavors. In: J. Janick (ed). *Horticultural Reviews*. John Wiley & Sons, New York, 16: 197-234.

Table 1. Physio-Chemical properties of apples by harvest date.

Golden Delicious									
day	SIV	pH	A	B	Firmness	Core ethylene (ppm)	Volatile ethylene (ppm)	TSS	Acidity
262	5.17±0.17	3.79±0.02	-16±0.34	40.5±0.42	8.03±0.12	20.2±18.8	0.05±0.02	11±0.31	5.12±0.18
269	5.44±0.19	3.73±0.02	-16±0.3	40.2±0.26	7.38±0.12	0.13±0.02	0.03±0.01	11.2±0.45	5.14±0.19
276	5.89±0.07	3.62±0.04	-15±0.31	39.2±0.45	7.06±0.32	0.16±0.05	0.03±0.01	9.99±0.54	4.88±0.43
283	5.39±0.16	3.73±0.03	-15±0.32	40.1±0.59	6.94±0.14	23.4±15.1	0.04±0.01	11±0.66	5.53±0.25
290	5.22±0.19	3.83±0.03	-14±0.67	41.9±0.74	6.85±0.19	9.54±6.78	0.03±0.01	11.7±0.5	5.53±0.26

GoldRush									
day	SIV	pH	A	B	Firmness	Core ethylene (ppm)	Volatile ethylene (ppm)	TSS	Acidity
270	1±0	3.3±0.02	-9.8±0.75	40.5±0.53	12.3±0.16	0.03 ±0	0.03±0.02	12.8±0.24	7.78±0.16
277	1.56±0.18	3.28±0.01	-7.2±1.37	41.3±0.3	11.5±0.13	0.04± 0.01	0.01±0	13.9±0.37	11.9±0.62
285	2±0	3.27±0.03	-4.7±1.33	42±0.36	10.7±0.16	0.1±0.04	0.02±0	13.9±0.26	9.97±0.54
291	2±0	3.21±0.01	-4.9±0.7	43.8±0.85	11.2±0.32	0.58±0.2	1.01±1	14.9±0.26	10.7±0.58
297	3±0	3.24±0.02	-6.1±1.24	45±0.53	10.8±0.27	0.41±0.12	0.01±0	14.9±0.45	10.3±0.85
304	3.33±0.17	3.18±0.01	-5.2±0.65	49±0.51	11± 0.2	1.46±0.17	0.01±0	16.9±0.2	13.9±0.39
311	4.56±0.18	3.25±0.03	-7.3±1.78	46.4±1.08	10.2±0.19	0.98±0.17	0.02±0	15.2±0.4	10.3±1.02
318	5±0	3.25±0.01	-4.7±0.54	49±0.53	10.8±0.18	0.68±0.1	0.01±0	15.4±0.13	9.2±0.35
322	5±0	3.33±0.01	-4.9±0.68	49.3±0.53	9.69±0.11	0.64±0.13	0.02±0	15.7±0.2	9.93±0.43
336	5.44±0.18	3.41±0.02	-5.7±1.2	44.2±0.43	9.38±0.25	5.89±1.84	0.05±0.01	14.7±0.31	9.06±0.44

SIV=starch index value= 1-6 range.

Total acidity expressed as g malic acid/100ml.

Table 2. Mean values of the response of four electronic sniffers to GoldRush apples based on three ripeness stages determined by starch test index.

stage of development	n	sensors			
		TGS-800	TGS-822	TGS-842	TGS-880
I	32	7.94a ¹	10.5a	14.7a	4.39a
II	21	8.72a	9.58 b	17.7 b	5.16 b
III	29	6.28 b	8.10 c	16.9 b	3.94 c

Mean values in the same column followed by the same letters are not significantly different by Duncan's Multiple Range Test (p= 0.5).

Table 3. Mean values of the response of four electronic sniffers to Golden Delicious apples based on three ripeness stages determined by calendar date.

stage of development	n	sensors			
		TGS-800	TGS-822	TGS-842	TGS-880
I	7	6.14a ²	7.18a	16.2a	4.00a
II	7	4.84a	7.77a	11.0 b	3.25 b
III	26	7.75 b	10.2 b	14.8 c	4.49 c

Mean values in the same column followed by the same letters are not significantly different by Duncan's Multiple Range Test (p= 0.5).

Table 2b. Mean values of the response of four electronic sniffers to GoldRush apples based on three ripeness stages determined by the Streif index.

stage of development	n	sensors			
		TGS-800	TGS-822	TGS-842	TGS-880
SI	32	7.94a ³	10.47a	14.75a	4.39a
SII	26	8.75a	9.61 b	17.87 b	5.16 b
SIII	24	5.73 b	7.76 c	16.56 c	3.70 c

SI, SII, and SIII: Ripeness stages determined by the Streif index.

Mean values in the same column followed by the same letters are not significantly different by Duncan's Multiple Range Test ($p=0.5$).

Table 3b. Mean values of the response of four electronic sniffers to Golden Delicious apples based on three ripeness stages determined by the Streif index.

stage of development	n	sensors			
		TGS-800	TGS-822	TGS-842	TGS-880
SI	14	5.49a ⁴	7.48a	13.59a	3.61a
SII	9	6.18a	9.82 b	13.60a	3.94a
SIII	17	8.57 b	10.36 b	15.36 b	4.77 b

SI, SII, and SIII: Ripeness stages determined by the Streif index.

Mean values in the same column followed by the same letters are not significantly different by Duncan's Multiple Range Test ($p=0.5$).

Table 4. Contingency table of cross validation evaluation for the discrimination of GoldRush (GR) and Golden Delicious (GD) apples into three ripeness stages

From/to stage	GoldRush			Golden Delicious		
	I	II	III	I	II	III
I	32	0	0	7	0	0
II	0	20	1	0	7	0
III	0	5	24	1	0	25

Table 4b. Contingency table of cross validation evaluation for the discrimination of GoldRush (GR) and Golden Delicious (GD) apples into three ripeness stages based upon the Streif index.

From/to stage	GoldRush			Golden Delicious		
	I	II	III	I	II	III
I	32	0	0	12	1	1
II	0	25	1	0	9	0
III	0	0	24	1	2	14

Table 5. Pearson correlation of measured properties from Golden Delicious apples

	SIV	pH	a	b	Firm- ness	C. Ethyl	V. Ethyl	TSS	acid- ity	TGS - 880	TGS - 822	TGS - 842	TGS - 880
date	.01	.10	.54*	.22	-.57*	.01	-.09	.10	.21	.61*	.69*	.19	.58*
SIV		-.13	-.03	-.44	-.17	.15	-.21	-.54*	-.40	.14	.27	.23	.10
pH			.11	.11	-.02	.11	.19	-.11	-.32*	.22	-.03	.27	.17
a				.25	-.31	-.00	-.11	-.38*	.12	.38*	.31*	.30	.41*
b					-.18	-.00	.21	.73*	.51*	.00	-.15	.11	.05
Firmness						-.09	-.11	-.06	.18	-.32*	-.45	.01	-.32*
C. Ethyl							.42*	-.16	-.02	.10	-.12	.23	.17
V. Ethyl								.04	-.07	-.04	-.20	-.01	.01
TSS									.64*	-.15	-.17	-.03	-.05
acidity										-.08	-.05	.03	.00
TGS-800											.77*	.66*	.94*
TGS-822												.37*	.78*
TGS-842													.77*

Table 6. Pearson correlation of measured properties from GoldRush apples

	SIV	pH	a	b	firmne ss	C. Ethyl	V. Ethyl	TSS	acid- ity	TGS- 800	TGS- 822	TGS- 842	TGS- 880
date	.96*	.29*	.23*	.63*	-.73*	.50*	-.05	.50*	-.04	-.30*	-.59*	.50*	-.26*
SIV		.31*	.13	.62*	-.69*	.42	-.08	.43*	-.13	-.30*	-.58*	.49*	-.23*
pH			-.47*	-.32*	-.33*	.29*	-.11	-.50*	-.67*	-.37*	-.17	-.06	-.47*
a				.46*	-.11	.09	.01	.69*	.52*	.15	-.05	.22*	.14
b					-.34*	.12	-.05	.79*	.39*	-.13	-.50*	.38*	-.03
firmness						-.35	.15	-.23*	.15	-.05	.13	-.54*	.01
C. ethyl							-.00	.13	-.02	-.31*	-.41*	.05	-.30*
V. ethyl								.03	.02	.05	.05	-.09	-.01
TSS									.70*	.01	-.33*	.38*	.12
acidity										.19	.03	.09	.27*
TGS-800											.85*	.55*	.09*
TGS-822												.27*	.73*
TGS-842													.62*

Figure 1. Canonical discrimination of apples to three ripeness classes using four channels of the electronic sniffer output.

Chemical Characterization of Apple Volatiles

Aroma volatiles of 'Golden Delicious' and 'GoldRush' apples: As part of the development of the electronic sniffer for apple ripeness, a preliminary study was conducted on the aroma volatiles from fresh ripe fruit of 'Golden Delicious' apples (*Malus × domestica* Borkh.) and 'GoldRush', a seedling of 'Golden Delicious' were recovered by a dynamic headspace sampling method and analyzed by gas chromatography and gas chromatography-mass spectroscopy. A total of 22 aromatic volatiles were identified with seventeen volatiles being common to both cultivars. Three volatiles (dipropyl disulfide, pentyl 2-methylpropionate, and 2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione), were found only in 'Golden Delicious' and two compounds (nonanal and nerolidol) were found only in 'GoldRush'.

Background on apple volatiles: Several previous reports have described apple aromatic volatiles in detail from fresh apples (Macleod and Pieris, 1981; Macleod and Troconis, 1982; Sagrero-Nieves et al., 1989; Wyllie et al., 1987; Yahia, 1994); apples under storage conditions (Yahia et al., 1990; Mattheis et al., 1991; Yahia, 1991; Brackmann et al., 1993; Mattheis et al., 1995); commercial apple essence (Flath et al., 1967); and also related to the biosynthetic pathway of apple volatiles (Anet, 1972; Olias et al., 1992; Yahia, 1994). The objectives of this study were to characterize the fruit volatile compounds responsible for the aroma of 'Golden Delicious' and 'GoldRush', the latter cultivar had not yet been examined. This information was important in order to better assess and understand the response of the sniffer to these apple cultivars with which we were studying. Though parallel studies with the sniffer were conducted on these same apples and over the entire harvesting season, that data is being analyzed. Here, we present the results of the aroma volatile portion of the study only at the stage where both fruit cultivars were classified as ripe.

Methods: Plant Material. Fresh ripe apple fruits of both 'Golden Delicious' (GD) and 'GoldRush' (GR) were manually harvested on 26 Sept., 1994 (GD) and 31 Oct., 1994 (GR) from trees growing at the Purdue Horticulture Farm, West Lafayette, IN, and immediately brought into the laboratory for sampling.

Sample preparation and recovery of volatiles. The aroma volatile compounds of the fruit from two cultivars were collected by using a modified dynamic headspace method. Single apples were placed in a 1000 ml sealed wide-mouth bottle, and air purified by first flowing through active charcoal filter, was introduced and subsequently exited passing through an absorbent trap tube (ORBOTM 32; Supelco, Bellefonte, PA). The volatile components were removed from the headspace of the bottle by gas purging with a vacuum pump and trapped onto an active standard charcoal trap tube. The collecting process was carried out for 2.5 h at a gas flow

rate of 30 ml/min and the adsorbent was placed in a 5 ml vial with 2 ml dichloromethane. The vial was placed on a sample agitator (Supelco, Bellefonte, PA) for 60 min, the dichloromethane was removed and concentrated to 500 µl with a gentle stream of argon at room temperature.

Analysis of volatiles. The concentrated solution and standard compounds were analyzed using a Varian 3700 gas chromatograph equipped with FID and a Varian electronic 4270 integrator (Varian, Walnut Creek, CA.). A fused silica capillary column (30 m × 0.25 mm i.d.) with the stationary phase SPBTM-5 (0.25µm film thickness, Supelco, Bellefonte, PA) was used. Chromatographic conditions were as follows: initial temperature, 35 °C for 5 min; program rate, 2 °C / min.; final temperature, 160 °C for 4 min.; injector temperature, 160 °C; detector temperature, 300 °C; carrier gas, helium (20: 1 split-vent ratio) (Charles and Simon, 1995).

The volatiles were also identified using gas chromatography/mass spectroscopy. The MS conditions were: ionization voltage of 70 eV; emission current of 40 µA; scan rate and range of one scan per second and 40 to 300 Da, respectively; and a source temperature of 160 °C (isobutane was used as the reagent gas in CI). The identification of volatile constituents were accomplished by retention time and comparing the mass spectra of each compound with different MS compound libraries for best fit (Stenhagen et al., 1974; Heller and Milne, 1978; Adams, 1989; Finnigan 4000 GC/MS. NBS Library 31,331 entries), and confirmed by notation MS based upon interpretation of mass spectral data.

Results and Discussion: A total of 22 aromatic volatiles were recovered, 19 from 'GoldRush', and 20 from 'Golden Delicious' apples (Table 1). Of these, 17 were common to both cultivars, three were unique to 'Golden Delicious' (dipropyl disulfide, pentyl 2-methylpropionate, and 2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione) and two (nonanal and nerolidol) were unique to 'GoldRush'. Seven of the 20 compounds identified in 'Golden Delicious' were not previously reported in this cultivar (Dimick and Hoskin, 1983; Olias et al., 1992; Brackmann et al., 1993; Yahia, 1994). The identified constituents contributed 92.7 % of the total detected volatiles of 'GoldRush' and 93.0 % for 'Golden Delicious'. The majority of the compounds were esters, contributing about 75 % of the total volatiles in both cultivars. The aroma volatile in highest concentration in 'GoldRush' was butyl acetate, while hexyl acetate was highest in 'Golden Delicious'.

The aromatic hydrocarbon compounds, toluene and xylene, were detected in both 'GoldRush' and 'Golden Delicious' apples. This was not expected, and we assumed contamination in the system. To test this assumption that toluene and/or xylene was not natural and was found as a contaminant in the solvent and/or the system, we tested the concentrated dichloromethane on the GC (2 ml dichloromethane was concentrated to 500 µl in the same manner as we concentrated the apple samples) and neither compound was present. We also examined the tubing used in the dynamic headspace sampling system and again did not find any traces of either compound.

Toluene and xylene (o-, m-, and p-xylene) have been reported as aroma volatiles in tomato (Petro-Turza, 1987). Toluene has been also reported as an aroma volatile in wood apple (*Feronia limonia*) by Macleod and Pieris (1981) and although reported in apple essence (Flath et al., 1967), it was presumed to be from solvent. We detected all three isomers of xylene (o-, m-, and p-), but as it was difficult to separate the three, the xylene reported in Table 1, is the sum of all three isomers. Toluene has a sweet-gassy odor, while xylene has a pungent, gassy, kerosene-like odor but not as powerful as that of toluene (Arctander, 1969). Further studies are required to determine whether xylene and toluene are naturally present in apples or where the compounds originated. Butyl acetate, an important aroma compound with high fruity and pear-like odor (Dimick and Hoskin, 1983; Maga, 1990), contributed 13.5 % and 15.1 % in total detected volatiles of 'GoldRush' and 'Golden Delicious' apples, respectively (Table 1). 2-methylbutyl acetate, another volatile associated with a high fruity and apple-like odor (Maga, 1990) contributed 6.85 % and 4.9 % of the total volatiles of 'GoldRush' and 'Golden Delicious', respectively. Hexyl butyrate (9-11 %) and hexyl hexanoate (6-8 %) were found in both cultivars in moderate concentration. These volatiles have been associated with "apple-peel smelling" esters (hexyl hexanoate) and were ranked by Cunningham et al. (1986) as two of the top ten most intense odor volatiles in apples. Other volatiles, including isobutyl acetate (2-methylpropyl acetate) with a fruity odor; cis-2-hexenal with a grass-like odor; and trans-2-hexenal with a green odor; which have been reported important to apple aroma intensity (Maga, 1990) were not detected in either cultivar. Whether this is due to a different ripeness stage or to cultivar differences is unknown.

Ethyl phthalate (also call diethyl phthalate) has been reported as an aroma volatile of fresh tree-ripened plum (Gomez and Ledbetter, 1994). Ethyl phthalate has not been identified in 'Golden Delicious'. Ethyl phthalate has been described as having a bitter taste but being virtually odorless when purified as "perfumery grade" commercial diethyl phthalate (Arctander, 1969).

Dipropyl disulfide, a sulfur-containing compound, was detected in headspace of 'Golden Delicious' but not 'GoldRush' apples. Sulfur-containing compounds generally contribute significantly to the aroma characteristics of fruit (Wyllie and Leach, 1992).

The sesquiterpene, α -farnesene, was detected in both 'Golden Delicious' and 'GoldRush' apples. α -Farnesene is rapidly auto-oxidized yielding simple carbonyl compounds including the major compound 6-methyl-5-hepten-2-one (Anet, 1972). The degradation product, 6-methyl-5-hepten-2-one was not detected in this study perhaps because we used a nondestructive headspace sampling of volatiles rather than other extraction techniques. α -

Farnesene has very mild, sweet, and warm, rather nondescript odor of good tenacity (Arctander, 1969). α -Farnesene can be chemically produced by the dehydration of farnesol or from nerolidol by heat with acetic anhydride (Arctander, 1969). Nerolidol was present in 'GoldRush', but not in 'Golden Delicious' (Table 1). Of the 22 volatiles, five volatiles were distinct between each cultivar, a difference of 23 %. The similarity of volatiles (17 volatile constituents in common) suggests a close genetic relationship between the two cultivars. The parents of 'GoldRush' are 'Golden Delicious' and 'Coop 17' (Crosby et al., 1994), and 'Coop 17' is 12.5 % 'Golden Delicious'. The volatile emissions of aromatic compounds allow aroma sensing to be possible in classifying apple ripeness, but are just one factor responsible for apple flavor and taste.

Table 1. Relative percentage of volatile constituents in order of elution present in the headspace of 'GoldRush' and 'Golden Delicious' Apple.

Volatile constituents	GoldRush	Golden Delicious
	Area $\pm$ SD	Area $\pm$ SD
	(%)	(%)
Toluene	8.18 $\pm$ 1.43	3.14 ^Z $\pm$ 0.33
Butyl acetate	13.49 $\pm$ 3.10	15.11 $\pm$ 2.51
Hexyl formate	2.46 $\pm$ 0.79	0.21 $\pm$ 0.21
2-Methylbutyl acetate	6.85 $\pm$ 1.16	4.92 $\pm$ 2.40
Xylene	0.98 $\pm$ 0.19	1.41 ^Z $\pm$ 0.48
Butyl propionate	2.23 $\pm$ 0.61	0.87 $\pm$ 0.13
Pentyl acetate	0.57 $\pm$ 0.25	0.84 $\pm$ 0.11
1-Methylpropyl butyrate	3.55 $\pm$ 0.48	2.99 ^Z $\pm$ 0.75
Hexyl acetate	10.95 $\pm$ 2.70	18.39 $\pm$ 2.25
Dipropyl disulfide ^X	ND ^Y	6.99 ^Z $\pm$ 2.03
2-Methylpropyl 2-methylbutyrate	1.14 $\pm$ 0.32	0.68 ^Z $\pm$ 0.32
Pentyl 2-methylpropionate ^X	ND ^Y	0.18 $\pm$ 0.07
Hexyl propionate	1.63 $\pm$ 0.49	1.70 $\pm$ 0.18
Nonanal ^X	1.84 $\pm$ 0.27	ND ^Y
Hexyl butyrate	9.69 $\pm$ 1.55	10.99 $\pm$ 1.74
4,7-Dimethyl undecane	0.59 $\pm$ 0.11	0.85 ^Z $\pm$ 0.24
Hexyl 2-methylbutyrate	7.95 $\pm$ 1.79	3.64 $\pm$ 0.97
Hexyl hexanoate	7.83 $\pm$ 0.98	6.29 $\pm$ 0.79
α -Farnesene	4.12 $\pm$ 0.63	3.23 $\pm$ 1.20
2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione ^{X,W}	ND ^Y	2.22 ^Z $\pm$ 0.93
Nerolidol ^{X,W}	1.82 $\pm$ 0.55	ND ^Y
Ethyl phthalate	6.84 $\pm$ 1.35	8.31 ^Z $\pm$ 1.39
Total	92.71	92.96

^Z Compounds detected in 'Golden Delicious' apple for the first time.

^Y ND = Not Detected.

^X Compound only detected in the one cultivar as listed.

^W Relative order of elution of these two compounds are not clear.

Aroma Sensing with an Electronic Sniffer for Quality Sorting in Strawberries

Abstract: An electronic semiconductor sniffer was evaluated for nondestructively determining the ripeness and quality of strawberries. Strawberry fruit emit aromatic volatiles increasingly as ripeness progresses, and with fruit damage and decay. The sniffer electronically senses, records and quantifies these volatile compounds. The sniffer requires less than one second to respond, and can be used during the sorting stage, or for objective inspection during quality control. In this study, we comparatively evaluated the electronic sniffer to quantitative and qualitative indices of fruit ripeness. Strawberries were first visually classified into six distinct ripeness classes, and the following ripeness indices were measured: color, soluble solids, titratable acidity, pH, and firmness. The output from the electronic sniffer correlated to fruit ripeness. The electronic sniffer was able to differentiate half-ripe, ripe and over-ripe at 1, 5, 10, and 30s, but was not able to separate immature fruit. Hue was able to distinguish between immature fruit, but was less efficient in separating mature fruit. Stepwise discrimination analysis indicated that hue, electronic sniffer at 10s and 30s, titratable acidity, soluble solids, pH together provided the most accurate ripeness classifiers. A combination of nondestructive assessment of aromatic volatiles coupled with hue enabled an accurate classification of strawberries into six ripeness classes. Our results demonstrate that the electronic sensing of aromatic volatiles can be used as a new technique in the quality sorting of strawberries.

Introduction: Strawberries (*Fragaria x ananassa* Duchesne) are aromatic and flavorful fruit that deteriorate rapidly once harvested. The fruit are generally visually sorted for ripeness, based upon fruit color, prior to marketing. Other parameters of fresh strawberry fruit quality include sweetness, sourness, firmness, texture, odor, freedom from damage and discoloration, and an overall subjective assessment of 'quality'. As the fruit can easily damage, packers are careful to minimize physical handling in grading and sorting. As fruit ripen, there generally is an associated increase in aromatic volatiles which contribute to the aroma and flavor (Pesis and Avissar, 1990). The presence of aromatic volatiles in strawberries has been extensively studied, and the volatile compounds associated with fresh strawberries identified (Douillard and Guichard, 1990; Hirvi, 1983; Lee and Lee, 1992; Shamaila et al., 1992). While monitoring aromatic volatiles appears to be an effective technique to assess ripeness and, in part, fruit quality, measuring aromatic volatiles nondestructively has been time consuming and difficult. In general, headspace gases surrounding a pack of fruits are sampled and then injected into a gas chromatograph for compound separation and total gas concentration. Recently, we developed an electronic sensory system (Benady et al. 1995) that indirectly measures the total aromatic volatiles via a semiconductor, and for some fruit such as cantaloupe, this 'electronic sniffer' was able to successfully classify the

ripeness stage of the fruits, at an accuracy at least equal to the traditional destructive quantitative indices of ripeness. Preliminary work with other small fruit suggested that the electronic sniffer could distinguish overripe or damaged fruit from ripe fruit. The electronic sniffer provided a rapid and nondestructive method to sort blueberry fruit quality (Simon et al. 1996). The sniffer detected soft and damaged blueberry fruit in packaged containers at a 5% level of damage, separated immature from mature fruit, and classified fruit into four ripeness classes. Sniffer response correlated well with blueberry firmness, pH, titratable acidity, and color, and detected cultivar differences (Simon et al., 1996). Successful application of aroma sensing technology as a discriminating tool has been reported on other food and beverage products. Electronic sensing of volatiles has detected alcohols and tobacco smoke (Shurmer et. al., 1989), and distinguished coffee odors (Gardner et al., 1990), flavors (Nakamoto et al., 1993) and beers (Bailey and Hammond, 1995; Pearce et al., 1993; Tyler et al. 1994).

As the aromatic volatiles of strawberries have been reported to be modified by postharvest storage, modified atmosphere packaging, and that off-odors from damaged or decaying strawberry fruit reflect changes in specific volatile constituents (Shamaila et al. 1992), we hypothesized that the electronic sniffer may also be able to distinguish the ripeness stage of strawberry and thus, have application in the nondestructive quality sorting process in strawberries.

Objectives: The objectives of this work was to determine whether the nondestructive monitoring of aromatic volatiles could be used as a ripeness discrimination for strawberries. A secondary objective was to compare the electronic sniffer with other traditional quantitative and qualitative ripeness indices.

Materials and Methods: Strawberries, cv. Earliglow, were hand-harvested in June 1993, and manually sorted into six ripeness classes based upon external appearance of the fruit. The following codes were used for ripeness classification: Green; White; Pink; Half-ripe; Ripe; and Overripe. The overripe class also included damaged fruit. The strawberries were then packed into 36 packs (six replications per each class) each consisting of 5 to 14 strawberries with total pack weight of approximately 31 g. For each pack, the following ripeness measures were acquired: (1) Head space aromatic volatile gas concentration; (2) External color; (3) Average firmness; (4) Total soluble solids (TSS); (5) Titratable Acidity (TA); and (6) pH.

Headspace volatiles were measured by an electronic sniffer (Benady et al. 1995) using a Compaq portable computer equipped with data acquisition board (Data Translation Marlboro, MA). The strawberries were placed in a glass jar, and sniffer response recording was initiated the instant the sniffer head was placed on top of the

open glass jar and thus exposed to accumulating volatiles. Sampling rate was 10 Hz, and data was smoothed by FFT low pass filter (Press et al. 1988). Headspace gases were sampled at 1, 5, 10 and 30 s. Glass jars were flushed with compressed air between samples. Response from the gas sensor when exposed to the volatile reducing gases of the fruit can be described in an exponential function model in which the sensor response (voltage output, mV), increases during a transitional period and then stabilizes. Empty glass jars were sampled and did not induce signal response by the sniffer. All calculations were performed with normalized sensor response value as R_s/R_0 , where R_s is the sensor resistance at different concentrations, and R_0 is the sensor resistance at 300 ppm ethanol, used as the reference standard.

External color was measured by a chroma-meter (Minolta CR200) using C illuminant, data was recorded in the CIE units L , a , b and hue was calculated from the a and b (McGuire, 1992). Average firmness for each ripeness group, was measured from three 2.5 cm fruit tissue slices using a 1.1 cm cylindrical plunger (ASAE standard S368.1) attached to a force gauge (Chatillon, Force Measurement, Greensboro, North Carolina).

Fruit samples were manually crushed into a puree. A 5 ml sample of the puree was diluted with 100 ml of boiled distilled water for pH and TA determination. The pH was measured with a Corning model 220 pH meter (Corning, NY) standardized to pH 7.0 and 4.0. TA was determined by titrating to pH 8.2 with 0.1 N NaOH and was expressed as g citric acid/100 ml. A Brinkman Digital Buret (Brand, W. Germany) was used for titrations. TSS of the puree was measured by a Reichert temperature compensated hand refractometer.

Results: The electronic sensing of aromatic volatiles after 1, 5, 10, and 30 seconds was recorded for each of the six subjectively classified ripeness stages (Fig. 1). The electronic sniffer was able to differentiate half-ripe, ripe, and over-ripe fruit (from 1, 5, 10, and 30 s), but was not able to separate immature fruit, i.e. green, white, and pink (Table 1). Similarly, subjective classification of ripeness was compared with the traditional physio-chemical properties: a , b , hue, firmness, TSS, TA and pH by a multiple range test (Table 2).

The correlation among all 11 parameters was summarized using factor analysis (Fig. 2). Parameters that are highly correlated form clusters in the factor space. There was a high correlation of sniffer output at four different sampling times (Fig. 2). Similarly, high correlation existed between hue and firmness (Fig. 2). Quadratic classification (cross validation) using all 11 parameters resulted in an agreement of $\kappa=0.73$ (Cohen, 1960). Since hue is a function of a and b , and in particular a was not highly correlated with other ripeness parameters (Fig. 2), both parameters were removed from subsequent analysis. Canonical discriminant analysis was conducted to evaluate possible discrimination of the sample packs into ripeness classes. Nine parameters were included as

discriminating variables: firmness, pH, TA, TSS, hue, and sniffer output at 1, 5, 10, and 30 seconds. The first two canonical variables are plotted in (Fig. 3). Strawberries can be clearly classified into five out of six predetermined classes. The ripe and overripe fruit can only be partially discriminated. Quadratic discrimination (cross validation) resulted in an agreement of $\kappa=.77$.

Stepwise discrimination was conducted to eliminate parameters which are insignificant for ripeness determination. The remaining parameters are (in order of significance): hue, sniffer at 30 s, TSS, pH, sniffer at 10 s, and TA. Summary of stepwise discrimination with the associated partial correlation is listed in Table 3.

Canonical discrimination of the strawberry packs to ripeness classes using only the six parameters selected in the stepwise analysis (Table 3) illustrates that the packs can be clearly discriminated to five out of the six predetermined classes. The ripe and the overripe groups are expected to be partially confused (Fig. 4). Discriminant analysis using the primary six parameters was conducted to evaluate classification. Cross validation summary shows that four of the six replications were classified accurately (66%), with coefficient of agreement $\kappa=.87$ (Table 4). Misclassification occurred only between ripe to overripe classes. A similar discriminant analysis was conducted to evaluate discrimination using only nondestructive parameters including hue, and the sniffer at 30 s as the discriminating variables. The first two canonical variables, using these two parameters, are plotted in Figure 5. Cross validation indicated that discrimination efficiency was reduced (coefficient of agreement: $\kappa=.77$) using hue and electronic sniffer at 30 s as the discriminating variables (Table 5).

Discussion: Strawberry fruit were first manually separated and classified into six ripeness classes based on visual fruit appearance. As such, it was expected and confirmed that hue was the closest parameter to agree with the human determination of the fruits to each ripeness stage. However, while hue correlated with ripeness, it was not able to discriminate between ripe and overripe strawberries.

The electronic sensing of aromatic volatiles in strawberries was successful in separating half-ripe, ripe, and over-ripe fruit. Means separation clearly showed this at all times tested (1, 5, 10, and 30s). Yet, when canonical discrimination analysis was performed to separate ripe and overripe fruits, such a clear separation was not as apparent. This suggests that although discrimination is possible, a high classification error is expected between these two ripeness groups. Improved separation between these two classes could be obtained when a considerably larger database is employed, as we have observed with blueberries (unpublished data), or by improving the efficiency of this initial prototype system. While the multivariate test evaluates the grouping of

class means, the canonical space illustrates the actual distribution of all the observations, showing also outlying observations. This conclusion is supported by the results of the quadratic classification summarized in Tables 4 and 5. Ripeness determination based on the subset of the six parameters (hue, sniffer at 30 s, TSS, sniffer at 10 s, and TA) was superior to discrimination using all 11 parameters. Use of only the two nondestructive parameters (sniffer and hue) produced similar coefficient of agreement as the 11 parameters classification.

In conclusion, electronic sensing based upon aroma sensing technology can be used in quality sorting of strawberries once fruit ripen, as it is able to distinguish half-ripe, ripe, and over-ripe fruit. This prototype system was not able to separate unripe fruit, presumably because at that early stage little to no aromatic volatiles are emitted by the fruit. While no single index can be used to accurately classify strawberries into these six ripeness classes, a combination of nondestructive assessment of aromatic volatiles with the sensing of color enabled an accurate classification of strawberries into six ripeness classes.

Literature Cited:

- Benady, M., J.E. Simon, D.J. Charles, and G.E. Miles. 1995. Fruit ripeness determination by electronic sensing of aromatic volatiles. *Trans. Amer. Soc. Agr. Eng.* 38:251- 257.
- Bailey, T.P. and R.V. Hammond. 1995. Applications for an electronic aroma detector in the analysis of beer and raw materials. *J. Am. Soc. Brew. Chem.* 53:39-42.
- Cohen, J. 1960. A coefficient of agreement of nominal scales. *Educ. and Psych. Measurement* 20(1):37-46.
- Douillard, C. and E. Guichard. 1990. The aroma of strawberry (*Fragaria ananassa*) Characterization of some cultivars and influence of freezing. *J. Sci. Food Agr.* 50:517-531.
- Gardner, J.W., H.V. Shurmer and T.T. Tan. 1992. Application of an electronic nose to the discrimination of coffees. *Sensors and Actuators B*6:71-75.
- Hirvi, T. 1983. Mass fragmentographic and sensory analyses in the evaluation of the aroma of some strawberry varieties. *Lebensm- Wiss. Technol.* 16:157-161.
- Lee, Y.C. and S.H. Lee. 1992. Flavor quality of concentrated strawberry pulp with aroma recovery. *J. Food Quality* 15:321-332.
- McGuire, R.G. 1992. Reporting of objective color measurements. *HortScience* 27(12):1254-1255.
- Nakamoto, T., T. Fukuda, and T. Moriizumi. 1993. Perfume and flavour identification by odour-sensing system using quartz-resonator sensor array and neural network pattern recognition. *Sensors and Actuators B*10:85-90.
- Pearce, T.C., J.W. Gardner, S. Friel, P.N. Bartlett and N. Blair. 1993. Electronic nose for monitoring the flavour of beers. *Analyst* 118:371-377.

- Pesis, E. and I. Avissar. 1990. Effect of postharvest application of acetaldehyde vapour on strawberry decay, taste, and certain volatiles. *J. Sci.Food Agric.* 52-385.
- Press, W.H., B.P. Flannert, S.A.V. and W.T. Vetterling. 1988. Numerical Recipes in C. The art of scientific computing. NY: Cambridge University Press, MA.
- Shamaila, M., W.D. Powrie and B. Skubra. 1992. Analysis of volatile compounds from strawberry fruit stored under modified atmosphere packaging (MAP). *J. Food Sci.* 57(5):1173-1176.
- Shurmer, H.V., J.W. Gardner and H.T. Chan. 1989. The application of discrimination techniques to alcohols and tobaccos using tin-oxide sensors. *Sensors and Actuators* 18:361-371.
- Simon, J.E., A. Hetzroni, B. Bordelon, G.E. Miles, and D.J. Charles. 1996. Electronic sensing of aromatic volatiles for quality sorting of blueberries. *J. Food Sci.* 61:967-972.
- Tyler, M., C. Bamforth, and G. Merry. 1994. Potential application for an electronic aroma detector within the brewing industry. Cincinnati, MBAA Convention:20.

Table 1. Electronic sniffer output of fruit aromatic volatiles (R_s/R_0) of strawberry packs at different ripeness stages over time (n=6 replications).

Ripeness	sniffer data (R_s/R_0)			
	1 sec	5 sec	10 sec	30 sec
green	.998a ^z	.986a	.965a	.900a
white	.995a	.982a	.960a	.881ab
pink	.994a	.980a	.953a	.850ab
half ripe	.993a	.972a	.941a	.826 b
ripe	.985 b	.944 b	.888 b	.719 c
overripe	.977 c	.902 c	.808 c	.615 d

^zMean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test ($p < .05$).

Table 2. Comparison of physio-chemical properties associated with the ripening of strawberry fruit (n=6 replications).

Ripeness	<i>a</i>	<i>b</i>	hue	firmness	TSS	pH	acidity
green	-1.0 d	24.8 b	92.3a	20.00a	6.17 b	3.39 b	1.16a
white	22.5 c	26.2 b	49.7 b	7.95 b	5.86 b	3.38 b	1.11a
pink	31.5 b	28.2a	41.9 c	6.22 bc	6.39 b	3.43 b	0.98 b
half ripe	37.4a	24.6 b	33.3 d	4.92 cd	7.35a	3.43 b	0.95 b
ripe	37.1a	17.6 c	25.4 e	4.20 cd	7.79a	3.55a	0.95 b
overripe	32.3 b	16.1 c	26.5 e	3.37 d	7.49a	3.60a	0.78 c

^zTSS values were arc sin transformed for statistical analysis. Actual mean %'s provided.

^yMean values in the same column followed by the same letters are not significantly different by Duncan's multiple range test ($p < .05$).

Table 3. Summary of stepwise discrimination analysis to select variables that contribute to ripeness discrimination of strawberry packs.

Variable	Partial r^2	F	Prob > F
hue	.98	269.5	.0001
sniffer 30 s	.70	13.7	.0001
TSS	.45	4.58	.0035
pH	.44	4.23	.0057
sniffer 10 s	.29	2.12	.0955
TA	.27	1.89	.1325

Table 4. Cross validation summary using linear discriminant function for classifying strawberries into six ripeness classes using: pH, TA, TSS, hue, electronic sniffer at 10 s, and electronic sniffer at 30 s as classification parameters. Coefficient of agreement $\kappa=0.87$.

from ripeness	to ripeness					
	green	white	pink	half-ripe	ripe	over-ripe
green	6	0	0	0	0	0
white	0	6	0	0	0	0
pink	0	0	6	0	0	0
half-ripe	0	0	0	6	0	0
ripe	0	0	0	0	4	2
overripe	0	0	0	0	2	4

Table 5. Cross validation summary using linear discriminant function for classifying strawberries into six ripeness classes using: hue and electronic sniffer at 30 s as the classification parameters. Coefficient of agreement $\kappa=0.77$.

from ripeness	to ripeness					
	green	white	pink	half-ripe	ripe	over-ripe
green	6	0	0	0	0	0
white	0	4	2	0	0	0
pink	0	0	6	0	0	0
half-ripe	0	0	0	6	0	0
ripe	0	0	0	1	3	2
overripe	0	0	0	0	2	4

Figure 1. Average sniffer response (R_s/R_o) of strawberry packs at different ripeness stages over time.

Figure 2 Correlation among ripeness parameters plotted as factor scores. Legend: $a = a$; $b = b$; firmness = c ; pH = d ; acidity = e ; TSS = f ; hue = g ; sniffer at 1 s = h ; sniffer at 5 s = i ; sniffer at 10 s = j ; sniffer at 30 s = k .

Figure 3. Discrimination of strawberry packs to ripeness stages using nine parameters (hue, firmness, TSS, pH, acidity, and the electronic sniffer at 1, 5, 10, and 30\,s), showing distribution in the canonical space.

Figure 4. Discrimination of strawberry packs to ripeness stages using only six parameters: hue, electronic sniffer at 30\,s, TSS, pH, electronic sniffer at 10\,s, and acidity, showing distribution in the canonical space.

Figure 5. Discrimination of strawberry packs to ripeness stages using two nondestructive parameters: hue and electronic sniffer at 30 s.

Applications of Aroma Sensing for Quality, Product Uniformity and Product Contamination

Flavor and aroma are important quality criteria from the standpoint of acceptability of food products. Two general types of flavor evaluation methods are presently used, namely, the consumer acceptance (preference) method and the panel difference method. In the food industry there, is a continued need to measure flavor reliably and objectively. Some objectivity has been introduced by employing a panel of trained assessors, using commonly derived terms to describe the various attributes and to assign intensity values (Jones et al. 1994). Measurements of the odor headspace of raw food materials or final products are extremely difficult even with gas chromatography due to the large number of chemical components present. Subtle changes in the relative concentrations of such components may cause large changes in the human perception of odor quality. Current nondestructive techniques for automating the fruit quality sorting process approach the problem by measuring one or two parameters and correlating the individual measured parameters to fruit quality. However, delineating quality requires measuring more than one parameter. Recently, the reliability of electronic aroma detectors has increased considerably with the significant advances achieved in the development and application of new gas sensing surfaces (Bailey et al. 1995; Gardner & Bartlett, 1994; Hobbs & Pain, 1995; Persaud & Pelosi, 1985).

Another major concern in food processing is spoilage in canned foods, filled hot or thermally processed after closing (Troy et al. 1963). Bacteria spoilage may occur in canned foods after they have been processed: first, under processing, the failure to destroy all bacteria capable of subsequent growth in the product during the heat process; and second, leakage, the contamination of the product after an adequate heat process. The presence of mold filaments in tomato products indicate the use of moldy raw material or contamination by unclean moldy equipment. A similar problem faces the fresh produce industry, in that during storage, fungal and bacterial pathogens can be responsible for high losses of perishable products particularly following fruit damage and bruising. In tomato for example, *Botrytis cinerea* is one of the most postharvest pathogens of tomato fruit which is highly susceptible to fungal rot during storage (Fallik et al., 1993). Because of the successful application of the electronic sniffer to fresh food products (apples, blueberries, melons, raspberries and strawberries), preliminary studies were conducted in our Laboratory to determine the specificity of individual electronic tin dioxide sensors to selected volatiles common to fruits and vegetables, and the ability of the same sensory system to detect changes in quality of stored processed products such as tomato juice. The sensors were selected from commercially available gas sensors to include those that pertain to volatiles produced from food products. This output was continuously recorded into computer files using a data acquisition system and results promising.

Ability of sensors to detect microbiological contamination in stored tomato juice: Tomato juice extracted from cultivar "Rutgers" was divided into eight glass jars of 300 g juice each. The jars were placed, open, on a counter

top in the lab for eight days (first day of treatment is day 0). Air temperature was 23 C and 70% RH during the whole period. The jars were divided into two treatments. Each treatment had four jars with 300g juice in each jar: (1) an experimental treatment (SA-), in which tomato juice is kept in open jar exposed to contamination from ambient room conditions and; (2) a control treatment (SA+), in which open jars are kept in the room, but suppressing development of microbiological contamination by adding 0.15% sorbic acid. The samples were tested every 24h for 8 days and for these tests each jar was placed inside the sniffer chamber (5400 cc). The chamber was flushed with nitrogen before placing a new jar inside. Results were grouped by treatments and time (days from start of experiment). Analysis of the means was conducted to evaluate differences, for each sensor, between treatments by day. Outputs by each of the gas sensor heads is depicted in Fig. 1. The sensor output for the control juice (SA+) showed a monotonous decline, while the output for the experimental juice (SA-) exhibited an increase three days after the treatment started. All the sensors included in this study showed the same trend. Total volatiles detected in GC analysis showed a major difference between the two treatments as major increase of total volatiles started in the experimental treatment (SA-), around the third day after treatment, and increased to the sixth day. The total volatile level in the control treatment (SA+) did not significantly change during this same time period. The gas sensors detected changes in volatiles by Day three after treatment. While we do not present the actual chromatograms, the three aromatic volatiles appearing at the time of increased sniffer response included: 3-methylbutyl 2-methylpropionate, 2-methylbutyl 2-methylpropionate, and 2-methylbutyl 3-methylbutyrate. The rise in these volatiles concurrent to the sniffer response warrants further study for confirmation that those compounds actually induced the sniffer response, but again supports the concept that the sniffer can be used as an early warning signal, and that the basis for signal induction can be traced back to specific aromatic volatiles. Carbon dioxide is not measured by these sensors. Mold growth was visible only from the fifth day onwards, but it was detected by a mold count on Day four after treatment, the same day that the total volatiles and the sensor response increased dramatically (Fig. 1). No mold growth was detected in control treatment (SA+), nor was there any significant change in pH level in either treatment. In short, the sniffer was able to detect mold prior to the onset of visible symptoms, and was effective in predicting the presence of mold right at the onset, as traditionally determined by mold count. In this preliminary analysis with only two treatments to tomato a single gas sensor was sufficient for early detection of microbiological contamination. If more treatments are present, e.g., different types and levels of contamination, then analysis by multiple sensors which differ in their sensitivity and concentration range to the predicted contaminants, will be required. These results in concert with our other work conducted during this project, strongly suggest that aroma sensing technology has application for continuous monitoring and evaluation of fresh and processed food products for quality, in particular for the detection of microbiological contamination as an early indicator of product deterioration and contamination.

Fig. 1. Output of gas sensing heads (TGS), and total volatile, as emitted by control (containing 0.15% sorbic acid, —■ SA(+)) experimental (without sorbic acid, —◆ SA(-)) tomato juice, and mold count from the experimental tomato juice, as measured for 8 days.

DEVELOPMENT OF ON-LINE MEASUREMENT METHODS FOR FIELD IMPLEMENTATION OF GAS SENSORS FOR RIPENESS DETERMINATION

Keywords: Cantaloupes, Gas sensing, Fruit ripeness, Sorting, Melons, Semiconductors

Abstract

This research provides on-line measurement methods for melon ripeness determination using gas sensors. Results indicate: 1) environmental influences on gas emission from the melons distort measurement results 2) it is sufficient to measure the melon's surface temperature when considering its influence on the gas emission (the internal temperature can be neglected). Measurement methods were developed for temperature and humidity compensation. A device for reducing measurement time was developed.

1. Introduction

One of the most important parameters that influence quality and shelf-life is the ripeness stage of fruit. In many fruit, ripening is associated with significant changes in respiratory rates (Leopald and Kriedemann, 1975). Aromatic volatiles accumulate during fruit ripening for both climacteric and nonclimacteric fruits. More than 60 aromatic volatiles have been identified in muskmelons and significant changes in the composition and relative concentration of aroma compounds occur during fruit ripening (Kemp et al., 1972; Horvat and Senter, 1987). A sniffer for determining fruit ripeness based on electronic sensing of gas emission has been developed (Benady et al., 1995) and successfully implemented for different fruits (Benady et al., 1994; Charles et al., 1995; Hetzroni et al., 1994, 1995; Simon et al., 1996). Previous research indicated a strong correlation between melon ripeness and the quantity of the emitted gases (Benady et al., 1995) using an off-the-shelf gas sensitive semiconductor sensor. However, measurement analysis time was too long for on-line application.

The adsorption of a gas molecule on the surface of a semiconductor results in the transfer of electrons due to the differing energy levels of the gas molecule and the semiconductor surface (Ihokura and Watson, 1994). The transfer of electrons from the donor level of the semiconductor to the layer of the absorbed gas results in decreased conductivity (increased resistance) of the semiconductor material. Temperature and humidity changes effect the sensitivity of the gas sensor (Figaro, 1990).

Hence, to obtain reliable results in field conditions, the influence of the ambient temperature and humidity on the gas emission must be determined. It is obvious that there are temperature differences between the surface of the melon and its inside: the external temperature of the melon is higher than its internal temperature in mid-day while, in the evening, the external temperature is lower than that inside the melon. So, when measuring

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the same melon in the morning and at noon, we would obtain different results, and these measurements could not provide reliable results indicating the degree of the melon ripeness. The objectives of this research were to:

1. determine environmental influences on the reliability of measurement of gas emission from melons
2. develop measurement methods to overcome environmental influences
3. develop methods to reduce gas analysis time.

2. Methods

2.1 Influence of environmental conditions on ripeness measurements

To investigate the influence of temperature on gas measurements a laboratory measuring device was developed using the TGS-822 FIGARO gas sensor. The system consisted of a power supply, a sensing head, and signal conditioning electronics (Benady et al., 1995) and data acquisition hardware. The first stage included two different laboratory investigations: 1) environmental temperature simulation achieved using heated and cooled airflow; 2) changing the temperature of stationary air using heated and cooled water. The second stage included field experiments performed on melons grown in a commercial greenhouse.

Laboratory investigations: Five ripe melons (*Cucumis melo* var. *reticulatus* Ser.) were measured in each experiment. Each melon was placed into an environmental chamber (Associated Testing Laboratories Model 8k-11-1, Wayne, New Jersey). A computerized system controlled the ambient temperature which was varied up to 45 C and controlled the mechanical device, which introduced the gas sensing device into the chamber and moved it close to each melon for several seconds while conducting the measurements. Ambient temperature, external temperature on the melon's surface and internal temperature in the cavity of the melon were continuously measured using Chromel-Alumel thermocouples.

In the second investigation each melon was placed in a glass vessel which, in its turn, was put in a tank filled with water. By first heating (up to 33 c) and then cooling the water, simulation of the natural temperature variation was achieved. The changing quantity of gas in the internal cavity of the melon was measured. For this purpose, a 2mm diameter needle was introduced into the internal cavity of the melon with a sensor connected to the other end of the needle. A second sensor was connected to the melon's external surface. Ambient, internal and external temperatures were measured using Chromel-Alumel thermocouples.

2.2 Influence of environmental conditions on sensor response

Dependency of the Figaro sensor on ambient humidity is documented as part of the sensor's specifications. Response time is sensitive to the ambient temperature: the higher the sensor temperature the faster is the response to change in gas concentration as well as recovery after removal from gas.

Temperature influence: The TGS 822 gas sensor was exposed to ethanol at different temperatures by heating the external surface of a closed glass vessel. The gas sensor was mounted on the porous lid through which the volatile gases were released. The sensor was exposed to the gas after the gas was heated to the desired temperature by placing the

sensor on the lid. After the desired exposure time, the sensor was removed from the chamber.

Humidity influence: Several studies were conducted to evaluate the effect of continuous rapid and slow humidity changes. A 170 cm³ chamber was designed with gas (Figaro TGS 822), humidity (Figaro NH1) and temperature (Chromel-alumel thermocouples) sensors. Air is pumped into the chamber through a removable wet filter. All humidity measurements were conducted relatively, based on the general calibration curve provided by the manufacturer. The influence of humidity on the gas sensor was evaluated in two modes. In both modes first humid air was pumped into the chamber. Change of humidity was achieved by changing the amount of water. In the rapid mode, the wet filter was then removed and ambient air pumped into the chamber. In the slow mode, the wet sensor was removed but air was not forced into the chamber, rather, free air flow was allowed through the inlet/outlet of the chamber.

3. Results and discussion

3.1 Influence of environmental conditions on ripeness measurements

Results of measurements in the first experiment (Fig. 1) indicate that gas emission from the melon depends to a greater degree on the temperature of its external surface than on its internal temperature. Results of the second experiment (Fig. 2) confirmed this conclusion. Furthermore, these results indicated that the gas quantity on the melon's internal cavity depends to a greater degree on the external temperature. Increase of external temperature causes an increase of the gas emission outside the melon and inside it. This substantiates what is already known about mango i.e., that in its maturity period, ethylene is concentrated in the outer mesocarp and in the ripening period-in the peel, but not in the inner mesocarp (Cua and Lizada, 1990).

These experiments indicate that to obtain reliable results, the external surface temperature must be measured and a method must be developed to compensate its influence on the quantity of the emitted gas.

3.2 Influence of environmental conditions on sensor response

Figs. 3-6 describe sensor sensitivity to gas temperature, rapid and slow humidity changes. Results indicate that both temperature variations and slow variations in humidity can be compensated. Rapid variations in humidity cannot be compensated, however, are very rare in agriculture conditions.

Development of measurement techniques for temperature and humidity compensation

To compensate temperature variations, we used a differential method. A pair of Figaro TGS-22 sensors with similar temperature characteristics were chosen and one of them was hermetically closed with a heat-conductive plug. By connecting both sensors in a differential circuit, the temperature influence can be compensated (BARD, 1995). To compensate for humidity, humidity was measured using a NH-1 Figaro. The sensing device consist of a mounted humidity-sensitive element and a printed thermistor for temperature influence compensation. Resistance of the humidity-sensitive element changes as a function of the relative humidity. The sensor resistance ratio can be obtained and

dependency on humidity calculated. The humidity sensor resistance can then be computed to correct the gas sensor's voltage according to the measured humidity.

4. Development of measurement techniques to reduce analysis time

To reduce analysis time, acceleration of the air flow based on the Venturi principle was applied. The designed system (Fig. 7) consists of a pump which causes air flow through a closed main pipe which is connected to a tube which holds the gas sensor. Actuation of the pump causes increase in air velocity and decrease of pressure in the main pipe. This decrease in pressure causes air flow from the sensor towards the tube. The difference from an ordinary pump consists in the fact that the accelerated air flow passes through the sensor only while the hood cap dome containing the sensor is tightly pressed to the melon surface. Only the portion of the gas contained in the cone at the measurement time is analyzed. To refresh the sensor, exit of the air flow must be disabled. Actuation of the pump increases the air pressure in the pipe and causes air flow towards the gas sensor thereby cleaning the sensor.

Results (Fig. 8) indicate that this method decreases analysis time by 2 to 3 times. To demonstrate the influence of the artificial suction, the following test was performed: the pump was sequentially switched on and off during the analysis. After the switching on the pump, the sensor output voltage rises rapidly up to saturation. After the pump is switched off, the sensor output voltage goes down and then rises as a function of the natural gas emission. Using this method, the melon ripeness analysis time can be reduced to 3-4 seconds which makes it applicable for on-line field testing.

5. Conclusions

Experimental results indicate influence of temperature and humidity on gas measurements using a gas sensor. Measurement techniques were developed to overcome these influences. Both temperature variations and slow humidity variations can be compensated by developing a sensing device which consists of three sensors: two equivalent gas sensors connected in a differential circuit and one humidity sensor and measuring their output using custom designed hardware or software. Rapid humidity changes cannot be compensated, however, are very rare. Sampling time can be significantly decreased by connecting the sensor to an air suction pump which is switched on and off alternatively. These developed measurement techniques enable application of the gas sensor for on-line melon ripeness determination in field conditions.

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References

1. BARD, 1995. Electronic Sensing of fruit ripeness based on volatile gas emissions. BARD Project No. US-2334-91R First and second annual reports. BARD, POB 6, Bet Dagan 50250 Israel.
2. Benady, M., J.E. Simon, D.J. Charles and G.E. Miles. 1995. Fruit ripeness determination by electronic sensing of aromatic volatiles. *Trans of the ASAE* 38(1): 251-257.
3. Cua, A.U. and M.C.C. Lizada. 1990. Ethylene production in the 'Carabao' mango fruit during maturation and ripening. *Acta Horticulturae* 269.
4. Figaro, 1990. Figaro Gas sensor TGS, General catalogue, Figaro Engineering Inc., 1-5-3, Senbanishi, Minoo City, Osaka 562, Japan.
5. Hetzroni, A., J.E. Simon, M. Benady, B. Bordelon. 1994. Electronic sensing of aromatic volatiles for quality sorting of strawberries. *ASAE Paper No.* 94-6029.
6. Hetzroni, A., D.J. Charles, J. Janick, J.E. Simon. 1995. Electronic sensing of apple ripeness based on volatile gas emissions. *HortScience* 30: 816.
7. Horvat, R.J., S.D. Senter. 1987. Identification of additional volatile compounds for canataloupes. *J. Food Sci.* 52(4): 1097-1098.
8. Kemp, T.R., L.P. Stoltz and D.E. Knavel. 1972. Volatile components of mukmelon fruit. *J. Agric. Food. Chem.* 20(2): 196-198.

INTERNAL AND EXTERNAL GAS OUTPUT DEPENDENCE ON TEMPERATURE

Figure 1 Gas output dependence on external and internal temperature (air temperature varied).

GAS QUANTITY DEPENDENCE ON MELON INTERNAL AND EXTERNAL TEMPERATURE

Figure 2 Gas output dependence on external and internal temperature (water temperature varied).

SENSOR OUTPUT DEPENDENCE ON TEMPERATURE. CALIBRATION CURVE

Figure 3 Sensor output dependence on ambient temperature.

Figure 4 Sensor output dependence on tested gas temperature.

GAS SENSOR VOLTAGE DEPENDENCE ON RAPID CHANGES OF THE HUMIDITY

Figure 5 Sensor output dependence on rapid humidity changes.

GAS SENSOR VOLTAGE DEPENDENCE ON SLOW HUMIDITY CHANGES

Figure 6 Sensor output dependence on slow humidity changes.

Figure 7 Design of an artificial suction device to increase sampling velocity of the gas sensor by using the Venturi principle.

Figure 8 Results of artificial suction device.

Continued Development of the Measuring Device

As part of the research conducted to develop on-line measurement methods for field implementation of gas sensors for melon ripeness determination (see the previous section), we recognized the limitations of the tin oxide sensors to environmental variations. The prior section depicted the sensor output dependence on temperature and relative humidity. To compensate for temperature variations and slow humidity variations, a differential method was used. A pair of sensors with similar temperature characteristics were selected and one was hermetically sealed with a heat-conducting plug. By connecting both sensors in a differential circuit, we were able to compensate for temperature influence. Humidity was measured using the NH-1 sensor of the Figaro Corp. The flow-chart of the sensor is shown on the following page (Fig. 9). The sensor consists of a mounted humidity-sensitive element (R_s) and a printed termistor (R_t) for temperature influence compensation. Resistance of the humidity-sensitive element changes as a function of relative humidity. Passing the AC signal through the sensor and measuring the voltage, the dependency on humidity can be determined. The processing of the measured data to obtain the final results may be achieved in either of two ways. When data evaluation is conducted using a computerized system, the calculation may be executed at the software level. The flow chart of this solution is presented in the following Fig. 10. Voltages from the gas and humidity sensors are detected and fed to the computer through a multi-plexed A-to-D converter (Fig. 10). The computer is set to compute the humidity sensor resistance and corrects the gas sensor's voltage in accordance with the measured humidity. A second method is based on humidity compensation and this could be performed at the hardware level. In this later case, an autonomous device must be developed, with a proposed flow-chart illustrated in Fig. 11. The principle of the device is expressed by the formula:

$$U_h = (U_{sum} - U_s) / U_{sum}$$

for implementation at the hardware level. The difference $U_{sum} - U_s$ is obtained by using a differential amplifier and the division is executed by a controlled divider circuit. When the device is calibrated in known humidity conditions, its output voltage is set to "0". If humidity levels differ from that in the process of calibration before the measurement, the device output voltage differs from "0" as a function of the humidity difference. Changing the gas sensor control voltage to "0", the humidity influence may be compensated. At this research stage, such compensation was performed manually by a potentiometer, but automatic operation is feasible to introduce.

$$\frac{R_t}{R_t - R_s} = \frac{V_{sum} - V_s}{V_{sum}}$$

Figure 9. A flow-chart of the sensor circuit.

GAS EMISSION AND HUMIDITY MEASUREMENT

Figure 10. A flow-chart of a computerized gas-measuring device.

GAS MEASUREMENT WITH HUMIDITY COMPENSATION

Annual Progress

In Year 1: A modified prototype of our original nondestructive electronic sensory system (sniffer) that responds to the volatile gases emitted by fruit during maturation was developed (the original sniffer is described in Benady et al., 1995). This system was tested during the summer of 1994 mainly for the determination of ripeness of three melon cultivars. This ripeness sensory system, based on a single gas sensor, was able to partially classify melons into five ripeness stages ranging from unripe to over-ripe. Headspace gas samples from the melons were analyzed using gas chromatography to evaluate chemical constituents. A multi-gas-sensor system was employed to determine ripeness of fruits using apples as a case study. Physicochemical measurements related to fruit maturity and quality indexing were conducted for all fruits to verify and establish a reference classification.

Sensor sensitivity was tested under different temperature conditions. The volatile emission of melons both in the seed cavity and through the rind, under continuously changing internal and external temperature was tested by the sniffer and the volatiles captured for chemical analysis. A continuous measurement by the sniffer of a single melon while it was ripening depicted both the dependency of the sniffer response to ambient temperature and the characteristic change of response as the fruit ripens. Studies were conducted to evaluate ways to shorten the sampling time by forcing air flow to carry the volatiles from the rind to the sensor. Improvements were made on the actual sniffer cup which houses the gas sensor and is placed on the fruit surface.

In Year 2: We focused on developing a multi-gas-sensing system rather than a single gas detector system, and sought to understand the specificity of each semi-conductor. To accomplish this objective, we used a Figaro control chamber modified to contain a series of semi-conductors on a removable lid, and later designed and constructed a stationary sniffer, containing three chambers which could be operated together or independently, with up to six semi-conductors built into each chamber top. The screening of eight semi-conductors to a large number of individual volatiles across a wide concentration range was done to assess the each of the semi-conductor's specificity and to ascertain whether selected gas sensors were more associated with a given chemical class of compounds or to specific volatiles. From these studies, we identified which matrix of sensors would be best to assess fruit ripeness and fruit quality and for placement in the stationary sniffer. Two major studies, the chemical characterization of melon and apple volatiles, and studying the relationship between the volatiles and sniffer response were conducted. Studies on the impact of relative humidity and temperature on sniffer response continued.

In Year 3 and the During the No-Cost Project Extension: Results in Year 2, provided the preliminary data that permitted us to modify the sniffer to compensate for temperature and relative humidity within the sniffing environment. During this final time period, sniffer modifications were completed, the stationary unit continued to be tested, data and ideas were exchanged between the Purdue and the Israeli groups, and the focus was on data analysis. While we made progress toward the developing the sensor fusion algorithm for accurately determining the degree of fruit ripeness and fruit quality based on the electronic sniffer response, environmental influences and cultivar specific data, it is in this area where more research will be required.

Future Work to be Addressed:

While both prototype systems are ready for commercialization, the following work still needs to be addressed:

1. Chamber needs to be further redesigned to allow for the simultaneous sampling of volatiles (aromatic volatiles, CO₂) as well as sniffer response.
2. Studies on the aging and potential degradation of the sensing surfaces need to be explored by accelerating the aging of the sensors and determining changes in response to controlled volatiles introduced at specific concentration ranges.
3. All systems need to be rebuilt with temperature and relative humidity compensation.
4. Further work on developing a generalizable pattern recognition system as well as specific algorithms for individual fruit need development.
5. The stationary system with three chambers was used successfully under industrial conditions. Such a prototype now is ready for commercialization, with an attached data acquisition and pattern recognition system.
6. The mobile single sensor unit is operational and would serve as an effective research and training tool, but needs to now include the sensor fusion system as an integral part of the unit, rather than as an attached laptop computer system.
7. Miniaturization of the system is now ready to be addressed for commercialization and research usefulness.
8. Further studies are recommended on using this approach to identify the presence of pathogens, and other contaminants in foodstuffs, as well as to detect malodors and adverse air quality in agricultural and food processing environments.
9. All such work needs to be refined to minimize any false positive signals and to introduce a system for sensor fusion to allow data base expansion.

Collaboration between the USA and Israel:

This project was a truly multidisciplinary project between plant physiologists, chemists, and engineers, and the collaboration led to the advances that we were able to achieve. The plant physiological studies were conducted primarily in the Laboratory for New Crops and Plant Products in the Department of Horticulture and Landscape Architecture, Purdue University. In this lab, most all the plant based studies were conducted and the sniffer units constructed. The engineering facilities in the Department of Agricultural and Biological Engineering at Purdue, via Prof. G.E. Miles and Prof. B. Engel were utilized in addition to that of the pilot shop. The engineering facilities of the Department of Testing and Instrumentation, Institute of Agricultural Engineering, Volcani Center, Israel, were used for most of the temperature and relative humidity compensation studies, studies on improvement of the sniffer head designs and complimentary studies on melon ripeness. Both groups were equally involved in the designs, project objectives, research direction, and the overall project implementation. The electronic sensing system developed at Purdue was the result of a strong ongoing collaboration between our research group at Purdue, with that of Dr. Hetzroni, Mr. Uri Peiper, Dr. Mizrach, Dr. Grinshpun and others at the Department of Testing and Instrumentation, Institute of Agricultural Engineering. The collaboration that occurred during the project was quite successful, and began before this BARD project was initiated, and the collaboration continues today even after the termination of this project. The opportunity to work together has lead to a longer term scientific relationship, one that is valued by both groups both personally and professionally. Naturally, the advances in electronic mail and file transfers via the internet have permitted a great and ongoing exchange of information between both groups. In addition, the visitation during the project between the Israel staff coming to Purdue and Purdue staff visiting Israel helped significantly toward a common vision of shared research goals.

Selected Publications and Presentations Resulting from this Research Project:

- Stroshine, R.L., G.G. Dull, J. Abbott, V. Bellon, D. Beaumelle, M. Benady, R. Cavalieri, P. Chen, W.R. Forbus, Jr., N. Galili, A. Mizrach, S. Nelson, Y. Sarig, D. Slaughter, J.E. Simon, and B. Zion. 1993. Nondestructive Sensing of Internal Composition. Proc. of BARD Workshop on Quality Sorting of Fruits and Vegetables. June, 1993, Seattle, WA.
- Ozer, N., B.A. Engel and J.E. Simon. 1994. An adaptive technique for multi-sensor fusion and classification of fruit. ASAE Paper No. 94-6025, June. Amer. Soc. Agric. Eng. 2950 Niles Rd., St. Joseph, MI:49085-9659.
- Hetzroni, A., J. E. Simon, Benady, M., B. Bordelon. 1994. Electronic Sensing of Aromatic Volatiles for Quality Sorting in Strawberries. Paper no. 94-6029. ASAE St. Joseph, MI 49085.
- Benady, M., A. Hetzroni, J.E. Simon, B. Bordelon. 1994. Electronic sensing of aromatic volatiles for quality sorting in blueberries. Presented at the annual meeting of the Amer.Soc for Hort.Sci. Corvallis, OR. Hortscience:(abstr.)
- Charles, D.J., A. Hetzroni and J.E. Simon. 1995. Electronic sensing of volatiles from tomato juice as a new technology for quality control and detection. HortScience 30(4):811.
- Hetzroni, A., D.J. Charles, J. Janick, and J.E. Simon. 1995. Electronic sensing of apple ripeness based upon volatile gas emissions. HortScience 30(4):816.
- Hetzroni, A., D.J. Charles and J.E. Simon. 1995. Electronic sensing of melon ripeness based upon volatile gas emissions. HortScience 30(4):766.
- Ozer N., B.A. Engel, and J. E. Simon, 1995. Fusion Classification Techniques for Fruit Quality Sorting. *Transaction of the ASAE* 38(6):1927-1934.
- Ozer N., B.A. Engel, and J. E. Simon. 1995. Neural Network for Quality Sorting and Grading of Cantaloupes by Multiple Nondestructive Sensors. *ASAE Paper No. 95-3218*. ASAE, 2950 Niles Rd., St. Joseph, MI:49085-9659.
- Charles, D.J., A. Hetzroni, P. Angers and J.E. Simon. 1995. Electronic sensing of aromatic volatiles as a technology for food quality. Presented at the International Society of Food Engineers, Chicago, Ill. November 4, 1995.
- Simon, J.E., A. Hetzroni, B. Bordelon, G.E. Miles and D.J. Charles. 1996. Electronic sensing of aromatic volatiles for quality sorting of blueberry fruit. J. Food Science 61(5):967-972.
- Grinshpun, J., Y. Edan, and A. Hetzroni. 1997. Development of on-line measurement methods for field implementation of gas sensors for ripeness determination. Proc. 3rd Int. Symposium on Sensors in Horticulture, Tiberias, Israel. Acta Hort: (in press).

- Hetzroni, A., D. Charles, B. Bordelon and J.E. Simon. 1997. Aroma sensing with an electronic sniffer for quality sorting in strawberries. *HortScience*: (accepted).
- Ozer, N., B.A. Engel and J.E. Simon. 1998. A multiple impact approach for non-destructive measurement of fruit firmness. *Trans. Amer. Soc. of Agric. Eng.*: (in press).

Selected Literature Cited (others listed in respective chapters):

- Adams, R.P. 1989. Identification of Essential Oils by Ion Trap Mass Spectroscopy. Academic Press: New York.
- Anet, E.F.L.J. 1972. Superficial scald, a functional disorder of stored apples. VIII. Volatile products from the autoxidation of a-farnesene. J. Agr. Food Chem. 23:605-8.
- Arctander S. 1969. Perfume and flavor chemicals, vols. 1 and 2. S. Arctander: Montclair, N.J.
- Arora, G., F. Cormier, B. and Lee. 1995. Analysis of odor-active volatiles in cheddar cheese headspace by multidimensional GC/MS sniffing. J. Agric. Food Chem. 43(3):748-752.
- Baldwin, E.A., M.O. Nisperos-Carriedo, and M.G. Moshonas. 1991. Quantitative analysis of flavor and other volatiles and for certain constituents of two tomato cultivars during ripening. J. Amer. Soc. Hort. Sci. 116(2):265-269.
- Benady, M., J.E. Simon, and G.E. Miles. 1992. Determining melon ripeness by analyzing headspace gas emission. ASAE. Paper No. 92-6055 ASAE St. Joseph, MI 49085-9659.
- Benady, M. 1993. Sensory Perception for an Agricultural Robot. Ph.D.. Purdue University, West Lafayette, IN.
- Benady, M., A. Hetzroni, J.E. Simon, and B. Bordelon. 1994. Electronic sensing of aromatic volatiles for quality sorting in blueberries. Annual Meeting of the Amer. Soc. for Hort.Sci (Abstract).
- Benady, M., J.E. Simon, G.E. Miles, and D.J. Charles. 1995. Fruit ripeness determination by electronic sensing of aromatic volatiles. Trans. of the ASAE :38(1):251-257.
- Brackmann, A, J. Streif, and F. Bangerth. Relationship between a reduced aroma production and lipid metabolism of apples after long-term controlled-atmosphere storage. J. Amer. Hort. Sci. 118(2):243-247, 1993.
- Buttery, R.G., R.M. Seifert, D.G. Guadagni, and L.C. Ling. 1971. Characterization of additional volatile components of tomato. J. Agr. Food. Chem. 19(3):524-529.
- Buttery, R.G.; Seifert, R.M.; Ling, L.C.; Soderstrom, E.L.; Ogawa, J.M., Turnbaugh, J.G. 1982. Additional Aroma components of honeydew melon. J. Agric. Food Chem. 30:1208-11.
- Buttery, R.G., R. Teranishi, and L.C. Ling. 1987. Fresh tomato aroma volatiles: a quantitative study. J. Agr. Food Chem. 35:540-544.
- Buttery, R.G., R. Teranishi, L.C. Ling, R.A. Flath, and D.J. Stern. 1988. Quantitative studies on origins of fresh tomato aroma volatiles. J. Agr. Food Chem. 36:1247-1250.
- Buttery, R.G., R. Teranishi, a. R.A. Flath, and L.C. Ling. 1990. Identification of additional tomato paste volatiles. J. Agr. Food Chem. 38:792-795.
- Caradec, P.L. and P.E. Nelson. 1985. Effect of temperature on the serum viscosity of tomato juice. J. Food Sci. 50:1497-1498.

- Charles, D.J., J.E. Simon, and K.V. Wood. 1990. Essential oil constituents of *Ocimum micranthum* Willd. J. Agr. Food Chem. 38:120-122.
- Charles, D.J., J.E. Simon, and M.P. Widrlechner. 1991. Characterization of essential oil of *Agastache* species. J. Agr. Food Chem. 39:1946-1949.
- Charles, D.J. and J.E. Simon. 1990. Comparison of extraction methods for rapid determination of essential oil composition of basil (*Ocimum* spp.). J. Amer. Soc. Hort. Sci. 115:458-462.
- Charles, D.J.; Simon, J.E. Characterization of essential oil of dill (*Anethum graveolens* L.). J. Essent. Oil Res., 1995, 7, 11-20.
- Corcoran, P. 1993. Electronic odor sensing systems. Electronics & Communication Engineering Journal 5(5):303.
- Crosby, J.A., J. Janick, P.C. Pecknold, J.C. Goffreda, and S.S. Korban. 1994. 'GoldRush' apple. HortScience. 29:827-828.
- Davide, F.A.M., C. Di Natale, and A. D'Amico. 1994. Self-Organizing multisensor systems for odour classification: internal categorization, adaptation and drift rejection. Sensors and Actuators B 18-19:244-258.
- Dimick, P.S. and J.C. Hoskin. Review of apple flavor - state of the art. Crit. Rev. Food Sci. Nutr. 18: 387-409, 1983.
- Edan, Y., H. Pasternak, D. Guedalia, N. Ozer, and I. Shmulevich. 1994. Multi-sensor quality classification of tomatoes. ASAE, St. Joseph MI 49085. Paper No. 94-6032.
- Ema, K., M. Yokoyama, T. Nakamoto, and T. Moriizumi. 1989. Odour-sensing system using a quartz resonator sensor array and neural network pattern recognition. Sensors and Actuators 18:291.
- Fallik, E., J. Klein, S. Grinberg, E. Lomaniec, S. Lurie and A. Lalazar. 1993. Effect of postharvest heat treatment of tomatoes on fruit ripening and decay caused by *Botrytis cinerea*. Plant Disease 77(10): 985-988.
- Flath, R.A., D.R. Black, D.G. Guadagni, W.H. McFadden and T.H. Schultz. 1967. Identification and organoleptic evaluation of compounds in Delicious apple essence. J. Agr. Food Chem. 15: 29-35.
- Gardner, J.W., H.V. Shurmer, and T.T. Tan. 1990. Application of an electronic nose to the discrimination of coffees. Sensors and Actuators B 6:71.
- Gardner, J.W. and P.N. Bartlett. 1991. Potential applications of electropolymerized thin organic films in nanotechnology. Nanotechnology 2:19-32.
- Gardner, J.W. and P.N. Bartlett. 1994. A brief history of electronic noses. Sensors and Actuators B 18-19:211-220.

- Gomez, E., and C.A. Ledbetter. 1994. Comparative study of the aromatic profiles of two different plum species: *Prunus salicina* Lindl and *Prunus simonii*. J. Sci. Food Agr. 65:111-5.
- Gould, W.A. 1991. Tomato Production, Processing and Technology. CTI Publications Inc., Baltimore, MD.
- Grinshpun, J., Y. Edan, and A. Hetzroni. 1997. Development of on-line measurement methods for field implementation of gas sensors for ripeness determination. Proc. 3rd Int. Symposium on Sensors in Horticulture, Tiberias, Israel. Acta Hort: (in press).
- Heller, S.; Milne, G. (Eds). 1978. EPA/NIH Mass Spectral Data Base. U.S. Department of Commerce: Washington, D.C.
- Hetzroni, A., J.E. Simon, M. Benady, and B. Bordelon. 1994. Electronic sensing of aromatic volatiles for quality sorting in strawberries. ASAE Paper No. 94-6029 ASAE, St. Joseph, MI 49085.
- Hines, E.L. and J.W. Gardner. 1994. Artificial neural emulator for an odour sensor array. Sensors and Actuators B 18-19:661-664.
- Ho, C. and N. Ichimura. 1982. Identification of heterocyclic compounds in the volatile flavor of fresh tomato. Lebensmittel-Wissenschaft und Technol. 15(6):340-342.
- Hobbs, P.J., T.H. Misselbrook, and B.F. Pain. 1995. Assessment of odours from livestock wastes by a photoionization detector, an electronic nose, olfactometry and gas chromatography mass spectrometry. J. Agric. Eng. Res. 60(2):137-144.
- Hobson, G.E. and J. Ambler. 1988. An improved automatic "Firmness Meter" in use at the glass house. Crop Res. Annual report Glass house Crop research institute 1986-7. :120-125.
- Homatidou, V.I.; Karvouni, S.S.; Dourtoglou, V.G.; Poulos, C.N. 1992. Determination of total volatile components of *Cucumis melo* L. variety *cantaloupensis*. J. Agric. Food Chem. 40:1385-8.
- Horvat, R.J.; Senter, S.D. 1987. Identification of additional volatile compounds from cantaloupe. J. Food Sci. 52: 1097-8.
- Ihokura, K. and J. Watson. 1994. The Stannic Oxide Gas Sensor. CRC Press, Inc., Boca Raton, FL.
- Jones, M., P.J. Wates, and R.J. Smith. 1994. A Mobile Dynamic Olfactometer for Feedlot Odor Studies. Applied Engineering in Agriculture 10(3):417-423.
- Jones, R.A. and S.J. Scott. 1984. Genetic potential to improve tomato flavor in commercial F1 hybrids. J. Amer. Soc. Hort. Sci. 109(3):318-321.
- Kader, A.A., C. Morris, L.M.A. Stevens, and M. Albright-Holton. 1978. Composition and flavor quality of fresh market tomatoes as influenced by some postharvest handling procedures. J. Amer. Soc. Hort. Sci. 103:6-13.
- Kemp, T.R.; Knavel, D.E.; Stoltz, L.P. 1971. Characterization of some volatile components of muskmelon fruit. Phytochem. 10:1925-8.

- Kemp, T.R.; Knavel, D.E.; Stoltz, L.P. 1972. Volatile components of muskmelon fruit. *J. Agric. Food Chem.* 20:196-8.
- Kemp, T.R.; Knavel, D.E.; Stoltz, L.P. 1972. Cis-6-Nonenal: A flavor component of muskmelon fruit. *Phytochem.* 11: 3321-2.
- Kemp, T.R.; Knavel, D.E.; Stoltz, L.P. 1973. Volatile *Cucumis melo* components: Identification of additional compounds and effects of storage conditions. *Phytochem.* 12:2921-4.
- Kemp, T.R.; Knavel, D.E.; Stoltz, L.P.; Lundin, R.E. 1974. 3,6-Nonadien-1-ol from *Citrullus vulgaris* and *Cucumis melo*. *Phytochem.* 13:1167-70.
- Lurie, S. A. Handros, E. Fallik and R. Shapira. 1996. Reversible inhibition of tomato fruit gene expression at high temperature. *Plant Physiology* 11: 1207-1214.
- McColloch, L.P., Cook, H.T. and Wright, W.R. 1982. Market diseases of tomatoes, peppers and eggplants. Agriculture Handbook No. 28. Agricultural Research Service, U.S.D.A., Washington D.C., 74 pp.
- Macleod, A.J., and N.M. Pieris. 1981. Volatile flavor components of wood apple (*Feronia limonia*) and a processed product. *J. Agr. Food Chem.* 29:49-53.
- Macleod, A.J., and N.G.D. Troconis. Volatile flavor components of Cashew 'apple' (*Anacardium occidentale*). *Phytochemistry* 21:2527-30, 1982.
- Maga J.A. 1990. Analysis of aroma volatiles. p. 103-4. In: M.H. Gordon. (eds.), Principles and applications of gas chromatography in food analysis. Ellis Horwood Limited, Chichester, England.
- Marin, A.B.; Acree, T.E.; Hotchkiss, J.H.; Nagy, S. 1992. Gas chromatography-Olfactometry of orange juice to Assess the effects of plastic polymers on aroma character. *J. Agric. Food Chem.* 40: 650-4.
- Mattheis, J.P., D.A.Buchanan, and J.K. Fellman. 1991. Changes in apple fruit volatiles after storage in atmospheres inducing anaerobic metabolism. *J. Agr. Food Chem.* 39:1602-5.
- Mattheis, J.P., D.A.Buchanan, and J.K.Fellman. 1995. Volatile compound production by Bisbee Delicious apples after sequential atmosphere storage. *J. Agr. Food Chem.* 43:194-9.
- Moshonas, M.G.; Shaw, P.E.; Baldwin, E.A.; Yuen, W. 1993. Volatile and nonvolatile components in Hami melon (*Cucumis melo* L.). *Lebensm.-Wiss.-Technol.* 26 (6): 577-80.
- Mutton, L.L., Cullis, B.R. and Blakeney, A.B. 1981. The objective definition of eating quality in rock melons (*Cucumis melo*). *J. Sci. Food Agric.* 32:385-391.
- Nakamoto, T., H. Takagi, S. Utsumi, and T. Moriizumi. 1992. Gas/odor identification by semiconductor gas-sensor array and an analog artificial neural-network circuit. *Sensors and Actuators B* 8:181-186.
- Nakamoto, T., T. Fukuda, and T. Moriizumi. 1993. Perfume and flavour identification by odour-sensing system using quartz-resonator sensor array and neural network pattern recognition. *Sensors and Actuators B* 10:85.

- Nakamoto, T., K. Fukunishi, and T. Moriizumi. 1990. Identification capability of odor sensor using quartz-resonator array and neural-networks pattern recognition. *Sensors and Actuators B1* :473-476.
- Nakamoto, T., Y. Higuchi, and T. Moriizumi. 1994. Study of fire-alarm system using plural semiconductor and quartz-resonator gas sensors. *Sensors and Actuators A* 40:155-158.
- Neuner-Jehle, N, and F. Etzweiler. 1991. The Measuring of Odours, pp. 153-212. In: Miller, P. M. and D. Lamparsky (eds.). *Perfumes: Art, Science & Technology*, Elsevier, NY.
- Olafsson, R., E. Martinsdottir, G. Olafsdottir, S.I. Sigfusson, and J.W. Gardner. 1992. Monitoring of fish freshness using tin oxide sensors. In: Gardner, J.W. and P.N. Bartlett (eds.). *Sensors and sensory systems for an electronic nose*. Kluwer Academic Publishers, Dordrecht.
- Olias, J.M., L.C.Sanz, J.J.Rios, and A.G. Perez. 1992. Inhibitory effect of methyl jasmonate on the volatile ester-forming enzyme system in Golden Delicious apples. *J. Agr. Food Chem.* 40:266-70.
- Ozer, N., B.A. Engel, and J.E. Simon. 1994. An adaptive techniques for multi-sensor fusion and classification of fruit. ASAE Paper No. 94-6025 ASAE At. Joseph, MI 49085. :13 pp.
- Ozer, N., B.A. Engel, and J.E. Simon. 1995. Fusion classification techniques for fruit quality sorting. *Trans. of the Amer. Soc. of Agric. Eng.* 38(6):1927-1934.
- Persaud, K.C. and P. Pelosi. 1985. An approach to an artificial nose. *Trans Am. Soc. Artif. Organs* 31:297-300.
- Petro-Turza, M. 1987. Flavor of tomato and tomato products. *Food Rev. Intl* 2(3):309-351.
- Pisanelli, A.M., A.A. Qutob, P. Travers, S. Szyszko, and K.C. Persaud. 1994. Applications of Multi Array Polymer Sensors to Food Industries. *Life Chemistry Reports* 11:303-308.
- Sagrero-Nieves, L., H.L.Pooter, and F.A.V. Wassenhove. 1989. Major volatile constituents from the fruit of Mammee Apple (*Mammea americana* L.). *J. Food Comp. Analysis* 2:382-5.
- Shewfelt, R.L. 1993. Stress physiology: A cellular approach to quality, pp. 257-276. In: Shewfelt, R.L. and Prussia, S.E. (eds). *Postharvest Handling: A Systems Approach*. Academic Press, San Diego, CA.
- Shewfelt, R.L. 1994. Quality characteristics of fruits and vegetables, pp. 171-189. In: Singh, R.P. and Oliveiria, F.A.R. (eds). *Minimal Processing of Foods and Process Optimization: An Interface*. CRC Press, Boca Raton, FL.
- Shurmer, H.V., J.W. Gardner, and H.T. Chan. 1989. The application of discrimination techniques to alcohols and tobaccos using tin-oxide sensors. *Sensors and Actuators* 18:361.
- Shurmer, H.V. and J.W. Gardner. 1992. Odour discrimination with an electronic nose. *Sensors and Actuators B* 8:1-11.
- Simon, J.E., A. Hetzroni, B. Bordelon, G.E. Miles and D.J. Charles. 1996. Electronic sensing of aromatic volatiles for quality sorting of blueberry fruit. *J. Food Science* Sept./Oct.: in press.
- Stecker PG. 1968. *The Merck Index*. Rahway: Merck & Co; 1102p.

- Stenhagen, E.; Abrahamsson, S.; McLafferty, F. (Eds). 1974. Registry of Mass Spectral Data, Wiley: N.Y.
- Stevens, M.A., A.A. Kader, M.A. Albright-Holton, and M. Algazi. 1977. Genotype variation for flavor and composition in fresh market tomatoes. J. Amer. Soc. Hort. Sci. 102(9):680-689.
- Thakur, B.R., R.K. Singh, D.M. Tieman, and A.K. Handa. 1996. Tomato product quality from transgenic fruits with reduced pectin methyltransferase. J. Food Science 61: 85-88.
- Thibould, M. 1991. Empirical Classification, pp. 253-286. In: Miller, P. M. and D. Lamparsky (eds.). Perfumes: Art, Science & Technology, Elsevier, NY.
- Tressler, D.K. and M.A. Joslyn. 1961. Fruit and vegetable processing technology. AVI Publishing Co., Westport, CT.
- Troy, V.S., J.M. Boyd, and J.F. Folinazzo. 1963. Spoilage of canned foods due to leakage. Continental Can Co., Metal Div. Res. Dev. Dep., Chicago, Ill.
- Tylor, M., C. Bamforth, and G. Merry. 1994. Potential application for an electronic aroma detector within the brewing industry. Cincinnati. MBAA Convention. 20.
- Villari, P., P. Costabile, G. Fasanaro, F. De Sio, B. Laratta, G. Pirone, and D. Castaldo. 1994. Quality loss of double concentrated tomato paste: Evolution of the microbial flora and main analytical parameters during storage at different temperatures. J. Food Processing and Preservation 18:369-387.
- Whitaker, T.W.; Davis, G.N. 1962. Cucurbits: Botany, Cultivation and Utilization. Interscience Publishing: New York.
- White, A.; Handler, P.; Smith, E.L.; Hill, R.L.; Lehman, I.R. 1978. Principles of Biochemistry. Mcgraw-Hill: NY.
- Wyllie, S.G., D.Cook, J.J. Brophy, and K.M. Richter. 1987. Volatile flavor components of *Annona atemoya* (custard apple). J. Agr. Food Chem. 35:768-70.
- Wyllie, S.G.; Leach, D.N. 1990. Aroma volatiles of *Cucumis melo* cv. Golden Crispy. J. Agric. Food Chem. 38: 2042-4.
- Wyllie, S.G.; Leach, D.N. 1992. Sulfur-containing compounds in the aroma volatiles of melons (*Cucumis melo*). J. Agric. Food Chem. 40: 253-6.
- Yabumoto, K.; Jennings, W.G.; Yamaguchi, M. 1977. Volatile constituents of cantaloupe, *Cucumis melo*, and their biogenesis. J. Food Sci. 42: 32-7.
- Yabumoto, K.; Yamaguchi, M.; Jennings. 1978. W.G. Production of volatile compounds by muskmelon, *Cucumis melo*. Food Chem. 3: 7-16.
- Yahia, E.M., F.W. Liu, and T.E Acree. 1990. Changes of some odor-active volatiles in controlled atmosphere-stored apples. J. Food Quality 13:185-202.

- Yahia, E.M. 1991. Production of some odor-active volatiles by 'McIntosh' apples following low-ethylene controlled-atmosphere storage. HortScience 26:1183-5.
- Yahia, E.M. 1994. Apple flavors. Hort. Rev. 16: 197-234.
- Yamaguchi, M.; Hughes, D.K.; Yabumoto, Y.; Jennings, W.G. 1977. Quality of cantaloupe muskmelons: variability and attributes. Sci. Hortic. 6: 59-70.