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Freeze damage detection in oranges using gas sensors

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Abstract

Three volatile sensing systems and two sampling methods were evaluated to determine their ability to detect freeze damage in Valencia oranges (*Citrus sinensis* (L.) Osbeck 'Valencia') 24 h after laboratory-simulated freezing. Carbon dioxide and ethanol were measured using hand-held gas sensors, and an electronic nose was employed to measure the overall headspace or internal gas profile of each orange. For carbon dioxide, best classification was obtained using internal gas sampling with a classification accuracy of 87 and 47% for unfrozen and partially frozen Valencia oranges. Discrimination using ethanol was best using headspace gas sampling and correctly classified 100% of sound fruit but only 37% of the partially frozen fruit. In general, sensing of the overall volatile composition using an electronic nose resulted in a more balanced classification with errors of similar magnitudes, than by sensing carbon dioxide and ethanol. The electronic nose correctly classified approximately 73 and 74% of sound fruit and 70 and 67% of the partially frozen fruit with headspace and internal gas sampling methods, respectively.

Keywords: Electronic nose; Carbon dioxide; Ethanol; Nondestructive; Fruit quality

1. Introduction

Freeze-damaged oranges display changes that make them unsuitable for consumption. Immediate visible changes include the appearance of spots on the surface of the fruit and the formation of white ice crystals in the interior of the fruit (Milliken et al., 1919). Ice crystal formation disrupts the pulp cells in frozen fruit and creates pathways for juice to exit the fruit, ultimately resulting in the dehydration of freeze-damaged oranges (Syvertsen, 1982). Adverse chemical changes include variation in volatile composition, formation of limonin, which causes the fruit to have a bitter taste, as well as a reduction in total soluble solids and total sugars (Sinclair, 1984).

According to US standards for grades of California oranges, 'injury', 'damage' and 'serious damage' are defined as any defect, which affects to varying degrees the appearance and edible or shipping quality of the fruit (United States Department of Agriculture, 1999). Accurate and efficient isolation of freeze-damaged fruit from sound fruit prevents undetected damaged fruit from passing US grade standards and going to the fresh market. This also allows the grower and packinghouse

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to make decisions on whether to pick the fruit for the fresh market or to put them to alternative use such as processing into juice (Wardowski et al., 1986).

The dryness cut method is typically used to estimate the volume percentage of freeze damage in a fruit. In this method, a slice is removed from the stem end of the fruit to expose the flesh and grade levels are based on the amount of visible freeze damage (Wardowski et al., 1986). Since the dryness cut method relies on visual inspection, it is subjective, inaccurate and destructive. An alternative method involves density separation of freeze-damaged fruit, since the specific gravity of the damaged fruit decreases as a result of dehydration. However, this method is only effective between a few weeks to 2 months after a freeze event, when the difference in specific gravity between damaged and undamaged fruit becomes apparent.

Several gas-sensing techniques have been used to study the post-harvest quality of oranges. Using gas chromatography, Davis (1970) determined that ethanol vapor production in the headspace of Hamlin and Valencia orange juice increased with increasing maturity of the fruit. Norman et al. (1967) studied the volatile production of Valencia oranges as a result of mechanical damage. They observed that the average amount of volatiles produced from injured oranges was 75 times more than that from uninjured fruit. In addition, volatiles produced from injured and uninjured oranges increased in number and concentration with increasing temperature. Recently, an electronic nose was used to evaluate the post-harvest quality of oranges. This device was sensitive to a range of volatiles that included aromatic compounds, alcohols and aldehydes, and was an efficient and convenient method of volatile detection (Natale et al., 2001). Since freeze-damaged crops undergo changes similar to those resulting from storage quality reduction and mechanical damage, it may be feasible to implement the electronic nose technology for freeze damage detection in citrus.

Accurate assessment of the degree of freeze damage in oranges helps orange growers to be better equipped in their fruit management strategies after a freeze event. Both the dryness cut and density separation methods have limitations. Sensors that take advantage of the chemical changes of the fruit have been shown to be highly sensitive and quantitative, and allow for accurate and nondestructive sensing of fruits.

The specific objectives of this project were to determine the optimum model to classify freeze-damaged and sound oranges using:

A. Three gas sensors
   (1) Electronic nose
   (2) CO_2
   (3) Ethanol
B. Two gas sampling methods
   (1) Headspace
   (2) Internal

2. Materials and methods

From May to August 2002, California Valencia oranges (*Citrus sinensis* (L.) Osbeck 'Valencia') grown at the Lindcove Research and Education Center (LREC) were shipped to our laboratory immediately after harvesting and stored in a low-temperature incubator (Model 307C; Fisher Scientific, Pittsburgh, PA) at 0 °C for 24 h before use. These oranges were handpicked and were not chemically treated, and each shipment of oranges was studied for at most 2 weeks. Only sound fruit of about 10 cm in diameter, with no visible physical defects, were used in this study. One hundred and forty Olinda and 78 Cutter Valencias were used for headspace gas sampling, and 257 Olinda Valencias were used for internal gas sampling in this study.

Citrus freezing was simulated in the lab using a modified household chest freezer (Model Kenmore 253; Sears Roebuck & Co., Chicago, IL). A wooden chamber containing a thin metal plate with holes cut into the plate was constructed to hold the orange samples inside the freezer. Fans situated on one side of the wooden chamber provided 50 cm^3/s of airflow down the length of the chamber. A 2.3A nichrome-wound heating element and a temperature controller (Model CN132; Omega, Stamford, CT) placed at the bottom of the freezer were used to maintain a constant −7.2 °C temperature in the chamber. Two rows of seven 3.8-cm holes were drilled into the left and right walls of the wooden container to allow the user to regulate the amount of air flowing into the top and bottom of the chamber. The bottom rows were sealed so that the portion of the orange that was located below the metal plate would be exposed to cooler air, while the portion

of the orange located above the plate would be exposed to warmer air circulated by the fans.

2.1. Sample freezing

Oranges were tested in batches that included 12 unfrozen and 12 partially frozen samples. Unfrozen oranges were stored in a 0 °C incubator before gas sampling. Oranges that were placed in the freezer were considered partially frozen when their temperature profiles exhibited latent heat release. Individual fruit temperature was recorded over the length of the freeze process at 1-min intervals using a datalogger (Model 21X; Campbell Scientific Inc., Logan, UT) and 30-gauge hypodermic needle temperature probes with Type T thermocouple elements (Omega, Stamford, CT). Thermocouples were taped to the fruit exterior surface approximately 2.5 cm from the stem end of each fruit, and the stem end of the fruit was exposed to cooler air than the stylar end to simulate natural freeze events. Samples remained in the freezer for 5–15 h after the onset of latent heat release. Subsequent testing of Olinda Valencia oranges frozen for headspace gas sampling indicated that this exposure produced approximately less than 30% ice mass as determined by differential calorimetry. After freezing, samples were removed from the freezer and held at room temperature. Gas sampling of unfrozen and partially frozen oranges was conducted 24 h after removing oranges from the freezer or incubator.

2.2. Headspace gas sampling

Glass jars (3.8 L) were used for orange headspace gas sampling, and lids of the jars were modified to allow for sample circulation through the electronic nose (Model Cyranose 320; Cyrano Sciences, Pasadena, CA) and the carbon dioxide sensor (Model CO_2 Analyzer 2820; Bacharach, Pittsburg, PA). Each fruit was sealed into a jar, and volatiles were allowed to accumulate for 1 h before gas sampling.

The electronic nose contained an array of 32 carbon-black organic polymer composite sensors. The output of each sensor was the ratio of the maximum resistance change of the sensor during sampling to the baseline resistance before sampling. Electronic nose sampling took 10 min with 1 min of baseline purge, 3 min of sampling and 6 min of air intake and sample gas purge. A silica gel desiccant cartridge was installed in the electronic nose to minimize humidity effects and environmental conditions were kept fairly constant to prevent baseline drift. The inlet port of the electronic nose was connected to the valve attached to the jar lid, and the outlet port of the sensor was connected to a 0.3 m length of polypropylene tubing via a plastic adapter (Fig. 1). While the electronic nose was in its sampling stage, ethanol was measured by inserting the needle of the ethanol sensor (Model Alco-Sensor IV; Intoximeters, St. Louis, MO) into a short piece of rubber tubing connected to the gas sampling chamber. At the end of the electronic nose gas purge, the CO_2 sensor was connected to the sampling jar and headspace gas was allowed to recirculate through it.

Fig. 1. Headspace gas sampling configuration.

2.3. Internal gas sampling

Internal gas sampling (Eaks and Ludi, 1960) was achieved using a custom 50 cm^3 volume glass syringe (Model Perfektum; Popper & Sons, Inc, New Hyde Park, NY), Fig. 2. A metal valve was attached to the syringe nozzle, and a 63.5 cm length of polypropylene tubing was connected to the valve. A 2.8-cm-diameter PTFE plunger was fabricated with a 3.2-mm-diameter

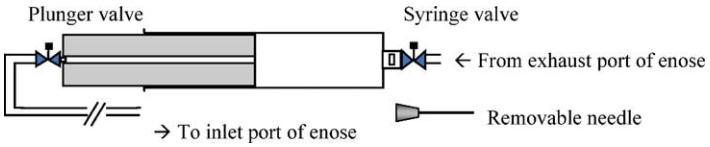

Fig. 2. Internal gas sampling configuration.

hole down the length of the plunger, which enabled gas recirculation. The opening of the plunger was fitted with another valve to which the other end of the tubing could be connected. A 30 cm^3 volume of gas was sampled from the core of each fruit by inserting a hypodermic needle into the center of the fruit. Measurements of gas trapped in the syringe were then taken using the electronic nose, the carbon dioxide sensor and the ethanol sensor. A short piece of rubber tubing was attached to the syringe to allow for ethanol measurement, while sampling with the electronic nose.

2.4. Data processing techniques

All statistical analyses were conducted using SAS (SAS Institute, Inc. v. 8.02. Cary, NC). Several methods for model development using the electronic nose sensor variables were evaluated for their ability to predict whether an orange was previously frozen or unfrozen. These included linear regression, discriminant function analysis, canonical discriminant analysis, stepwise discriminant analysis and partial least squares regression. Linear regression was used to identify electronic nose sensor variables that produced high coefficient of determination values. Classification error rates were then determined for these variables using canonical discriminant analysis of the calibration set. Leave-one-out cross-validation (Lachenbruch and Mickey, 1968), which involves an iterative process of training all but one observation and validating the resulting classification criterion with the omitted observation, was then performed on these models using canonical discriminant analysis. The final model was then selected based on lowest classification error and the proximity of the classification accuracy on the calibration set to that of leave-one-out cross-validation. Ethanol and carbon dioxide classification accuracies were also determined by performing leave-one-out cross-validation using canonical discriminant analysis.

3. Results and discussion

3.1. Headspace gas sampling

Using all 32 electronic nose sensors (labeled s1-s32), preliminary analysis showed that unfrozen Cutter Valencia oranges could be discriminated from unfrozen Olinda Valencia oranges with 100% accuracy. A four-sensor mathematical model to detect partially frozen oranges was developed for each batch of Cutter and Valencia oranges. The sensors that were most often selected in these models differed for the two varieties. Since these results showed that varietal differences were significant, statistical analysis was performed separately for Cutter and Olinda Valencias instead of combining the two varieties into a single data set. The optimum model for headspace gas sampling of Olinda Valencia oranges using the electronic nose employed 12 variables and correctly classified 71% of the fruit, Table 1. Models were not developed for Cutter Valencia oranges due to insufficient replicate numbers for leave-one-out cross-validation.

Mean carbon dioxide and ethanol levels were significantly different ($p \leq 0.05$) for partially frozen and unfrozen Olinda Valencias, Table 2. Ethanol and carbon dioxide concentrations were significantly higher

Table 1
Optimum electronic nose model[a] for headspace gas sampling of Olinda Valencia oranges

Treatment	Classification accuracy[b] (% correct)
Unfrozen	73
Partially frozen	70
Overall correct classification	72

[a] Sensors in model include s6, s7, s9, s10, s14, s18, s19, s23, s24, s27, s29 and s30.

[b] Classification accuracy based on leave-one-out cross-validation using canonical discriminant analysis. A total of 70 unfrozen and 70 partially frozen Olinda Valencia oranges were included in the classification.

Table 2
Ethanol and carbon dioxide values for frozen and unfrozen Olinda Valencias and their classification using headspace gas sampling

Treatment	Ethanol		Carbon dioxide	
	Mean[a] (mg/L)	Classification accuracy[b] (% correct)	Mean[a] (%)	Classification accuracy[b] (% correct)
Unfrozen	0.000a	100	0.168c	68
Partially frozen	0.054b	37	0.210d	46
Overall correct classification		69		57

[a] Mean values with the same letter are not significantly different at the alpha = 0.05 level.
[b] Classification accuracy based on leave-one-out cross-validation using canonical discriminant analysis. A total of 70 unfrozen and 70 partially frozen Olinda Valencia oranges were included in the classification.

Table 3
Optimum electronic nose model[a] for internal gas sampling of Olinda Valencia oranges

Treatment	Classification accuracy[b] (% correct)
Unfrozen	74
Partially frozen	67
Overall correct classification	71

[a] Sensors in model include s1, s4, s9, s12, s13, s14, s15, s19, s20, s21, s23 and s27.
[b] Classification accuracy based on leave-one-out cross-validation using canonical discriminant analysis. A total of 132 unfrozen and 125 partially frozen Olinda Valencia oranges were included in the classification.

for frozen fruits and there was no detectable ethanol in unfrozen oranges. Only 37% of the partially frozen oranges produced enough ethanol to be detectable by the ethanol sensor, which had a minimum detection level of 0.03 mg/L. The electronic nose classified the partially frozen and unfrozen fruit more accurately than the carbon dioxide sensor. The electronic nose and ethanol sensor had similar overall classification accuracies. However, the errors for the electronic nose were balanced, while the errors for ethanol were not.

3.2. Internal gas sampling

Internal gas sampling of Olinda Valencia oranges with the electronic nose had a similar classification accuracy compared with headspace gas sampling, Table 3. The comparison is slightly biased because the number of headspace gas samples was almost half that of the internal gas samples. Because both gas sampling methods resulted in similar classification accuracies, the decision of which method to use should be based on the specific application. Internal gas sampling allows immediate gas sampling, but headspace gas sampling is nondestructive.

Both ethanol and carbon dioxide values were significantly different between the partially frozen and unfrozen treatments, Table 4. The classification accuracies using the carbon dioxide sensor were 5% higher for partially frozen and 13% higher for unfrozen oranges than those using the ethanol sensor. Classification accuracy using internal gas sampling of ethanol dropped by 3% for partially frozen and 18% for unfrozen samples compared with headspace gas sampling of ethanol. Classification accuracy using internal gas sampling of carbon dioxide improved 19% for unfrozen samples and 1% for partially frozen samples compared

Table 4
Ethanol and carbon dioxide values for frozen and unfrozen Olinda Valencias and their classification using internal gas sampling

Treatment	Ethanol		Carbon dioxide	
	Mean[a] (mg/L)	Classification accuracy[b] (% correct)	Mean[a] (%)	Classification accuracy[b] (% correct)
Unfrozen	0.014a	82	0.552c	87
Partially frozen	0.026b	34	1.403d	47
Overall correct classification		58		67

[a] Mean values with the same letter are not significantly different at the alpha = 0.05 level.
[b] Classification accuracy based leave-one-out cross-validation using canonical discriminant analysis. A total of 132 unfrozen and 125 partially frozen Olinda Valencia oranges were included in the classification.

with headspace gas sampling. Internal gas sampling of ethanol showed less promise in successful classification than internal gas sampling using both the electronic nose and carbon dioxide sensor. While both the carbon dioxide and ethanol measurements showed improved classification of unfrozen oranges as compared to the electronic nose, the electronic nose still performed better in classifying partially frozen samples. Based on overall accuracy, internal gas sampling of carbon dioxide was only 4% lower in classification accuracy than internal gas sampling using the electronic nose but errors for the electronic nose were more balanced.

4. Conclusions

Model selection was affected by differences in varieties. Using all 32 electronic nose sensors, unfrozen Olinda Valencias were discriminated with 100% accuracy from unfrozen Cutter Valencias. In order for such clear discrimination, the volatile profile of sound Olinda Valencias must be significantly distinct from that of sound Cutter Valencias.

Overall classification accuracy of the electronic nose with headspace gas sampling was comparable to internal gas sampling at approximately 71–72%. Thus, the choice of gas sampling method would depend on the application, with headspace gas sampling method being nondestructive, and internal gas sampling method not requiring 1 h gas accumulation period.

Classification accuracy of headspace gas sampling of ethanol was superior to internal gas sampling of ethanol for both unfrozen and partially frozen oranges. The ethanol sensor was able to correctly classify sound fruit 100% of the time using headspace and 82% of the time using internal gas sampling. However, it experienced difficulty in accurately classifying partially frozen fruit. Only 37% of the partially frozen fruits were classified correctly using headspace gas sampling compared with 34% using internal gas sampling.

Carbon dioxide displayed better classification accuracies than ethanol when sampled from the interior of the fruit. With the internal gas sampling method, 87 and 47% of the unfrozen and partially frozen Olinda Valencias were classified correctly. However, classification accuracy of unfrozen Olinda oranges decreased by 19% when carbon dioxide was sampled from the headspace of the fruit compared to internal gas sampling.

Depending on the gas sampling method, sensing individual gas components such as ethanol and carbon dioxide produced comparable overall classification accuracies to sensing an array of volatiles using an electronic nose. With the headspace gas sampling method, the electronic nose and ethanol sensor displayed similar overall classification accuracies. Using internal gas sampling, the overall classification accuracy for the electronic nose was comparable to that for the carbon dioxide sensor. With both headspace and internal gas sampling, the electronic nose resulted in a more balanced classification than the carbon dioxide and ethanol sensors.

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