Southern Region Small Fruit Consortium Research Proposal

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TITLE

Blueberry Shelf Life Prediction and Postharvest Diseases Detection Using the Electronic Nose

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OBJECTIVE

To develop a new sensing approach using an electronic nose for blueberry quality control and fungal disease detection.

JUSTIFICATION

Blueberry industry in Georgia has grown by leaps and bounds with 170% increase of its economic value between 2000 and 2005 (Boatright and McKissick, 2006). It has surpassed peaches to become Georgia's most important fruit crop since 2004 with the total value of $60 million and acreage of 9,000 acres, equivalent to 56% of total Georgia fruits farm gate value for 2005. Nationwide, cultivated blueberries are only second to strawberries as the most important berry crop (Krewer and NeSmith, 2002; Fonsah et al., 2005).

However, blueberries are also a highly perishable fruit and usually more than 20% blueberries are lost before they get to consumers (Salunkhe and Desai, 1984). The increasing mechanical harvesting and improper postharvest handling (such as delayed cooling after harvesting) cause a lot of damage (Northeast Regional Agricultural Engineering Service, 1992). When blueberries are packaged, these invisible bruises may further develop and hence reduce blueberries shelf life (Eck, 1988). The shortened shelf life not only reduces the consumers' satisfaction, but also causes more waste during the shipping. Thus, the accurate prediction of the packaged blueberries shelf life has become extremely important. Furthermore, the damages make blueberries more susceptible to certain number of fungal diseases such as grey mold (*Botrytis cinerea*), anthracnose (*Collecotrichum gloeo-sporoides*) and alternaria fruit rot (*Alternaria spp.*) (Strik, 1993; Austin, 1994), which cause huge blueberry economic losses. With so much at stake, it is imperative to develop a non-destructive sensing method to predict the blueberry shelf life and detect its diseases.

METHODOLOGIES

Blueberries tested in this study were the rabbit eye "Brightwell" variety, a common variety grown in Georgia. They were organically grown and hand harvested in Alma, Georgia, in June and July, 2008. After harvesting, they were immediately stored in a cold room at 2±1^{o}C and 80% relative humidity at a research lab, Tifton Campus of University of Georgia before being inoculated and tested. In each experiment, blueberries with roughly the same size and maturity were hand selected. To avoid unintentionally inducing unknown natural diseases, blueberries were surface sterilized by dipping in 70% ethanol for 10 min. Sterile distilled water was used to rinse blueberry surface for three times to remove ethanol residues before inoculation.

Cultures of *Botrytis cinerea*, *Collecotrichum gloeo-sporoides*, and *Alternaria tenuissima* were provided by the Plant Pathology Laboratory at Athens Campus University of Georgia. The fungi were grown and maintained at the potato dextrose agar and stored in the refrigerator at 5^{o}C. Spore suspensions of three fungi culture were obtained with sterile distilled water. Each individual blueberry was poked by a needle on its stem side where it is attached to the bush to create a slight wound to facilitate infestation. Blueberries in the Control group were treated in a similar manner. Blueberries in three treatment groups were dipped in corresponding spore suspensions to create three fungal inoculations. Berries samples with 55 g in each replicate were then stored in a 500 ml glass bottle with Teflon coated septa lined cap (I-Chem, New Castle, USA) and held at an air conditioned room under 25±2^{o}C. The electronic nose measurements were performed during 6-10 days after inoculation (dai) when inoculated berries began to show molds on their surface. The *B. cinerea* and *A. tenuissima* infested berries showed similar symptom with grey and blackish molds, whereas the *C. gloeo-sporoides* appeared as pink fungal mold.

Headspace samples from control and three pathogen infested blueberries were analyzed with the Cyranose 320 electronic nose (Smith Detection Inc., Pasadena, CA). The electronic nose is essentially a gas sensor array consisting of 32 individual thin-film carbon-black polymer composite chemiresistors. Two independent experiments using blueberries harvested at two different times were performed in order to test the repeatability of the electronic nose. In experiment 1, blueberry samples were tested on 7 and 10 dai; while in experiment 2, samples were measured on 6 and 7 dai. Four categories, i.e., Control group and three pathogen infested groups, were prepared and each group has 10 replicates. Each sample was measured two times and an average of these two measurements was calculated as the third measurement. Therefore, 30 data sets from each category were obtained and total 120 data sets from four categories were acquired for each individual testing day. In each experiment, total 240 data sets were collected from two testing days. Blueberries were held in a 500 ml glass bottle sealed by Teflon coated septa lined cap. The headspace gas was accumulated for 12 h before each sampling. The E-nose sampling needle was inserted through the septum to draw headspace volatiles while another needle (provider,) was inserted through the septum in parallel to avoid making headspace in glass bottle vacuum. Each sampling took about 2 minutes. The sensor chamber was purged using the ambient air between each measurement.

RESULTS

A representative three-dimensional PCA score plot was presented in Figure 1 by analyzing blueberry data at 7 days after inoculation in group 2 data set. Duplicate data plus their average are plotted for each berry category. Blueberry data in other days show the similar pattern and

were not shown here. The first three most important principal components, accounting for 99.7% of the total variance, were utilized for this three-dimensional PCA plot. It is clearly seen that four distinct groups were formed representing control and three pathogen groups. The relative locations of four categories of blueberry data in the PCA plot reflect the response of the gas sensor array, which is based on distinct volatile profiles from four categories of blueberry treatments. For instance, the data points of Alternaria are closer to those of Botrytis category than to control or Colletotrichum category. Visual observation proved that blueberries inoculated by Alternaria and Botrytis were much more alike than the other two groups: both Alternaria and Botrytis inoculated berries showed grey mold, while the Colletotrichum appeared to be pink mold, and the control berries remained clean without any mold.

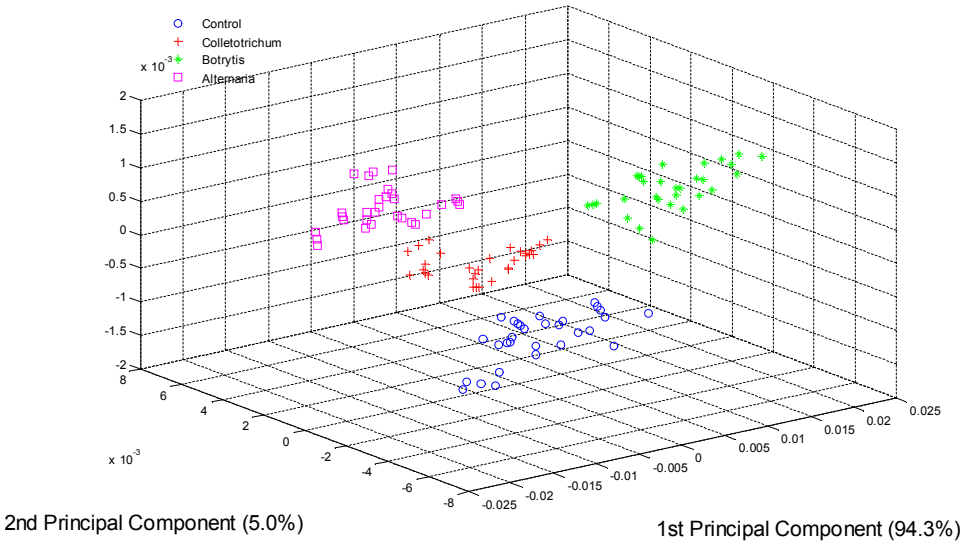

Figure 1. PCA score plot using the first three principal components based on the blueberry data at 7 days after inoculation for four categories (control, Alternaria, Botrytis, and Colletotrichum).

A pattern recognition algorithm linear Bayesian classifier was developed and used for data classification. Group 1 data (combining data from 7 dai and 10 dai) were used for classification test. A 25% of the total 240 data sets (60 vectors) were randomly selected and used as testing data sets and the remaining 75% data (180 vectors) were used for training. Among these 60 testing data sets, 16 are control samples, 15 Alternaria samples, 11 Botrytis samples, and 18 Collectotrichum samples. The linear Bayesian classifier was trained and tested on these data sets and the classification confusion matrix was shown in Table 1. It shows that all 16 control samples were correctly classified; among 15 Alternaria data sets, only 13 were correctly classified; two Alternaria samples were misclassified as Botrytis, while 2 Botrytis samples were misclassified as Alternaria group; 9 out of 11 Botrytis samples were correctly classified; 2 Colletotrichum were misclassified as Botrytis and the remaining 16 Colletotrichum were correctly classified. If the correct recognition rate is defined as the number of correct classifications divided by the number of total testing samples, the overall correct recognition rate is 90% (54/60). The similar infection mechanism of Alternaria and Botrytis may contribute to the erratic results between these two groups.

Table 1. Confusion matrix of classification result by linear Bayesian classifier

	Estimated Labels				
True labels	**Control**	**Alternaria**	**Botrytis**	**Colletotrichum**	**Totals**
Control	16	0	0	0	**16**
Alternaria	0	13	2	0	**15**
Botrytis	0	2	9	0	**11**
Collectotrichum	0	0	2	16	**18**
Totals	**16**	**15**	**13**	**16**	**60**

CONCLUSION

In summary, a conducting polymer sensor based electronic nose was successfully applied to detect the presence and differentiate three types of fungal diseases in blueberries. A three-dimensional PCA plot clearly demonstrated that four treatments of blueberries formed four distinct clusters based on their relationship and similarity. In two repeated experiments, a linear Bayesian classifier achieved satisfactory performance in classifying four categories of blueberries in both cases. Most misclassifications occurred between Botrytis and Alternaria categories due to the similarity of these two categories.

IMPACT STATEMENT

This olfactory based non-destructive sensing method showed great promise for blueberry quality control and fungal disease detection. It may provide a useful tool for blueberry postharvest quality sorting during storage and potentially reduce postharvest losses in blueberry industry. Marketers may be able to determine which pallets of blueberries should be sent to the most challenging destinations and which should be shipped and sold quickly. The success of this project will not only benefit consumers and blueberry industry which is the most important fruit industry in Georgia, but also provide a new alternative non-destructive sensing method for small fruits postharvest quality control and shelf life prediction in southern region of the United States.

CITATIONS ARISING FROM THE PROJECT

Current a manuscript titled “A Gas Sensor Array for Blueberry Fungal Pathogens Detection and Classification” is under review.

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