Discrimination of storage shelf-life for mandarin by electronic nose technique
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Abstract
Over the past years, electronic nose technology opened the possibility to exploit information on aroma attribute to assess fruit ripening stage during storage. The objective in this study was to evaluate the capacity of electronic nose in monitoring the change in volatile production of mandarin during different storage treatments, and storage shelf-life of mandarin was evaluated by measuring the change in volatile production of mandarin using electronic nose device (PEN 2). By comparing, storage shelf-life of mandarin was better distinguished using Linear Discriminant Analysis (LDA) than Principal Component Analysis (PCA). PCA and LDA analysis were efficient to classify mandarin with the same storage time in its respective groups; but the methods are not efficient to separate the mandarins by different storage times. The correlation between the measured and predicted values of fruit quality attribute (such as soluble solid content, acidity and compression force) shows poor to reasonable prediction performance by means of electronic nose signals. The results prove that sensors 2, 7 and 9 in the electronic nose PEN 2 for mandarin have a higher influence in the current pattern file. Hence, nearly a subset of few sensors can be chosen to explain all the variance. This result could be used in further studies to optimize the number of sensors and find better performance.

Keywords: Electronic nose; Discrimination; Mandarin; Quality; Shelf-life

1. Introduction
In recent years, there has been a considerable increase in demand for better quality fruit due to globalization of market. Consequently, it is important to evaluate fruit maturation stage and storage shelf-life. Many methods of monitoring maturation and shelf-life have already been proposed. The main disadvantages of these methods are that they are not practical for cultivars or storage stations, and most of them require the destruction of the samples used for analysis. This may be the reason that optimal harvest date and predictions of shelf-life are mainly based on practical experience (Wang, Teng, & Yu, 2004). Leaving these critical decisions to subjective interpretation implies that large quantities of fruit are always harvested too soon or too late and reach consumer markets in poor condition.

A strategy for determining the maturation and shelf-life consists of sensing the aromatic volatiles emitted from fruit by using electronic olfactory systems (Benady, 1995). Metabolic changes are mostly due to the following four items: post-harvest ripening, respiration, fermentation and phenolic oxidation (Young, Gilbert, Murria, & Ball, 1996). Aroma is an important food quality attribute. The aroma of a food product is detected when its volatiles enter the nasal passages at the back of the throat and is perceived by receptors of the olfactory system (Oshita et al., 2000; Sohn, Smith, Yoong, Leis, & Galvin, 2003). Concerning the exploitation of the information contained in the headspace of fruit, they have been studied in the recent past with the conventional analytical chemistry equipment, and the correlation between the state of over-ripening and the fruit aroma has also been found both in quantitative and in qualitative terms. Besides, some specific compounds have been identified to be responsible of the aroma of particular fruit.

In the last decade, the electronic nose technology has opened the possibility to exploit, from a practical point of
conduct, respectively. The electronic nose offers a fast and nondestructive alternative to sense aroma, and, hence, may be advantageously used to predict the optimal harvest date. Commercially available electronic noses use an array of sensors combined with pattern recognition software. There have been several reports on electronic sensing in environmental control, medical diagnostics and the food industry (Keller, Kangas, Liden, Hashem, & Kouzes, 1995; Schaller, Bosset, & Escher, 1998). Some authors reported positive applications of electronic nose technology to the discrimination of fruit of different qualities, with oranges (Gomez, Wang, Hu, & Pereira, 2006), tomatoes (Berna, Jeroen, Stijn, Corrado, & Nicolaï, 2004), apples (Young, Rossiter, Wang, & Miller, 1999; Brezmes, Llobet, Vilanova, Saiz, & Correig, 2000; Brezmes et al., 2001; Saevels et al., 2003) and cherry (Toivonen, Kappel, Stan, McKenzie, & Hocking, 2006), but as yet few literatures refer to control signals.

The objectives in this study were: (1) to evaluate the capacity to measure the change in volatile production of mandarin during storage using a specific electronic nose device (E-nose, PEN 2), (2) to study Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) to obtain whether the electronic nose is able to distinguish and classify different mandarin storage states; and (3) to evaluate the mandarin parameters such as firmness, soluble solids content and pH on the base of electronic nose signals.

2. Materials and methods

2.1. Experimental material

Chinese variety, Satsuma mandarin “Zaojin Jiaogan” (C. reticulata), was selected for the experiment. The experimental samples were hand harvested from the experimental orchard in Department of Horticulture, Zhejiang University. Mandarins were harvested on 3 October 2004.

Fruit was selected according to an approximately uniform size and weight. Because fruit were harvested from different trees, pooled, then randomized, the experimental design was completely randomized with each fruit as an experimental unit. All fruit were individually numbered. Three hundred mandarins were divided into three groups (100 samples each group) and three storage treatments were conducted, respectively.

For cross validation, the same variety, Satsuma mandarin, was selected for the experiment and harvested on 3 October 2004 (the same day). But the samples were hand harvested from the other orchard (Jingde orchard, Zhejiang), 12 km far from the experimental orchard in the Department of Horticulture. One hundred and fifty mandarins were divided into three groups (50 samples each group) and the same three storage treatments were conducted, respectively.

2.2. Storage conditions

Different storage treatments were conducted in laboratory conditions and in refrigerator. The mandarin fruit stored in laboratory conditions were placed in 4 folded plastic bags and 4 carton boxes with 20 mandarins each group, respectively; they were stored for 12 days at 20±1 °C, relative humidity (RH) 50–60%. Mandarins (a bag/a box) were removed from storage at days 3, 6, 9 and 12 (day 3 is the third day since harvest, day 0 is the picked day) and evaluated using electronic nose.

During refrigerator storage, 4 folded plastic bags with 20 mandarins each group were kept in the refrigerator at temperature 4±0.5 °C, 85–90% RH; one bag was removed from storage at days 15, 30, 45 and 60 and mandarins were evaluated.

For cross validation, the same three treatments were conducted for all mandarins from the other orchard. All mandarins were evaluated at the picked day (day 0) using electronic nose technique before being divided into four groups and 50 samples each group. One group was evaluated by E-nose at picked day, and other four groups were stored and evaluated.

2.3. Electronic nose data acquisition and analysis

An electronic nose device PEN2 (E-nose), provided by WMA Airsense Analysentechnik GmbH, Schwerin, Germany, was used. The portable electronic nose PEN2 has an array of 10 different metal oxide sensors positioned into small chamber (V = 1.8 ml). Fig. 1 shows a schematic diagram of the electronic-nose measurements and gas flow of PEN 2 during the experiments. Table 1 lists all the sensors used and their main applications. This table contains current known or specified reaction.

Each fruit was placed into an airtight glass jar with a volume of 11 (concentration chamber). The glass jar was then closed and the headspace inside it was equilibrated for 1 h. Preliminary experiments showed that after 0.5 h of equilibration, the headspace reached a steady state and experiments were conducted after 0.5 h of equilibration. One luer-lock needle (20 g) connected to a Teflon-tubing (3 mm) was used to perforate the seal (plastic) of the vial and to absorb the air accumulated inside it, during the measurements. The headspace gas was pumped over the sensors of the electronic-nose; during the measurement process, three different phases can be distinguished: concentration, measurement and stand-by. The electro valves, controlled by a computer program, guide the air through different circuits, depending on the measurement phase. No matter the phase, airflow is always kept constant throughout the measurement chamber. During the measurement phase, the bomb pushes the volatiles through a closed loop that includes the measurement and concentration chambers. No air enters or exits the loop. The measurement phase lasts 60 s, time enough for sensors to reach a stable value. The collected data interval was 1 s.
When a measurement is completed, a stand-by phase is activated (60 s). The main purpose is to clean the circuit and return sensors to their baseline. Clean air enters the circuit, crosses the measurement chamber first, the empty concentration chamber afterwards, and pushes the remaining volatiles out of the circuit.

E-nose was used at the temperature of 20 ± 2 °C and 50–60% RH during all experiments. When the sensors are exposed to volatiles, during the measurement phase, the computer records the resistance changes that the sensors experience. When the measurement was completed, the acquired data were properly stored for later use.

The set of signals of all sensors during measurement of a sample is a pattern. Pattern of multiple measurements dealing with the same problem are stored in a Pattern File and act as the Training Set. The pattern data were recorded, checked visually and analysed using WinMuster (version 1.5.2.4 June 2003, copyright 1996–2002 WMA Airsense Analysentechnik GmbH 2003).

### 2.4. PCA, LDA

Using the PCA, the measurement data, previously trained, will be transformed into two-dimensional (2D) or 3D coordinates. This is carried out through the data reduction that extracts the most important information from the database as a result. The results of training phase can be displayed in a 2D view. PCA is based on a linear project of multidimensional data into different coordinates based on maximum variance and minimum correlation. Training pattern from measurements of similar samples will be located close to each other after transformation. Hence, the graphical output can be used for determining the difference between groups and comparing this difference to the distribution of pattern within one group.

The LDA is the first step of the Discriminant Function Analysis (DFA). The LDA calculates the discriminant functions and similar to the PCA—a 2D or 3D display of the training set data. The difference between PCA and LDA is that PCA does not care about the relaton of data points to the specified classes, while the LDA calculation uses the class information that was given during training. The LDA takes care about the distribution within classes and the distances between them. Therefore, the LDA is able to collect information from all sensors in order to improve the resolution of classes.

The sum of displayed variances is higher; the further principal components also contain discriminant information using PCA and PCA.
2.5. Quality attribute measurement

2.5.1. Compression test and fruit firmness

The fruit firmness was evaluated by maximum compression force \( (C_F) \). The maximum compression force required to compress a fruit by 3% of its medium diameter (Equator) was recorded at a deformation rate of 0.0016 m/s (Wang, Teng, & Yu, 2006). The maximum compression force of all individual fruit was measured on the three positions along the equator approximately 120° between them, perpendicular to the stem-bottom axis. The measurements were carried out by a Universal Testing Machine (Model 5543 Single Column, Instron Corporation, Canton, MA, USA). The test was performed using parallel plates for compression test.

2.5.2. Soluble solid content (SSC) and pH measurement

SSC of juice for each fruit were measured with temperature compensating refractometer (Digital refractometer WYT-J 0–32% Beijing, China). pH of juice was measured by a pH meter (Manufacturer: Sartorius AG, PB-20 (PB-s), Geottingen, Germany, pH/mv = 0 ± 0.3 mV).

2.6. Evaluation models for E-nose prediction

For fruit quality indices, different calibration models were used by partial least square (PLS). The quality indices of the calibration model were quantified by standard error of calibration (SEC), standard error of prediction (SEP) were used by partial least square (PLS). The quality indices were used in the model and noise was modeled. SEC and SEP, a high correlation coefficient. A large number of LV were resulted in an “overfitted” model, while fewer produced an “underfitted” model. The RMSEP is defined as follows:

\[
RMSEP = \frac{1}{I_p} \sum_{i=1}^{I_p} (\hat{y}_i - y_i)^2.
\]  

To assess the electronic nose prediction, the results by E-nose response were compared with those derived from well-established traditional techniques such as pH, soluble solid content and compression test. The calculations were carried out using ‘The Unscrambler V8.0.5 1986–2003’ (CAMO, PROCESS, AS, OSLO, Norway), a statistical software package for multivariate calibration. Before the calibration, the relative conductance variation of the data was analysed by PCA and defective sensor response was eliminated, and PLS was used to build the prediction models. The latter is a projection method, which uses the independent variable and the dependent variables to regress the dependent variables on the LV (factors) (De Jong, 1993).

3. Results and discussions

3.1. Electronic nose response to fruit aroma

Fig. 2 shows a typical response of ten sensors during measuring mandarin fruit. The data obtained are the changing ratio of conductivity between \( G \) and \( G_0 \) (the conductivity of the sensors when the sample gas or zero gas blows over). Each curve represents a different sensor transient. The curves represent sensor conductivity of one sensor of array against time due to electro-valve action when the volatiles from the fruit reach the measurement chamber. In that transition, the clean airflow that reaches the measurement chamber is substituted by airflow that comes from the concentration chamber, closing a loop circuit between both chambers. It can be seen that after
an initial period of low and stable conductivity (when only clean air is crossing the measurement chamber), conductivity increases sharply and then stabilizes after 30 s. The each sensor signal generally stabilizes and was considered to use in analysis of electronic nose. In this research, the signal of each sensor at response 42 s was used in analysis of electronic nose.

3.2. Signal analysis

Fig. 3 shows the change of the signals generated by the sensor array to different storage mandarin. Each line represents the mean signal variation of mandarins for one sensor, linking to the measurements of conductance increase or decrease experienced by the sensor each time. It was presented that E-nose sensor response would change for mandarin during three storage treatments.

During the mandarin storage, the respiration led to a decrease of the vapors generated by the fruit; it reached in less quantity of the sensor array during measurement process (Fig. 3). This result was contrary to those obtained by Brezmes, Llobet, Vilanova, Saiz, and Correig (2000) testing peaches and pears.

This result is due to special changes of chemical composition in citrus during storage process. Erkan and Pekmezci (2000) reported that the respiration rate of oranges held at 20°C (shelf-life conditions) showed initially an increase during a short period of time (within 2 days) in respiration, which subsequently decreased (after 2 days). Whereas, the respiration of orange stored at 3–7°C decreased continually.

For three storage treatments, the decrease was more steeper for the mandarin stored in carton boxes facilitated by the contact between fruit and atmosphere, and the opposite result was obtained for mandarins kept in refrigeration, where the low temperatures led to a lower decrease of volatile.

In all treatments, some sensors with minor response to fruit volatile production have a small erratic behavior during measurements.

3.3. Classification of mandarin using PCA and LDA

3.3.1. Mandarin stored in box

PCA and LDA analyse were performed, and the principal component 1 (PC1) and principal component 2 (PC2) and first and second linear discriminant LD1 and LD2 are shown in Fig. 4. Imaged ellipses correspond to the 95% confidence intervals.

PCA in Fig. 4 shows the score plot inside the ellipses and represents the variation around different storage times in box for mandarin. The processed data show an erratic shift of different storage times along the first principal component, PC1, which explains 90.90% of the total variance with value 98.38%. The tendency is not constant on the axis-x (PC1); different groups were less separated by different storage time. The measurements values were overlapped for groups between day 0 and day 9, day 0 and day 12, and day 6 and day 12. The second principal component (PC2) explains 7.48% of the variation and shows no particular trend with the storage time.

After analysing the same data set using LDA, five groups were more distinguished than using PCA (Fig. 5). In this plot, about 57.20% of the total variance of the data is displayed. Functions 1 (LD1) and 2 (LD2) accounted for 41.21% and 15.99% of the variance, respectively. LDA analysis showed the variation of group of day 0, day 3 (or day 6) and day 9 along the axis-y (LD2) with a decrease tread. The group of day 6 has a clear separation in negative sense away from the rest of the groups on the axis-x (LD1). The sample of day 0 (or day 3), 6, 9 and 12 would be approximately distinguished using LDA analysis.

Cross-validation analysis was performed using mandarins from the other orchard mandarins (Jingde orchard) by LDA. Fig. 4 showed that the classification for day 0 had three unclassified samples (not located into the original ellipse), two of them were classified into the group of day 3, another were classified into the group of day 12; the group of day 12 also had one unclassified sample, this representing 10% of the total (n = 10). This result represents 14% of the total samples in this group (n = 50). The other groups of day 3, 6 and 9 contain inside all corresponding samples.

Fig. 3. Mean value of each sensor’s response to different storage times of mandarin in bag (a), box (b) and cool storage (c) ■: sensor 1; ●: sensor 2; ▲: sensor 3; ▼: sensor 4; ◆: sensor 5; ▼: sensor 6; ◄: sensor 7; ●: sensor 8; ★: sensor 9; ○ sensor 10.
3.3.2. Mandarin stored in folded plastic bag

Fig. 5 shows a shift of different storage time along the first principal component, PC1, which explains 90.72% of the total variance with value 96.61%, and consecutive different groups of fruit separated by storage time did overlap (days 0, 3, 6 and 9).

PCA analysis showed the variation of five groups along of the axis-x (PC1) with a less increment from day 0 to day 9, but the group of day 12 showed an advance in negative direction on the function 1. The second principal component (PC2) explains 5.88% of the variation and showed no particular trend with the storage time. No clear shift on the axis-y (PC2) was shown. The sample of day 12 would be distinguished from the sample by using PCA analysis.

After analysing the same data set using LDA, a clear separation was achieved among the group of days 0, 12 and the remaining groups, but groups of days 3, 6 and 9 overlap each other (Fig. 5). In this plot, only 65.11% of the total variance of the data is displayed. Functions 1 (LD1) and 2 (LD2) accounted for 53.49% and 11.62% of the variance, respectively. The samples were better distinguished using LDA than using PCA.

Cross-validation analysis was performed with mandarins from the other orchard (Jingde orchard) by using LAD. Fig. 5 showed that the classification for day 0 had three unclassified samples; groups of days 6, 9 and 12 each had one unclassified sample. All samples in group of day 3 only were correctly classified. This result represents 12% of the total samples in this group. LDA analysis was able to classify each group, meaning that this method is efficient to classify mandarin with the same storage time in its respective group; but this method was not efficient to separate clearly mandarin groups according to different storage times for folded packing bag.

3.3.3. Mandarin stored in refrigeration

Fig. 6 shows PCA and LDA analysis, separately, to mandarin kept in cool storage. Day 0 is representing measurements of all samples in the beginning of the observation.

Fig. 6 shows the score plot inside the ellipses and represents the variation around each maturity state in the space in correspondence with the storage time. The processed data show a less shift erratic of different storage
Along the first principal component, PC1, which explains 78.50% of the total variance with value 96.65%, and consecutive different groups separated by storage time did overlap, although there is a clear distinction among days 15, 30 and 60, or among days 0, 45 and 60. The second principal component (PC2) explains 18.15% of the variation and shows no particular trend with storage time, beside group of day 60 has a clear upward displacement along function 2, getting away from the remaining groups.

When analysed the same data set using LDA, five groups were clearly distinguishable, except between groups of days 45 and 60 (Fig. 6). In this plot, about 59.83% of the total variance of the data is displayed. LDA function 1 (LD1) and function 2 (LD2) accounted for 44.071% and 15.76% of the variance, respectively. LDA analysis showed the variation of each group along the abscissa (LD1) with an increment; however, the groups of days 45 and 60 showed an advance in negative direction on function 1.

Cross-validation analysis was performed with mandarins from the other orchard (Jingde orchard) by using LAD. LDA analysis was able to classify a 100% of the total samples in each respective group, but the method is not efficient to separate mandarin groups by different cool storage times in bag. The two groups of day 45 and 60 have similar space location in the score plot.

### 3.4. Prediction of fruit quality attributes

In order to compare the electronic nose performance with fruit quality attributes, olfactory system measurements were coupled with the values obtained from quality indices at the same measurement session.

The relative conductance values for each sensor at 42 s were related to each fruit quality characteristic such as soluble solid content (SSC), pH and maxim puncture force.

Twenty mandarins were separated randomly into two groups (each group of three storage treatments): a calibration set used to develop the calibration models (12 mandarins), the remaining samples of the population were used for prediction set (external validation) (8 mandarins); also the calibration models were validated using full cross validation. Measurements spanned over 12 days after harvest, i.e. in each measurement (each group of three storage treatments), 12 samples in 20 mandarins were removed from shelf-life at days 3, 6, 9 and 12 and used to develop the calibration models, and 8 samples in 20 mandarins were used to prediction set.

In the validation method, some samples are kept out of the calibration and used for prediction. This is repeated until all samples have been kept out once. Validation residual variance can then be computed from the prediction residuals. In segmented cross validation, the samples are divided into subgroups or “segments”. One segment at a time is kept out of the calibration. There are as many calibration rounds as segments, and predictions can be made on all samples. A final calibration is then performed with all samples. In full cross validation, only one sample a time is kept out of the calibration.

The correlation between the measured and predicted values of fruit parameters shows poor to reasonable prediction performance with values between 0.705 and 0.786 during the calibration model construction and with values between 0.686 and 0.759 for the internal validation set (Table 2).

PLS prediction results for soluble solid content, acidity, and compression force are presented in scatter plots (in Fig. 7). In figure, the ordinate and abscissa axes represent the predicted and measured fitted values of the appropriate parameters, respectively. During the prediction (external validation), poor correlation coefficients were obtained with values between 0.659 and 0.725; the best correlation coefficient was obtained for SSC and the worst value for pH.

In the previous research work, Saevels et al. (2003) in apples, a poor correlation between fruit quality indices (firmness, acidity and soluble solids) and nose signal response was also obtained, with correlation coefficient values between 0.66 and 0.76. However Brezmes et al. (2001), using electronic nose signal to predict firmness and pH in pink lady apple, obtained reasonable well
prediction performance with correlation coefficient values of 0.94 and 0.84, respectively.

Although it may seem surprising that physical measurements such as firmness measured by compression force can quite be predicted with sensor responses to organic volatiles generated by fruit, such results are meaningful since the physiological characteristics of fruit are closely related to chemical processes that take place during the ripening process of fruit. In other words, the electronic nose does not measure firmness directly; it actually measures volatiles that are well correlated with the firmness of the fruits.

4. Conclusions

(1) Storage shelf-life of mandarin was evaluated by measuring the change in volatile production of mandarin using electronic nose device (PEN 2). Upon comparing, storage shelf-life of mandarin was better distinguished using LDA than using PCA.

(2) PCA and LDA analyses are efficient in classifying mandarin with the same storage time in its respective groups; but the methods are not efficient in separating the mandarins by different storage times.

(3) The results prove that sensors 2, 7 and 9 in the electronic nose PEN 2 for mandarin have a higher influence in the current pattern file. Hence, nearly a subset of few sensors can be chosen to explain all the variance. This result could be used in further studies to optimize the number of sensors and find better performance.

(4) The correlation between the measured and predicted values of fruit parameters shows poor to reasonable prediction performance on the base of electronic nose signals.

Table 2
Results of calibration, cross validation and prediction for fruit quality property on the base of electronic nose signal

| Parameter | LV | Calibration | | | | Cross validation | | | | Prediction | | |
|-----------|----|-------------|---|---|---|---|---|---|---|---|---|---|---|
|           |    | r | SEC | RMSEC | Bias | r | SEP | RMSEP | Bias | r | SEP | RMSEP | Bias |
| SSC       | 4  | 0.786 | 0.557 | 0.553 | -4.97e-7 | 0.748 | 0.60 | 0.596 | -0.78e-3 | 0.726 | 0.694 | 0.739 | -0.265 |
| pH        | 3  | 0.705 | 0.095 | 0.094 | -5.11e-8 | 0.686 | 0.098 | 0.097 | -0.65e-3 | 0.659 | 0.128 | 0.129 | -0.019 |
| Fc        | 3  | 0.781 | 3.511 | 3.485 | -4.22e-7 | 0.759 | 3.656 | 3.63 | -0.73e-2 | 0.733 | 3.974 | 3.968 | -0.329 |

Fig. 7. Prediction results from the PLS models for soluble solid content, acidity and compression force.
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