

Prediction of Newhall navel orange internal quality based on digital microscopy

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Abstract: The traditional technology for internal quality detection of citrus fruit is believed to be destructive, inefficient, and error-prone. To address these issues, a novel method has been developed to evaluate nondestructively the sugar-acid ratio (SAR) of soluble solids by using the oil glands density (OGD). In this study, a total of 584 samples with different widths were collected. The sample data were correlated that the SAR and OGD decreased with time during storage. The relevance with time was significant between SAR and OGD of citrus positively correlated in the calibration group ($R^2=0.82$), which indicated that OGD could be used to predict the internal quality of citrus. It indicated that a new generation of digital microscopy could provide an alternative to predict nondestructively the internal quality of citrus fruit, as well as some theoretical insight for further studies of online quality detection in the future.

Keywords: citrus, nondestructive, oil glands, internal quality, storage, microscopy

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1 Introduction

Fruits quality characteristics, including external quality (such as color, size, and shape)^[1] and internal quality (such as soluble solids, titratable acid, and firmness)^[2], are the basic factors affecting the consumers' desire of purchasing^[3,4]. Nowadays, consumers' pursuit of fruits quality is not only limited to external quality but also extends to internal quantity. Citrus is planted widely in the world for its rich in vitamins, carotenoids, minerals, and dietary fibers^[5-8], which are essential for normal human growth and development and overall nutritional health. Soluble solids content (SSC) is one of the most crucial internal attributes determining consumers' purchasing desire also plays an important role in guiding orchard management. Because sugar contents

generally determine the flavor of fruits. After harvest, the texture of citrus fruit changes constantly, and the flavor deteriorates^[9]. These factors severely restrict the improvement of citrus economic benefits and its market competitiveness. As the world is changing at a rapid pace, driven by science and technology, the general public has drawn their attention to the issue of food quality and safety^[10]. Thus detection has performed a key role in improving fruit quality, enhancing fruit market competitiveness, and increasing the income of farmers^[11]. However, the traditional testing technology of SSC is destructive, time-consuming and high energy consumption, which is not suitable for the demand of fast and large amounts of fruits grading industry^[2].

Nondestructive testing technology has the advantages of real-time, low energy consumption and minimal sample preparation. In recent years, it has been extensively studied and accepted as an effective method for the detection of fruits maturity, shelf life, quality sorting, and even internal disease. In general, nondestructive testing technologies include electronic nose^[12-14], visible/near-infrared (Vis/NIR) spectroscopy^[15-17], ultrasonic sensing technique^[18,19], machine vision sensing technique^[20,21].

To be frank, the electronic nose is well-known that sample preparation and sampling are error-prone steps for e-nose measurements. Gas sensors are very sensitive to temperature, humidity, pressure, gas velocity, and vapor concentration. Lyu^[22] et al. combined NIR spectrometer with electronic nose technology to detect SSC of Ehime Prefecture Jell-O orange No. 38 more accurately. The R and RMSE of the validation set model were 0.8872 and 0.4709 respectively and the result shows that PCA-BPNN based on the fusion data has better SSC prediction ability. However, sample preparation of e-nose sensing is also

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very challenging since the number of volatiles released from foods depends on many factors such as temperature, pressure, and humidity. High repeatability and precision of e-nose measurement require strict control of sample preparation and sampling environment^[23].

Visible and near-infrared reflectance spectroscopy has become a powerful tool for the non-destructive monitoring and prediction of multiple quality and safety attributes of agro-food products^[24], Wang et al.^[25] had compared three kinds of spectral acquisition methods, diffuse reflection, transmission and diffuse transmission, to realize the detection of SSC in navel orange. The results showed that no matter how the sample is peeled or not, the transmission mode had the best result, and adding visible light region data will reduce the prediction accuracy of the model. However, it is difficult to the high-speed movement of the samples.

Ultrasound is in the wide ranges of frequency and power, easy usage and the safety of this technology for humans, made it an appropriate technique to apply in the different fields of fruits, juices and dairy industries including analyzing, control and processing, Mizrach has developed a non-destructive ultrasonic testing system, which used a set of low-frequency probes to measure the ultrasonic signals sent and received through the distance for evaluating the maturity and hardness of fruits^[26], but researches in the field of ultrasound in the food science are conducted under controlled laboratory conditions, and it cannot be expected that the same obtained experimental results would be accessible on an industrial scale^[27].

The advantage of machine vision is that provides rapidness, persistence, and non-destructiveness^[28].

Above all, the method of machine vision was used to observe citrus epidermis, secretory cavities occur naturally in all species of^[16] the family Rutaceae^[29]. In the genus citrus, they are commonly referred to as oil glands and occur in the stem, mesophyll of leaves, all parts of the flower except the stamens, and the fruit, where they are positioned in the exocarp or 'flavedo' layer of the rind amongst compact subepidermal parenchyma tissue and Knight found that the thickness of oil gland layer increased and the density of oil gland^[30-34] decreased with the fruit enlargement^[33]. Guo et al.^[35] measured the content of citrus peel essential oil under different storage conditions and showed that the content of citrus peel essential oil decreased with the increase of storage time, based on this, the decrease of essential oil leads to the decrease of oil gland density under machine vision. What is more, little information has been reported on the correlation between oil glands density (OGD) in the citrus epidermis and citrus internal quality. After the experiment in the study, there is a certain statistical law between the density of citrus oil gland and SSC.

In this study, a new nondestructive detection method, namely, continuous detection of the changes in oil glands in citrus epidermis to predict the changes in soluble solids in citrus was proposed. This method can be used to predict the internal quality of citrus by the morphological changes in oil glands. With further related research, this method will be part of a potential rapid online detection method.

2 Materials and methods

2.1 Sample preparation

Newhall navel orange (*Citrus synesis*) samples were collected from 15-year-old commercial fruit orchards in Gigue County, Hubei Province, China. Fruits with diameters of 80 mm and above (A), 75 mm to 80 mm (B), 70 mm to 75 mm (C), and 65 mm

to 70 mm (D) were picked when they reached commercial maturity in early December. On the same day, the fruits were transported back to Huazhong Agricultural University, Wuhan, Hubei, China. The fruits were graded based on the four diameter ranges, and fruits with deformities and surface defects were eliminated. After a thorough cleaning with clean water, the fruits were air-dried. After 24 h of air-drying process, the fruits were packed in polyethylene bags with a thickness of 0.01 mm and stored at room temperature in a ventilated warehouse. The storage temperature was 12 °C-20 °C, and the relative humidity was 80%-85%. After 24 h storage, sampling, observation and detection were conducted for the first time and then every 10 d for an additional 6 times. The specific sampling methods were as follows: Twenty fruits were selected from each of the four diameter ranges. Eight circular areas with a diameter of 1.5 cm were selected along the equator of each fruit to detect the oil glands changes. A total of 584 samples were selected with 504 (63 samples from each category of four different diameters) subjected to respiratory intensity (252 samples) detection and soluble solid (252 samples) detection, while another 80 samples were used to detect the density of oil glands in the epidermis. The detection was performed for every 10-d interval.

2.2 VHX-6000 imaging system and measurement method

The system of VHX-6000 (shown in Figure 1) consisted of a highly sensitive, high-speed 50-frame CMOS camera, TRIPLE'R wireless RZ lens, small high-speed motor, high-resolution plus high-speed XYZ electric platform, and REMAX VI imaging software. SPSS 17.0 and Origin 9.0 software were used for data processing. The Optoelectronic 3D color microscope could automatically measure areas, count oil glands, and calibrate the photographed images with an automatic elimination of man-made errors. During the detection, VH-Z20R/Z20T super miniature high-performance zoom lenses were used with a stable light source and the observation distance was 25.5 mm.

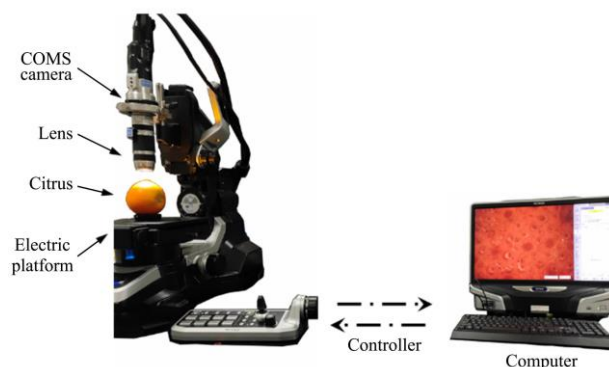


Figure 1 VHX-6000 imaging system

The flow chart of nondestructively predicting the internal quality of citrus fruit is shown in Figure 2. During the storage of citrus, the essential oils in the oil glands were absorbed or volatilized, which caused some characteristics of the oil glands to change. According to the experimental data, analyze the correlation between sugar-acid ratio (SAR) and OGD.

2.3 Determination of physical and chemical indexes

The basic morphological parameters and quality of navel orange samples were measured. Citruses with four different diameters were measured by Deli high precision electronic digital Vernier caliper (model DL91150, Deli Group Ltd., China). The sample fruits with different diameters (equatorial circumference) were weighed using a 1% electronic balance scale (model MP31001, Shanghai Hanging Scientific Instrument Co., Ltd., China). The content of total soluble solids (TSS) was determined

by PAL-1 (pocket refractometer PAL-1, Atagi, Japan). The content of titratable acid (TA) was determined by acidity meter according to the manufacturer's instructions^[36]. Nine fruits of the same size were combined into one group, and three fruits from the

same group were squeezed into juice together. Then, 0.3 mL of the juice was added to 30 mL of distilled water. After blending, the TA content was determined using a digital acidity meter (GMK-835, G-WON HITECH Co., Ltd., Korea)^[37].

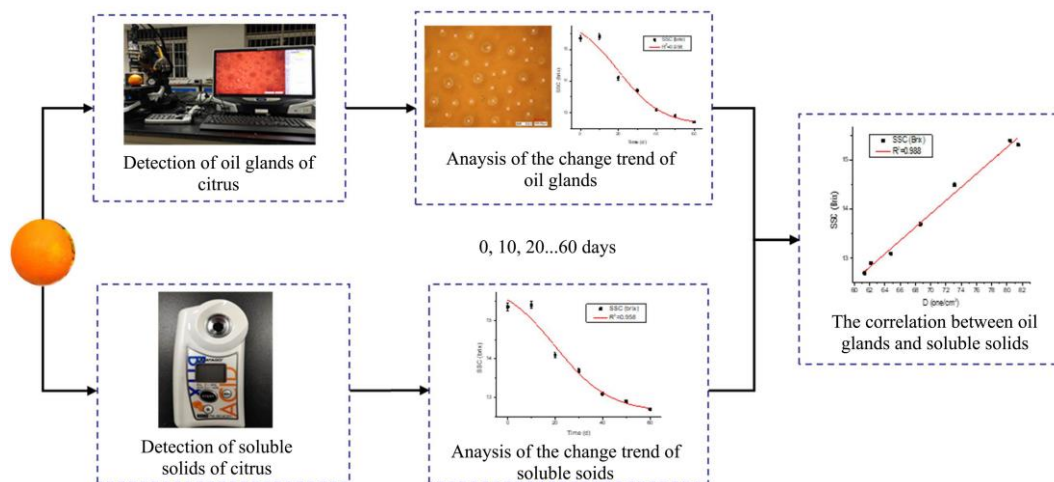


Figure 2 Flow chart of nondestructively predicting internal quality of citrus experiment

Three fruits from one group were selected and weighed, and this activity was repeated three times. A total of 36 fruits from 4 groups were sealed in a 2.6 L box for maintaining freshness and placed at room temperature for 2 h. Gas was extracted from the sealed box with a 1 mL syringe. The concentration of CO₂ in the 1 mL of the extracted gas was determined by gas chromatography (Agilent 7890A, USA)^[37].

3 Results and discussion

This study found that the number of oil glands decreased gradually with the prolongation of storage time^[37], as shown in

Figure 3. It was observed that the number of oil glands was (35±5) at 0 d, (33±4) at 10 d, (27±3) at 20 d, (26±3) at 30 d, (24±3) at 40 d, (23±2) at 50 d, and (21±2) at 60 d, and the detection area was $3.78 \times 10^{-5} \text{ m}^2$. The analysis showed that the decreasing trend of oil glands was obvious within day 10 to day 20 and that the changing trend of oil glands slowed down from 20 d to 60 d. At the beginning of storage, the OGD per unit area of citrus was high. With the prolongation of storage time, the number of oil glands and the size of oil glands decreased gradually. Therefore, a parameter was introduced to describe the change in OGD in the epidermis of citrus during storage.

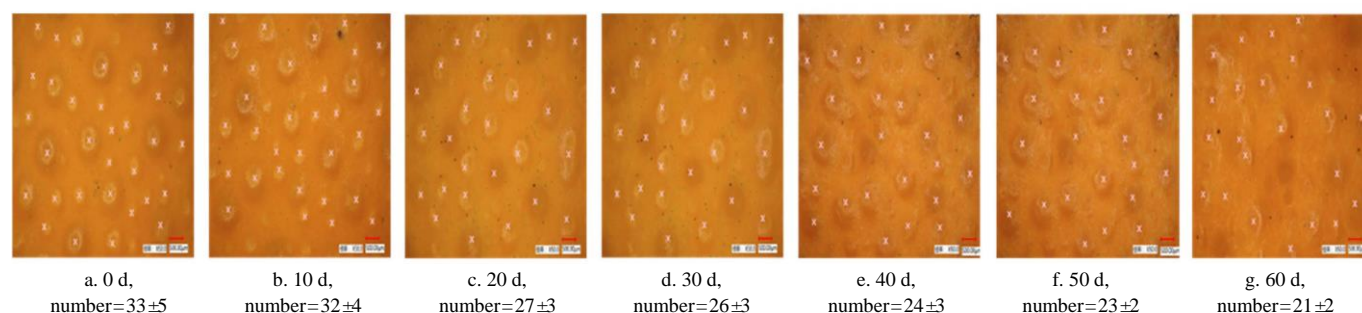


Figure 3 Example images of oil glands number changes with storage time

Figure 3 shows that the oil glands of fresh citrus epidermis exhibited obvious characteristics. With increasing storage time, the number of oil glands gradually decreased. The OGD was calculated as follows:

$$\text{OGD} = \frac{\text{Number}}{\text{Area}} \quad (1)$$

where, the number of oil glands referred to the total number of oil glands in the detection area, and the area was the projected area of the detection area ($3.78 \times 10^{-5} \text{ m}^2$). OGD indicates the number of oil glands per unit area, m^{-2} .

The detection area was divided into eight equal circular areas with a diameter of 1.5 cm distributed evenly on the equator of each citrus fruit.

The results show that the SAR of citrus also exhibited a decreasing trend with increasing storage time. As shown in Figure 4, the SAR increased temporarily within 0 d to 10 d, which

might be explained by the fact that after the citrus was picked from the tree, the acid content in the citrus decreased at a faster speed than the sugar content in the citrus in the early storage period. However, the SAR significantly decreased rapidly within 10 d to 20 d. During 20 d to 60 d, the decrease in the SAR gradually slowed down, which was similar to the trend in the OGD shown in Figure 4a. Based on the observed similar trends, the correlation between OGD and SAR was analyzed to establish a model for describing the internal relationship between these two parameters.

The correlation between SAR and OGD of the four fruit diameters is shown in Figure 5. The correlation R^2 of fruit diameters A, B, C and D is 0.733, 0.765, 0.791 and 0.937, respectively.

It can be seen from Figure 5 that the size of fruit diameter has a negative correlation with the correlation between SAR and OGD. The smaller the fruit diameter is, the higher the correlation between SAR and OGD.

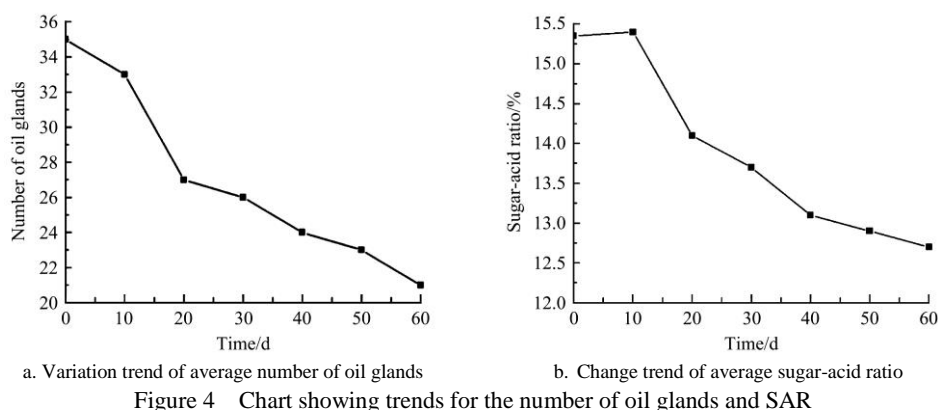


Figure 4 Chart showing trends for the number of oil glands and SAR

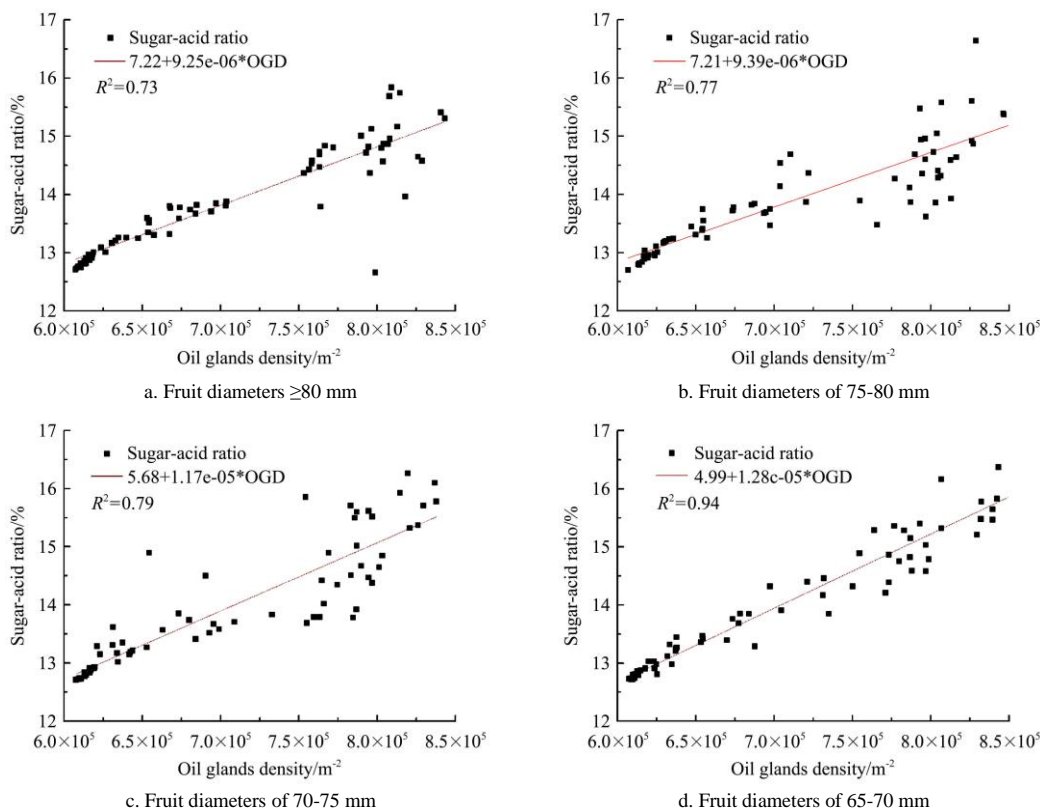


Figure 5 Correlation fitting curve of SAR and OGD for four diameters of navel orange

Citrus is a non-climacteric fruit, as can be seen from Figure 6. Although respiration decreased, the consumption of essential oil by oil glands was lower than expected within 0 d to 10 d of storage. Therefore, there was no significant change of OGD in 0 d to 10 d of storage. From 10 d to 40 d, the respiratory intensity decreased rapidly. However, the respiratory intensity remained at a relatively high level compared with that in the late period of storage (40-60 d). Affected by the change in respiratory intensity, the OGD decreased gradually. With the increase in storage time (from 10 d to 40 d), the small oil glands gradually disappeared and the OGD obviously decreased. From 40 d to 60 d, the decrease in OGD gradually slowed down, which might be attributed to the fact of slowly decreasing in respiratory intensity. Then the activity of oil glands in the citrus epidermis decreased, which resulted in the decrease in OGD was gradually slowed.

The physical parameters of citrus fruits, i.e., the OGD and SAR, are listed in Table 1. The OGD and SAR were measured in the citrus samples of the calibration group.

Figure 7 shows the fitting curves of SAR and OGD of all samples, and it is obvious that they have a strong correlation. Correlation coefficient R^2 is 0.82.

The fitting curve equation of the OGD and SAR was as follows:

$$SAR = 6.20114 + (1.09307 \times 10^{-5}) OGD \quad (2)$$
 where, SAR is the calibrated sugar-acid ratio, %; OGD is the density of oil glands (number of oil glands per unit area), m^{-2} .

The correlation analysis results showed that the OGD was highly positively correlated with the internal quality with an $R^2=0.82$.

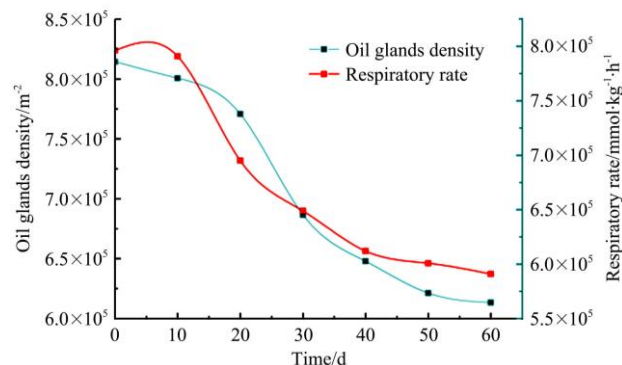


Figure 6 Diagram of respiratory rate, OGD of the calibration group

Table 1 OGD and SAR physicochemical parameters of navel orange samples in the calibration group

Time/d	Range		Mean		Standard deviation	
	OGD/m ⁻²	SAR	OGD/m ⁻²	SAR	OGD/m ⁻²	SAR
0	784507-859105	13.78-15.71	787205.89	15.54	19573.00	0.85
10	763284-846108	13.79-15.39	779568.72	15.37	26220.26	0.58
20	754958-803785	13.69-15.05	743705.61	15.00	22231.73	0.55
30	699067-703806	13.58-14.54	653754.89	14.00	17563.71	0.36
40	641798-685623	13.15-13.82	614012.94	13.38	18038.94	0.25
50	617260-632891	12.89-13.23	588541.22	13.03	8530.17	0.16
60	609732-615817	12.73-12.84	579257.94	12.81	5259.54	0.09

Note: OGD represents the oil glands density; SAR represents the sugar-acid ratio.

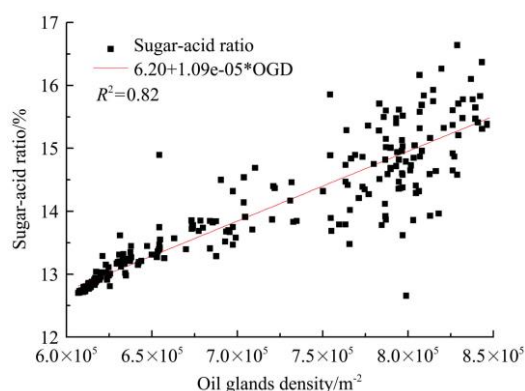


Figure 7 Correction group SAR and OGD fitting curve

4 Conclusions

Overall, the correlation analysis results showed that the SAR in the citrus was highly positively correlated with the OGD in both the calibration group ($R^2=0.82$), which indicated that the OGD could be used to predict the internal quality of citrus. Further research can establish more accurate models for different fruit diameters and explore corresponding models for different varieties. In addition, this study developed a new digital microscopy-based method for nondestructively predicting the internal quality of citrus fruit. It also provides a theoretical basis for further investigation of the nondestructive detection of citrus quality.

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