The Differential Degree Test: a Novel Methodology for Electronic Tongue Applications

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A novel methodology for electronic tongue applications based on the differential degree test was proposed in this study. Five basic tastes, namely, sour, sweet, bitter, salty, and umami, were detected by a novel voltammetric electronic tongue. Results proved that the differential degree test is applicable to intelligent instruments, and the $D_d$ (differential degree) value was confirmed to be the most useful to distinguish the differences between samples quantitatively. At the same time, the theoretical foundation for electronic tongue applications was advanced. The $D_d$ value is proportional to the logarithm of concentration ($r > 0.98$), and the sensors’ responses to the stimulus were similar to Fechner’s law in the field of psychophysics. On the basis of these results, the $D_d$ value was applied for the quantitative distinction of Chinese rice wine of different ages (1, 3, 5, and 8 years); the $D_d$ value increased with age. In addition, the changing quality of an orange juice beverage was indicated by the $D_d$ value; the electronic tongue proved useful in quality control.

1. Introduction

Sensory evaluation techniques were advanced on the basis of the perceived attributes of human beings on anything dependent on the sensory organs, such as eyes, ears, nose, mouth, and hands. These were developed and improved on the basis of the major laws of psychophysics, such as Weber’s law, Fechner’s law, and Stevens’ law.(1) The key issue in sensory evaluation is the difference test, such as the paired comparison test, triangle test, duo-trio test, “A”-“not A” test, ranking, classification, scoring, and scaling, widely applied to the analytical evaluation, comparison, and quality control of different products and gradual expressions of significance. However, sensory evaluation is
time-consuming, expensive to operate, and most often subjective, thereby reducing the accuracy of results. In an effort to address the above-mentioned concerns, intelligent sensory instruments are being developed.

Currently, several intelligent sensory instruments are derived by imitating human sense organs, such as the electronic eye, electronic ear, electronic hand, electronic nose, and electronic tongue. These have played important roles in the progress of human society. The electronic tongue system is characterized by an array of nonspecific or low-selectivity sensors and a computer as an alternative to the biological organization of the taste system and the brain in mammals. The sensor array is the main part that detects the different chemical substances and collects various types of signal information, which are then recorded in a computer. Utilizing special software, the computer easily distinguishes and recognizes the overall characteristics of the substances of different natures. The electronic tongue was developed on the basis of the principles of physical chemistry. However, limited information has been found on the type of rules observed, or whether it is systematic or unsystematic. This is a problem that must be solved urgently, and the result of this research would be important input to the theoretical foundation in the development of the electronic tongue.

The electronic tongue was produced concurrent with the development of sensory evaluation methods. This device is characterized by a nondestructive process for the samples and designed for rapid detection. Less than 30 years since its development, it has been proven to be an ideal alternative to traditional chromatographic techniques and in the analysis of food. It has been successful in the discrimination and classification of food and beverages. Its great application potential has been expressed gradually; it has not only succeeded in distinguishing and identifying basic taste substances, but also in penetrating into various fields of food research, e.g., discrimination and adulteration testing of milk and dairy products, distinction of tea, wine, apple juice and beverages, and control of the fermentation process. All of these studies have been mainly based on principal component analysis (PCA), artificial neural networks (ANNs), regression analysis, and multidimensional projection, for example. The purpose of the development and use of the electronic tongue is to make sensory evaluation scientific, objective, and quantitative, with the end view of using it in an artificial sensory evaluation. Material distinction is not enough; the difference between samples must be quantified and resolved for a wider application of the electronic tongue in the future. Although researchers have proposed using the distance to characterize the relative difference between two types of sample, this research remains as a concern worthy of further investigation. On the basis of the previous corresponding investigations, a novel methodology for electronic tongue applications was studied in this work.

This research was inspired by a related study in the sensory field. In the intelligent sensory field, issues arise from the following aspects: first, the research applicability of the classical law of psychophysics to electronic tongues and the exploration of the theoretical foundation by means of the difference test; second, the confirmation of the methodology used in the differential degree test for electronic tongue applications to qualify differences in the samples, establish the differential degree, and characterize differences between two samples scientifically. The current research thus aimed to validate the science behind sample differences. This study mainly embarked from the
practical application angle, and resolved actual existing questions on the electronic tongue using as a handy, rapid, and objective method.

2. Materials and Methods

2.1 Electronic tongue

Independently developed by the laboratory of the College of Food Science and Biotechnology, Zhejiang Gongshang University, PR China, the electronic tongue has been commercialized and characterized as a novel voltammetric device (see Fig. 1). It is based on multifrequency large-amplitude pulse voltammetry (MLAPV) controlled by a developed actuator and an array of sensors with low-selectivity metal electrodes, such as platinum, gold, palladium, tungsten, titanium, and silver electrodes. The detailed introduction of MLAPV has been reported.\(^{16}\) It adopted three frequencies, 1, 10, and 100 Hz, which are useful in the voltammetric electronic tongue for discriminating samples. The waveform of each frequency had the maximal value at 1.0 V and the minimal value at −1.0 V, with a decreased amplitude of each pulse at 0.2 V, and the interval between different successive frequencies was 5 s with 0 V. The electronic tongue displayed excellent ability in the discrimination of six different Chinese liquors and seven Chinese Longjing teas.\(^{16}\)

2.2 Samples

2.2.1 Taste substances

For the analysis, different taste substances were purchased from Hangzhou Huipu Chemical and Apparatus Co., Ltd.: citric acid and tartaric acid (sour), acesulfame (sweet),

Fig. 1. Structure of electronic tongue.
quinine sulfate (bitter), sodium chloride and potassium chloride (salty), and sodium glutamate and glycine (umami). A series of solutions were prepared with deionized water by the dilution method. Taking the minimum concentration solution (marked as R) as a control, other solutions (marked as 1, 2, 3, ..., i) were compared with R.

2.2.2 Chinese rice wine

Chinese rice wines were purchased from Hangzhou Century Lianhua supermarket and stored at room temperature. Pretreatment was not carried out before testing. The Kuaijishan wines, with different ages of 1, 3, 5, and 8 years, were produced by the Kuai Ji Shan Shaoxing Wine Co., Ltd.

2.2.3 Orange juice beverage

The orange juice beverage was also purchased from Hangzhou Century Lianhua supermarket and stored at room temperature. The orange juice beverage was produced by President Enterprise (China) Investment Co., Ltd.

When stored at high temperature in summer, the quality of the orange juice beverage degrades rapidly. Therefore in this study, we determined the quality change of the orange juice beverage over a long period. The first group of orange juice beverage was divided into 10 sterile bottles, 100 mL each, by a strict aseptic manipulation, and the bottles were placed in a 5°C refrigerator as the control sample. The second group was divided into 10 sterile bottles, 100 mL each, by a strict aseptic manipulation. These were placed in a homoeothermic incubator at 37°C as the heat-treatment sample. The processing periods were 0, 2, 5, 8, 12, 24, 30, 36, 48, and 60 h. The samples were detected by the electronic tongue regularly, and one bottle was selected each time. Before testing, the temperature of the samples was determined as high or low, and the samples were subjected to a water bath to ensure that the temperature remained at 24±1°C.

2.3 Electronic tongue detection

The samples were detected directly by the electronic tongue without any pretreatment. After ensuring constant room temperature (about 24±1°C), the group of 20 mL samples with consistent temperature were poured into a 25 mL beaker, and were then scanned. After detection, the sensors were rinsed with deionized water and transferred to another cup of deionized water for electrochemical cleaning to improve the stability of the electronic tongue. Water was removed using filter paper, and the sensors were readied for detection. Each sample was detected six times. Two types of differential degree test methods were used: paired comparison and sequence order. Paired comparison involved two different samples detected alternately by the electronic tongue. The sequence order test involved detecting the samples with the electronic tongue in the following order: 1, 2, 3, ..., i; 2, 3, ..., i, 1; 3, ..., i, 1, 2; ..., to avoid the sequence effect.

2.4 Data analysis

2.4.1 PCA

PCA is the most common multivariate data analysis method for electronic tongues, and has been shown to be effective for qualitative sample discrimination. It is a traditional linear technique of dimensionality reduction and a quantitatively
rigorous method for simplification. This method generates a new set of variables, called principal components, and each principal component is a linear combination of the original variables. In this study, the data obtained from the electronic tongue were evaluated using PCA via Matlab 7.11 (Mathworks Inc., Natick, USA).

2.4.2 Discrimination index (DI) value

The DI value is a parameter to characterize the distinction of the samples. In order to draw a clear calculating process, we described the details of this method and proposed two different ways to calculate the DI value according to whether the samples were separated on PCA score plot.

(i) Each area is separated clearly:

\[ DI = (1 - \frac{\sum S_i}{S_{all}}) \times 100\% , \] (1)

where \( S_i \) is the area occupied by one group of samples, and \( S_{all} \) is the area occupied by entire samples.

(ii) Overlap occurs:

\[ DI = - (\frac{\sum S_i}{S_{all}}) \times 100\% . \] (2)

(iii) The area is calculated as follows.
1. Connect two points by a straight line.
2. Repeat the first step for all points.
3. Determine the maximal area defined by the connection lines.
4. This area is \( S \).

The maximum DI value is 100%, which indicates the best separation of the samples. When the DI value is negative, it indicates that the samples were not be separated. The DI value was calculated using Matlab 7.11.

2.4.3 Differential degree

(1) \( E_d \) value

The commonly used \( E_d \) (Euclidean distance, \( E_d \)) is the true distance between two points in the dimension space. In a two-dimensional or three-dimensional space, the \( E_d \) is the distance between two points. The coordinates of each sample on the PCA plot, comprising six points \((X_1, Y_1), (X_2, Y_2), (X_3, Y_3), (X_4, Y_4), (X_5, Y_5), \) and \((X_6, Y_6)\), were calculated using Matlab 7.11. Afterward, according to the following formula, the center coordinates \((X, Y)\) are calculated:

\[ X = \frac{X_1 + X_2 + X_3 + X_4 + X_5 + X_6}{6}, \] (3)

\[ Y = \frac{Y_1 + Y_2 + Y_3 + Y_4 + Y_5 + Y_6}{6}. \] (4)

Two samples, \( R \) and \( i \), were compared to obtain the center coordinates \((X_R, Y_R)\) and \((X_i, Y_i)\), in accordance with the following formula, which is used to calculate the \( E_d \) value between \( R \) and \( i \):
(2) $D_d$ value

Several scientists used the separation term $(d_{ab})$ to indicate the distance between two samples. On the basis of the separation term calculation method, we proposed $D_d$ (differential degree) value to indicate that how great the difference was between two samples according to the distance: the larger the $D_d$ value, the greater the difference, and vice versa. Figure 2 presents a sketch map of the differential degree test in electronic tongue detection.

$$E_d = \sqrt{(X_R - X_i)^2 + (Y_R - Y_i)^2}. \quad (5)$$

![Diagram](image.png)

Fig. 2. Sketch map of the differential degree test of electronic tongue detection.
tongue detection. The $D_d$ value is calculated using the formula, $\Delta m$, between the mean values of two different types, $R$ and $i$, in a score plot, divided by the mean standard deviations ($\sigma_a$ and $\sigma_b$) of these types along the mean difference vector.

In this study, $R$ was considered as the control sample, and $1, 2, 3, \ldots, i$ indicated a series of test samples. The $D_d$ value of each test samples and control sample was calculated. By comparing the $D_d$ values, the difference between test samples can be known indirectly at any time and any place.

2.4.4 Fitting formula and coefficient of determination

The best-fitting curve, fitting formula, and the coefficient of determination $R^2$ were derived according to the relationship between the differential degree and concentration. $R^2$, the square of the correlation coefficient, is an important index to evaluate the fitting model and to determine whether the regression equation is good or not. Both the coefficient of determination and correlation coefficient were used to present the extent of the relationship between the two variables. The fitting formula and coefficient of determination were obtained using the curve fitting tool of Matlab 7.11.

3. Results

3.1 Difference test

A series of solutions (citric acid, sodium chloride, and sodium glutamate) were detected by the electronic tongue. The paired comparison test was conducted using a control sample (marked as $R$) and another series in the solution (marked as $1, 2, 3, \ldots, i$). The concentration of the control was 2 mM. The $DI$, $E_d$, and $D_d$ values of two samples (control and others) were calculated (see Table 1).

| $I$ (mM) | $DI$ (%) | $E_d$ | $D_d$ | $DI$ (%) | $E_d$ | $D_d$ | $DI$ (%) | $E_d$ | $D_d$ |
|----------|----------|-------|-------|----------|-------|-------|----------|-------|-------|
| 2.05     | -91.0    | 7.29  | 1.24  | -126.6   | 5.91  | 1.02  | -89.6    | 7.85  | 1.47  |
| 2.1      | -51.2    | 9.75  | 1.74  | 34.6     | 14.20 | 2.81  | -92.5    | 10.41 | 1.94  |
| 2.2      | 47.3     | 17.10 | 3.80  | 66.7     | 18.38 | 4.21  | 56.6     | 16.37 | 3.67  |
| 2.3      | 62.2     | 18.49 | 4.36  | 84.6     | 20.47 | 5.00  | 67.4     | 19.02 | 4.65  |
| 2.4      | 71.7     | 21.44 | 5.66  | 85.1     | 21.37 | 5.61  | 76.0     | 20.31 | 5.38  |
| 2.5      | 81.2     | 22.59 | 6.34  | 88.3     | 21.88 | 5.86  | 75.9     | 20.98 | 5.79  |
| 2.6      | 72.4     | 22.77 | 6.74  | 89.7     | 22.54 | 6.48  | 72.0     | 20.99 | 5.90  |
| 3        | 82.7     | 21.13 | 9.37  | 83.2     | 21.25 | 8.83  | 88.7     | 21.02 | 9.54  |
| 4        | 88.2     | 21.59 | 14.28 | 88.2     | 21.67 | 8.14  | 93.4     | 21.35 | 11.72 |
| 6        | 93.1     | 21.80 | 21.14 | 91.5     | 22.57 | 8.82  | 94.5     | 21.48 | 12.76 |
| 8        | 96.7     | 21.94 | 26.74 | 91.8     | 22.85 | 10.15 | 96.6     | 21.61 | 14.21 |
| 10       | 95.4     | 21.97 | 31.05 | 94.0     | 23.18 | 11.62 | 95.9     | 21.73 | 16.47 |
The DI value increased as the concentration of the sample solution changed from negative to nearer to 100%, but increased slowly from 85%. The Ed value had the same trend of change as the DI value; it increased with the concentration at the beginning, but increased slowly from a certain concentration. The Dd value was positively correlated with the concentration; it increased with the concentration.

3.2 Relationship between Dd value and concentration

Eight different types of basic taste substance were observed, and a series of solutions of citric acid and tartaric acid (sour), acesulfame (sweet), quinine sulfate (bitter), sodium chloride and potassium chloride (salty), and sodium glutamate and glycine (umami) (see Table 2) were detected by the electronic tongue with the sequence order R, 1, 2, 3, ..., i; 1, 2, 3, ..., i, R; 2, 3, ..., i, R, 1; 3, ..., i, R, 1, 2; ... ....

As mentioned earlier, the PCA method was used to calculate the data generated from the electronic tongue. On the basis of the PCA, the Dd values of the control and each test samples were calculated. The fitting curve, fitting formula, and the coefficient of determination between the Dd value and concentration were determined (see Figs. 3(a) –3(c)). In Fig. 3, the left showed a logarithmic relationship between the Dd value and concentration, and the right showed a linear relationship between the Dd value and the logarithm of concentration. The fitting curves of tartaric acid (a), sodium chloride (b), sodium glutamate (c), and the other five substances showed the same trend. The Dd value is proportional to the logarithm of concentration (see Table 3).

3.3 Discrimination of Chinese rice wine and quality control of orange juice beverage using Dd value detected by the electronic tongue

3.3.1 Discrimination of Chinese rice wine of different ages

Samples of Chinese rice wines from Kuaijishan, with ages of 1, 3, 5, and 8 years, were detected by the electronic tongue using paired comparison. Rice wine aged for 1 year was taken as the control sample, and samples of rice wine aged 3, 5, and 8 years

Table 2

| No.            | R   | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    |
|----------------|-----|------|------|------|------|------|------|------|------|------|
| Citric acid    | 2   | 2.2  | 2.4  | 2.5  | 2.6  | 3    | 4    | 5    | 7    | 10   |
| Tartaric acid  | 2   | 2.2  | 2.5  | 3    | 4    | 6    | 8    | 10   | 14   | 20   |
| Acesulfame     | 2   | 2.1  | 2.4  | 2.6  | 3    | 4    | 6    | 8    | 10   |      |
| Quinine sulfate| 1   | 1.1  | 1.2  | 1.3  | 1.5  | 2    | 2.5  | 3    | 4    |      |
| Sodium chloride| 2   | 2.2  | 2.5  | 3    | 4    | 6    | 8    | 10   |      |      |
| Potassium chloride| 2   | 2.2  | 2.5  | 3    | 4    | 6    | 8    | 10   |      |      |
| Sodium glutamate| 2   | 2.2  | 2.5  | 3    | 4    | 6    | 8    | 10   | 14   |      |
| Glycine\(^a\)  | 0.1 | 0.15 | 0.2  | 0.25 | 0.4  | 0.5  | 0.6  | 0.8  |      |      |

\(^a\)The concentration unit is M (mol/L).
Fig. 3. Relationship between $D_d$ value and concentration: (a) citric acid (b) sodium chloride and (c) sodium glutamate.
were distinguished from the control. Figures 4(a)–4(d) present the results. In Fig. 4(d), the \( D_d \) value is shown to change with age. The \( D_d \) value between 1 and 8 years was the largest, followed by that between 1 and 5 years, and then that between 1 and 3 years, indicating that the \( D_d \) value increased with age.

### 3.3.2 Quality control of orange juice beverage

An orange juice beverage containing sugar, acid, and concentrated fruit juice with some additives was tested. A series of changes occurred, and quality degraded when the beverage was stored at high temperature. On the basis of the paired comparison test, the \( D_d \) value of the samples stored at 37°C with the control (5°C) for different times was calculated, and a two-dimensional plot of the \( D_d \) value and time (see Fig. 5) was obtained. The \( D_d \) values of samples stored at 37°C were compared with the control sample and found to increase with storage time to 1.92 for 2 h, 2.67 for 5 h, and 6.01 for 60 h. Thus, high-temperature storage accelerated change in the orange juice beverage.

### 4. Discussion

The differential degree between two samples detected by the electronic tongue was studied. In the comparison, the \( D_I \) and \( E_d \) values were used as references to indicate the difference between two samples. However, the \( D_d \) value was more quantitative for indicating the differential degree between two samples detected by the electronic tongue. According to the results, a larger \( D_d \) value indicated a greater difference. Therefore, further research of the \( D_d \) value was conducted and used in the next section.

Eight different types of basic taste substance were detected by the electronic tongue, and the relationship between the \( D_d \) value and concentration was studied. In conclusion, the \( D_d \) value was proportional to the logarithm of stimulus intensity \((r > 0.98)\), and the stimulus intensity increased at a geometric rate, whereas the differential degree increased at an arithmetic rate.

The following summarizes the formula of the relationship between the \( D_d \) value and the concentration:

\[
D_d = a \cdot \log I + b, \tag{6}
\]
Fig. 4. PCA score plots and $D_d$ values corresponding to Chinese rice wines of different ages (1, 3, 5, and 8 years) detected by the electronic tongue: (a) 1 and 3 years (b) 1 and 5 years (c) 1 and 8 years, and (d) $D_d$ value changed with age.

Fig. 5. $D_d$ value change with time of orange juice beverage stored at 37°C.
where \( D_d \) is the differential degree value, \( I \) is the concentration, i.e., stimulus intensity, and \( a \) and \( b \) are constants.

This phenomenon is similar to Fechner’s law in the field of psychophysics. Fechner’s law states that sensation is proportional to the logarithm of stimulus intensity, and stimulus intensity increases at a geometric rate, whereas sensation increases at an arithmetic rate.\(^{(1)}\)

Above all, the relationship between the \( D_d \) value and concentration laid a theoretical foundation for electronic tongue detection.

Compared with other studies in which the discrimination function analysis method and PCA were used to distinguish Chinese rice wines of different ages,\(^{(22,31)}\) we used the numerical \( D_d \) value to indicate the difference between the samples, thereby making the result clearer and more easily visualized. A larger \( D_d \) value implies a greater difference between the rice wines. Furthermore, the \( D_d \) value increased with age. It is possible to estimate the age of rice wine by analyzing the \( D_d \) value.

The changing quality of orange juice beverage was clearly and distinctly indicated by the \( D_d \) value. High-temperature storage accelerated the change in the orange juice beverage. A quality control point of, for instance, 5 h, was set when the \( D_d \) value was greater than 2.67, which indicates the quality of the orange juice was degraded. Thus, electronic tongue distinction could be used for quality control.

5. Conclusions

In short, in this study, we presented a new concept of differential degree and the difference test method, including paired comparison and the sequence order test for electronic tongue applications. On the basis of the differential degree test, the \( D_d \) value was tested quantitatively by comparing the test samples with the control sample to achieve a quantitative comparison of different samples and different batches. The methodology was also extended to the comparison of different instruments, thereby expanding the areas of electronic tongue applications. The control sample had a notable importance in this research; other samples were compared with it. With regard to differential degree, a confirmed new idea for the experimental design constituted a new vision and new way of ensuring the science of electronic tongue applications. Thus, a more thorough study must be conducted. The present study opened a new perspective for the future of electronic tongue applications, all of which would provide potentially great contributions to daily life.

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