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Discrimination of Chinese teas according to major amino acid composition by a colorimetric IDA sensor

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ABSTRACT

An efficient liquid sensor array was presented for tea differentiation through analysis of amino acid composition based on indicator displacement assays (IDA). After construction and deduction upon response to theanine and six other amino acids abundant in tea leaves, the sensor is capable of distinguishing 70 tea samples within four categories. Statistical analysis including Hierarchy Cluster Analysis (HCA) and Principal Component Analysis (PCA) suggested that successful discrimination of teas depended not only on overall amino acid concentration but also composition of related amino acid. The PCA results even indicated a negative correlation between the loading value in the first principal component and the amino acid content in tea leaves. Besides, differentiation of tea samples of the same category with different quality grades and geographical origins was also realized, demonstrating the versatility of the presented sensor array. Our study offered an alternative method to construct colorimetric sensor for cost-effective surveillance and control of tea related products.

1. Introduction

Tea, together with coffee and cocoa, are among the top three most popular beverages in the world. According to difference in fermentation degrees and processing techniques, they can be classified as green tea (unfermented tea), Oolong tea (semi-fermented tea), black tea (fully fermented tea) and pu-erh tea (post-fermented tea) [1]. Generally, the price of tea is closely related to their quality, of which the discrimination poses an important skill to avoid fake or adulterate tea products, yet remains a problematic issue for ordinary consumers. Daily differentiation of the quality and variety of tea depends mainly on organoleptic evaluation, and inevitably would suffer from drawbacks such as personal subjectivity and bad reproducibility. Recently, a growing number of research work have been reported on the analysis of chemical composition in the teas. Those studies suggested that abundance in tea polyphenol, plant alkaloid, proteins, amino acids, vitamins, sugars, trace metals, mineral substance and other chemicals endows tea promising potential benefit for human health [2,3]. Further investigation on the relationship between the chemical composition with nutritive value of tea have also been explored using related analytical techniques including HPLC [4], GC–MS [5,6], electric nose [7], electric tongue [8], FT-NIR [9] and so on [10].

As an important group of nutritive component, amino acids are the main metabolite from nitrogen cycle of tea trees, basic constituent of proteins in tea leaves, and more importantly, one of the three key components (tea polyphenol, amino acid, and caffeine) that determine the taste of tea [11]. Accordingly, there are 26 kinds of amino acids recognized in the tea leaves and they constitute 1%-4% the composition, in which theanine, glutamic acid, aspartic acid, serine and arginine cover more than 50% the amount of free amino acids [12]. The species and amount of amino acids in the tea leaves exert the determinant influence on the flavor and taste of tea, and therefore determine their quality and economical value [13]. Interestingly, Rouba Horanni and coworkers detected 19 kinds of amino acids in 86 tea leaf samples using HPLC-UV after derivatization with FMOC-CL, which indicated that there was a reversed correlation between the fermentation degree and the amount of amino acids [14].

Although techniques based on large equipment provide various reliable protocols with good accuracy and sensitivity, the

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problems is that they usually suffer from shortcomings like complicated pretreatment procedures, time-consuming operations, high cost and requirements for professional operators. In view of those drawbacks, analysis methods based on colorimetric sensor arrays offer an effective alternative to deal with the dilemma and attract growing attention among researchers. Given merits of cost-effective and easy to operate, they have been widely applied to discriminate a great variety of matters including volatile gases [15], Chinese liquors [16], beers [17,18], proteins [19] and etc. Previously, our group reported a colorimetric sensor array to differentiate Chinese green teas of different quality degrees and geographical origins [20]. Apart from very good recognition ability demonstrated, synthesis of chromophore porphyrins limited the popularity of its application. Practically, it appears that the most powerful chromophores are always accompanied by complicated synthesis procedures and high cost for mass production let alone potential hazardous environmental impact originated from organic solvents. This problem can be partly solved by the indicator displacement assay (IDA). After introduction of targets of higher binding ability, indicators that binds to receptors in advance would be displaced as a result of competitive binding and thus give specific color response. Those assays have been successfully applied to analyze anions [21], phosphate [22], amino acids [23,24], flavonoids [25], and other chemicals [26–28].

In former studies, Sun et al. reported the detection of histidine based on competitive binding to Ni^{2+} with murexide [29], Khajehsharifi introduced the recognition of cysteine based on competition with zincon to bind Zinc ions [30], and Qian present the discrimination of 20 kinds of natural amino acids using pyrocatechol violet and Cu^{2+} through IDA [31]. Herein, we try to turn those proof-of-concept IDAs into practical applications and use it to prepare colorimetric sensor array to discriminate different kinds of teas. Seven kinds of main amino acids in tea have been selected as target analytes to design a final 3×3 sensor array, and totally 105 tea samples within 21 species would be analyzed to testify the practicability of the sensor.

2. Experimental section

2.1. Materials and reagents

Zincon (2-carboxy-2′-hydroxy-5′-sulfoformazylbenzene), pyrocatechol (PV), murexide, N-(2-hydroxyethyl)piperazine-N0-2-ethanesulfonic acid (HEPES), and seven amino acids including theanine (The), serine (Ser), glutamic acid (Glu), arginine (Arg), aspartic acid (Asp), cysteine (Cys), and histidine (His) were purchased from Aladin Reagent Co. Ltd (Shanghai, China). $ZnSO_4$, $CuSO_4$, $NiCl_2{\cdot}6H_2O$ and other chemicals are obtained from Kelong Reagent Company (Chengdu, China). All aqueous solutions used in the present study were prepared with deionized water (Mini Q, 18.2 mΩ). Concentrations of stocking solutions are as following: 2 mM Zincon, 4 mM PV, 2 mM Mur, 4 mM $ZnSO_4$, 4 mM $CuSO_4$, 4 mM $NiCl_2$, and amino acid of 1 mM. HEPES buffer of 50 mM was prepared using 5 mM NaOH to adjust pH.

2.2. Selection of sensitive elements to construct sensor array

Different combination of Zincon-Zn^{2+}, PV-Cu^{2+} and Mur-Ni^{2+} solutions were used to prepared three different 4×4 sensor arrays for subsequent selection of sensitive indicator displacement arrays. For each kind of assay, for different metal ion concentration were used including 1.6, 1.0, 0.6 and 0.1 mM (represented by 1, 2, 3 and 4 in following-up analysis). Different dye/metal ion ratios were used for different assays. Typically, for zincon-Zn^{2+}, the ratios included 2:1, 1:1, 2:3 and 1:2. For PV-Cu^{2+}, it was 2:1, 3:2, 1:1 and 1:2, and for Mur-Ni^{2+}, 2:1, 5:3, 1:1 and 1:2. HEPES buffer/dye/metal ions with a ratio of 7:1:1 was added into a 96-well plate, and shake gently for 5 min before initial color profiles were obtained using an Epson scanner. After that, 10 μM theanine of similar volume to dye solution was added and allow for reaction for 10 min before the post-reaction colorful images scanned. The top 16 sensitive combinations from those three sensor arrays were selected to fabricate a new 4×4 sensor array.

2.3. Simplification of the sensor array to improve selectivity

In a second step, the new 4×4 sensor array was further optimized into a 3×3 version to improve selectivity using seven major amino acids as targets. With a similar procedure, HEPES buffer/dye/metal ions/amino acid with a ratio of 7:1:1:1 was added into the 96-well plate. After reaction for 10 min, the final color profiles were obtained for subsequent data analysis. The top nine IDAs were selected to fabricate the final 3×3 sensor array.

2.4. Discrimination of tea samples

105 tea samples within four categories were purchased from local supermarkets in Chongqing, China. They were kept in tanks sealed by tin foil at 4 °C before evaluation. Upon test, tea samples were first dried in oven for 2 h at 35 °C, after which they were crashed into powders and sieved (40 mesh). 1 g powder was weighed into 100 mL boiled water and kept in thermostatic water bath (100 °C) for 30 min. The solutions were separated and diluted to 100 mL as final tea infusions. After that, HEPES buffer/dye/metal ions/tea infusion with a ratio of 7:1:1:1 was added into a 96-well plate and analyzed using the same procedure mentioned above.

2.5. Data analysis

Colorimetric evaluation of the sensor arrays was conducted using a 96-well plate, and scanned by an Epson Perfection V10 scanner. The image profiles were processed automatically with a self-made software to obtain color information before following-up statistical analysis. Typically, the colorimetric response of each indicator displacement assay was measured as an average intensity value of corresponding assays. The sensor response was calculated as the color change (grey value or RGB value) via deducting the intensity value after reaction by that before. It should be noted that every sample was measured for at least three times and the average response was used for final quantitative analysis. Hierarchical cluster analysis (HCA), principal component analysis (PCA) and linear discriminant analysis (LDA) were performed using a SPSS software (version 22).

3. Results and discussions

3.1. Fabrication of the liquid sensor array

Discrimination of teas within different quality degrades and geographical origins have been reported using protocols based on large equipment and also sensory methods [32]. Given that composition of free amino acids in tea fusions differed in term of varied categories [2,33], and facile detection and discrimination of amino acids can be easily realized by indicator displacement assays with commercial pigments [30,31], the presented study was designed to construct a reliable cost-effective sensor to discriminate different tea fusions based on prior reports.

Fig. 1 illustrates the overall procedure to fabricate the sensor array. Indicator displacement assays were first selected according to former studies and re-combined to obtain high sensitivity to