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An Electrochemical Fingerprint Approach for Direct Soy Sauce Authentic Identification Using a Glassy Carbon Electrode

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An electrochemical technique for identifying soy sauce authenticity using a glassy carbon electrode is described. Due to the presence of salt in soy sauce, a three-electrode system can be directly inserted into soy sauce without the addition of electrolyte. The free amino acids and ATP-related compounds in soy sauce can be oxidized during differential pulse voltammetry (DPV) to form a fingerprint pattern. By using the presence/absence of peaks as criteria to calculate ratios, the DPV profiles can be used for identifying sixteen different soy sauces in this work. We believe this proposed methodology can be further extended to other food quality screening applications.

Keywords: Electrochemistry; Soy sauce; Glassy carbon electrode; Cluster analysis; Food safety ; Fingerprint

1. INTRODUCTION

Soy sauce is a traditional seasoner in east Asia. Soy sauce is mainly produced from beans and grains using microbial enzyme-assisted fermentation and extraction processes. Due to global trade and migration, soy sauce is now widely used in every country. To date, much work has focused on the compositional analysis of soy sauce. For example, Yamamoto et al. [1] studied the metabolite profile of soy sauce. Low-molecular-weight hydrophilic components were separated using gas chromatography with time-of-flight mass spectrometry. Additionally, the sensory attributes of different compounds were discussed. Kaneko et al. [2] studied aroma compounds and their sensory attributes in Japanese soy sauce.

On the other hand, many food scientists have investigated the microbial composition during the soy sauce fermentation process. Yan et al. [3] investigated the microbial composition of Chinese soy sauce during the Koji-making process. Nan et al. [4] performed a microbial composition analysis of Korean soybean pastes using a massive sequencing approach. Wei et al. [5] used PCR-denaturing gradient gel electrophoresis for bacterial diversity analysis during soy sauce fermentation.

In addition to sensory and microbial analyses, the soy sauce grade and brand identification are also important for food safety. In China, the amino acid nitrogen (AAN) content has been used to determine the grade of soy sauce [6]. According to Chinese National Standard GB/T 2717-2003, there are four soy sauce grades based on ANN content. Therefore, several techniques have been established for measuring the AAN content in soy sauce. For example, Qin and co-workers used near-infrared spectroscopy for measuring the AAN content [7]. Frerot and Chen identified and quantified the ANN content in soy sauce based on ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) [8]. Very recently, Hu and co-workers developed a Vis-NIR spectroscopy method combined with wavelengths selected by a PSO algorithm for detecting the ANN content [9]. In addition to the ANN content, Hu and co-workers also measured the salt content, total acid content and colour ratio. Although these methods can be used to determine the quality and authenticity of soy sauce, the complicated sample preparation process and need for expensive instruments restricts their field application. In contrast, electrochemical analysis is an alternative approach that can be used for food quality evaluation. This technology has been well developed for determining food colorants [10,11], additives [12,13], and toxic compounds [14]. However, traditional electroanalytical methods cannot be directly used for fermented food analysis due to their complicated composition.

The recent development of electrochemistry allows the recording of the electro-active profile of plant tissues [15–18], minerals, antiques [19–24], food [25] and micro-organisms [26]. Electrochemically active substances in the analyte contribute current values at different potentials due to redox reactions. This electrochemical fingerprint depends on the types and concentrations of electro-active compounds. Therefore, this method has high potential for use in the identification of analytes. Our previous works demonstrated that this method can be used for determining plant species based on plant tissue extracts [27–36]. Therefore, based on our experiences, we would like to extend this methodology for identifying liquid food. In this work, we attempted to use a glassy carbon electrode (GCE) for recording the electrochemical fingerprints of soy sauce of different brands and grades. This method allowed the direct insertion of the GCE into the soy sauce for fingerprint recording without the addition of electrolyte. Sixteen different soy sauce samples were used as real samples. The difference between the fingerprints could be used for determining the soy sauce brand, which has high potential for screening food authenticity in the field.

2. EXPERIMENTAL

All electrochemical determination processes were carried out using a portable CHI760 electrochemical workstation. A commercial glassy carbon electrode (GCE), an Ag/AgCl electrode and a Pt electrode were used as the working electrode, reference electrode and counter electrode, respectively. All sixteen soy sauce were purchased from the local supermarket without any treatment. Table 1 shows the information of all sixteen samples.

A differential pulse voltammetry (DPV) scan was used for electrochemistry fingerprint recording. Glassy carbon electrode was firstly polished using alumina slurry after water wash. Then, the three-electrodes system was inset into a 5 mL of soy sauce. The electrochemical voltammogram recording was conducted at 0–1.4 V, with a pulse amplitude of 50 mV, a pulse width of 0.05 s and a pulse period of 0.5 s. After the first scan, the second scan was recorded using the same parameters without pulling out the electrodes.

Table 1. Information of sixteen soy sauce samples.

Brand (abbreviation)	**Grade**	**Ingredients**	**Origin**
Chubang-Meiweixian (C.M.)	Super	Water; Non-transgenic soybean; Wheat flour; Edible salt; White granulated sugar; Sodium glutamate; 5'-scented nucleotide disodium; Sucralose; Caramel colour; Potassium sorbate; Fructose syrup; Edible spices; Tripotassium glycyrrhizinate; Yeast extract	Yangjiang Guangdong
Donggu-Yipinxian (D.Y.)	Super	Water; Non-transgenic defatted soybean; Wheat flour; Edible salt; Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; Potassium sorbate; Fructose syrup; Sucralose	Heshan Guangdong
Haitian-Tejijinbiao (H.T.)	Super	Water; Non-transgenic soybean; Wheat; Edible salt; White granulated sugar;Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; 5'-inosine monosodium; Sucralose; Yeast extract	Foshan Guangdong
Liuyuexian (LYX.)	Super	Water; Non-transgenic defatted soybean; Wheat; Edible salt; White granulated sugar; Sodium glutamate; Potassium glycyrrhizinate; 5'-inosine monosodium; Yeast extract	Yantai Shandong
Aomendoulao (AMDL.)	First level	Water; Non-transgenic soybean; Wheat flour; Edible salt; White granulated sugar; MSG; 5'-scented nucleotide disodium; Xanthan gum; Caramel colour; Potassium sorbate; Fructose syrup; Drinking water; Ansai Honey; Ethyl maltol	Taishan Guangdong
Haitian-Jinbiaoshengchou (H.J.)	First level	Water; Non-transgenic defatted soybean; Wheat; Edible salt; White granulated sugar; Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; Ammonium glycyrrhizinate; Non-transgenic soybean; 5'-	Foshan Guangdong

		inosine monosodium; Sucralose; Yeast extract	
Haitian-Haixianjiangyou (H.H.)	First level	Water; Non-transgenic soybean; Wheat; Edible salt; White granulated sugar; Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; Scallop; 5'-inosine monosodium; Sucralose	Foshan Guangdong
Zhiwei-Songshengyuan (Z.S.)	First level	Water; Non-transgenic soybean meal; Wheat; Edible salt; White granulated sugar; Sodium glutamate; 5'-scented nucleotide disodium; Caramel colour; Potassium sorbate	Shaoxing Zhejiang
Huyang-Xianjiejiangyou (H.X.)	Secondary level	Water; Non-transgenic defatted soybean; Wheat; Edible salt; Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; Caramel colour	Huzhou Zhejiang
Lijinji-Boyanshengchou (L.B.)	Secondary level	Water; Non-transgenic soybean; Wheat flour; Edible salt; White granulated sugar; Sodium glutamate; 5'-disodium guanylate; Lactic acid; Caramel colour; Potassium sorbate; 5'-inosine monosodium; Yeast extract	Jiangmen Guangdong
Chubang-Caogulaochou (C.C.)	Secondary level	Water; Non-transgenic soybean; Wheat flour; Edible salt; Straw mushroom; Sodium glutamate; Caramel colour; Potassium sorbate	Yangjiang Guangdong
Chubang-Danyanjiangyou (C.D.)	Secondary level	Water; Non-transgenic defatted soybean; Wheat; Edible salt; Edible spices; Sodium glutamate; 5'-scented nucleotide disodium; Potassium sorbate; Fructose syrup; Yeast extract	Zhongshan Guangdong
Haitian-Laochouwang (H.L.)	Third level	Water; Non-transgenic defatted soybean; Wheat; Edible salt; White granulated sugar; Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; Caramel colour; Non-transgenic soybean; Wheat flour; 5'-inosine monosodium; Sucralose；	Foshan Guangdong
Huyang-Hongshaowang (H.S.)	Third level	Water; Non-transgenic defatted soybean; Wheat; Edible salt; Bran; Sodium benzoate; Caramel colour;	Huzhou Zhejiang
Lijinji-Jinzhenshengchou (L.J.)	Third level	Water; Non-transgenic soybean; Wheat; Edible salt; 5'-disodium guanylate; Sodium glutamate; Wheat flour; Defatted soybeans; Caramel colour; Potassium sorbate; Fructose syrup; 5'-inosine monosodium; Sucralose	Jiangmen Guangdong
Haitian-Xianweishengchou (H.X.S.)	Third level	Water; Non-transgenic soybean; Wheat; Wheat flour; Sodium glutamate; 5'-scented nucleotide disodium; Sodium benzoate; Caramel colour; 5'-inosine monosodium; Sucralose	Foshan Guangdong

Fingerprint standardization was carried out for establishing quantitative criteria of recognition [37], where the ratios between the current and the maximum peak current were obtained at different potentials. PCA and cluster analysis was performed using R based on the recorded electrochemistry fingerprint.

3. RESULTS AND DISCUSSION

The direct DPV responses of all sixteen soy sauces on the GCE are shown in Figure 1. Five individual scans were recorded for each soy sauce. Five scans exhibited consistent responses, suggesting that the direct insertion of a three-electrode system into soy sauce is capable of recording a stable electrochemical fingerprint. This stable response can be ascribed to the salt present in the soy sauce [38], which acts as the electrolyte during the electrochemical reaction.

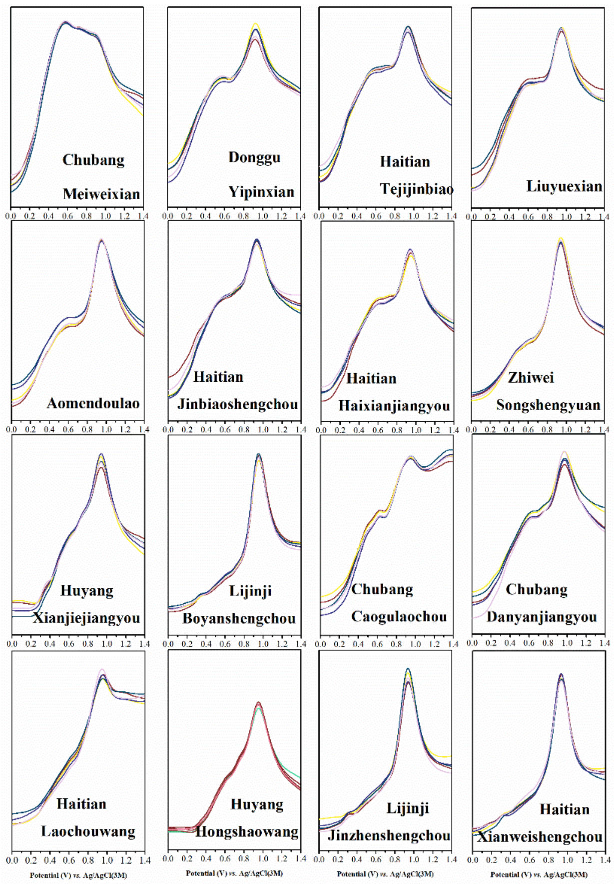

Figure 1. The first scan of DPV curves of sixteen soy sauce recorded on a GCE.

All voltammetric scans of soy sauce exhibited oxidation peak currents between 0 and 1.4 V, indicating that some of the compounds were electrochemically oxidized during the scan. Based on a previous compound analysis of soy sauce, a variety of free amino acids were formed during the fermentation process [39]. Among them, several amino acids, such as tyrosine [40] and cysteine [41], showed clear electro-activity. In addition to these amino acids, some of the ATP-related compounds formed during the fermentation process were also electrochemically active, such as inosine [42], hypoxanthine [43], xanthine [44] and uric acid [45,46]. All these compounds led to a voltammetric curve that reflected the composition and the ratio between these compounds. Although it was difficult to separate the peak into individual components—some of these compounds have very similar structures—the overall voltammetric pattern could be used for identifying soy sauce authenticity. An interesting phenomenon could be observed during DPV, in which the different soy sauce products from the same brand showed similar voltammetric patterns, which was probably due to the similar fermentation process conducted at the factory.

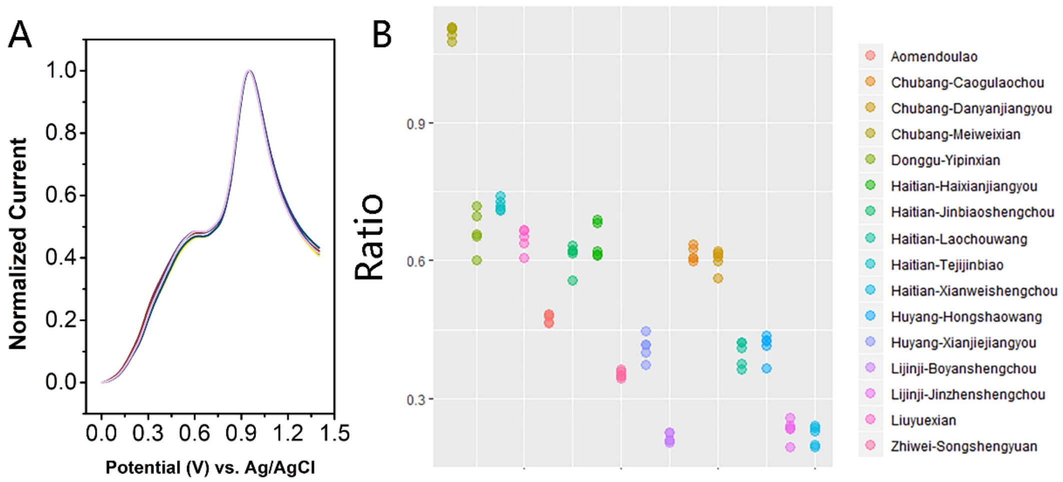

Figure 2. (A) Normalized DPV profile of Donggu-Yipinxian, (B) Plots of $i_{(+0.6\ V)}$ *vs.* $i_{(+0.95\ V)}$ for soy sauce samples in this study using the normalized current values measured in DPV.

Based on the above recorded DPV curves, data normalization was carried out before the chemometric analysis. Two major peaks at approximately 0.60 and 0.95 V could be found in the majority of samples (Figure 2A shows an example). The ratio between the peak current at 0.60 and 0.95 V was calculated as a factor for identifying soy sauce authenticity (Figure 2B). As shown in the figure, Chubang-Meiweixian showed the highest peak ratio among the samples. The overall trend showed a decrease in the peak ratio from the super grade to the third grade (grade information is supplied in Table 1). A low ANN content might be responsible for the low current response around the 0.60 V peak [9]. However, some exceptions were noticed, such as Lijinji-Boyanshengchou, Chubang-Caogulaochou and Chubang-Danyanjiangyou. Therefore, the peak ratio is insufficient for the direct identification of soy sauce authenticity.

As shown in Figure 1, the DPV curves also indicated that Lijinji-Boyanshengchou, Lijinji-Jinzhenshengchou, and Haitian-Xianweijiangyou had a small oxidation peak at approximately 0.30 V. Therefore, we conducted a second scan of all samples. Since the oxidized compounds already fouled the GCE surface, the electrochemical pattern of each sample showed a significant change. However, some additional features could be revealed. As shown in Figure 3, an oxidation peak at approximately 0.30 V could be observed with Haitian-Jinbiaoshengchou, Donggu-Yipinxian, Liuyuexian and Chubang-Danyanjiangyou. Moreover, an oxidation peak at 0.80 V could be observed in Haitian-Xianweijiangyou, Lijinji-Jinzhenjiangyou and Huyang-Xianjiejiangyou. Among these three samples, Haitian-

Xianweijiangyou also showed an oxidation peak at 0.20 V. Based on these observations, along with the features recorded in the first scan, we proposed a cascade scheme for the sequential discrimination of different soy sauces (Figure 4). By using the presence/absence of peaks as criteria to calculate peak ratios (shown in Figure 2B), sixteen soy sauces could be identified with this cascade scheme.

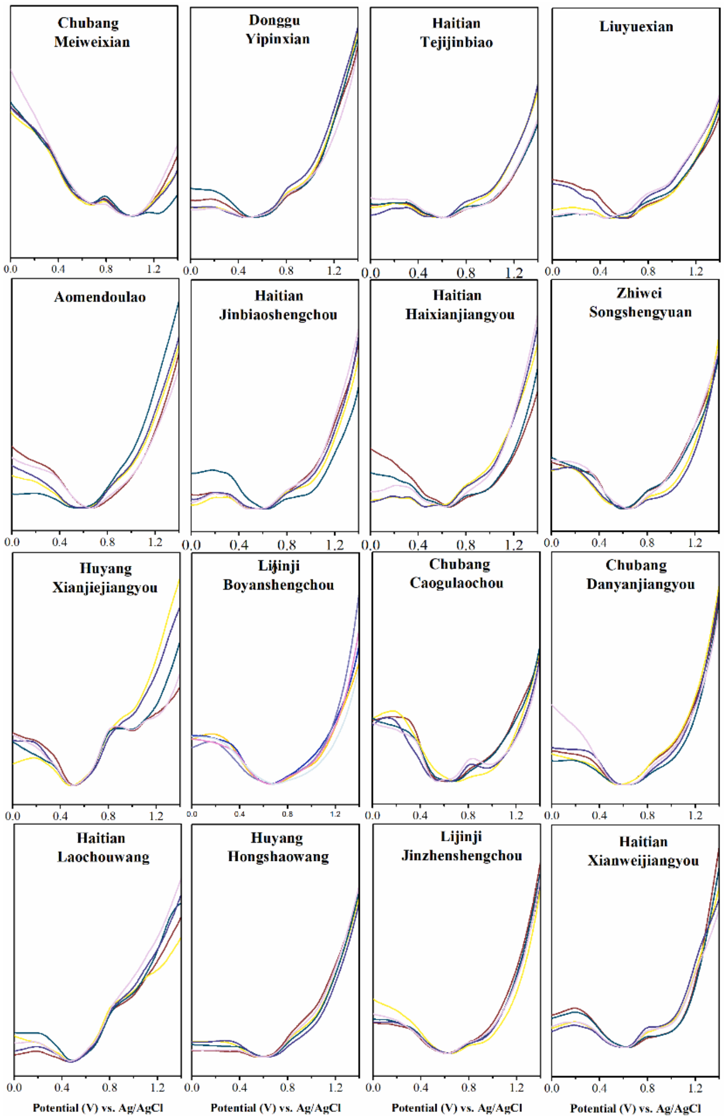

Figure 3. The second scan of DPV curves of sixteen soy sauce recorded on a GCE.

Although the cascade scheme was successfully used for discriminating sixteen soy sauces, this methodology only shows practical value when a limited number of samples are involved in the investigation. Moreover, the direct recognition of DPV curves is subjective by hand; therefore, a statistical method can more accurately identify the soy sauce authenticity. Therefore, we submitted the voltammetric data for principal component analysis (PCA). As shown in Figure 5, the PCA result showed several groupings of soy sauces. The PCA extracted two components that explained approximately 65% of the variation in the data. Based on the suitable separation results shown in the PCA analysis, hierarchical cluster analysis was then attempted for discriminating soy sauces. As shown in Figure 6, among the 90 tested samples, only 3 samples clustered at wrong positions, suggesting that the voltammetric data from soy sauce could be used as a database and subsequently used for unknown sample identification.

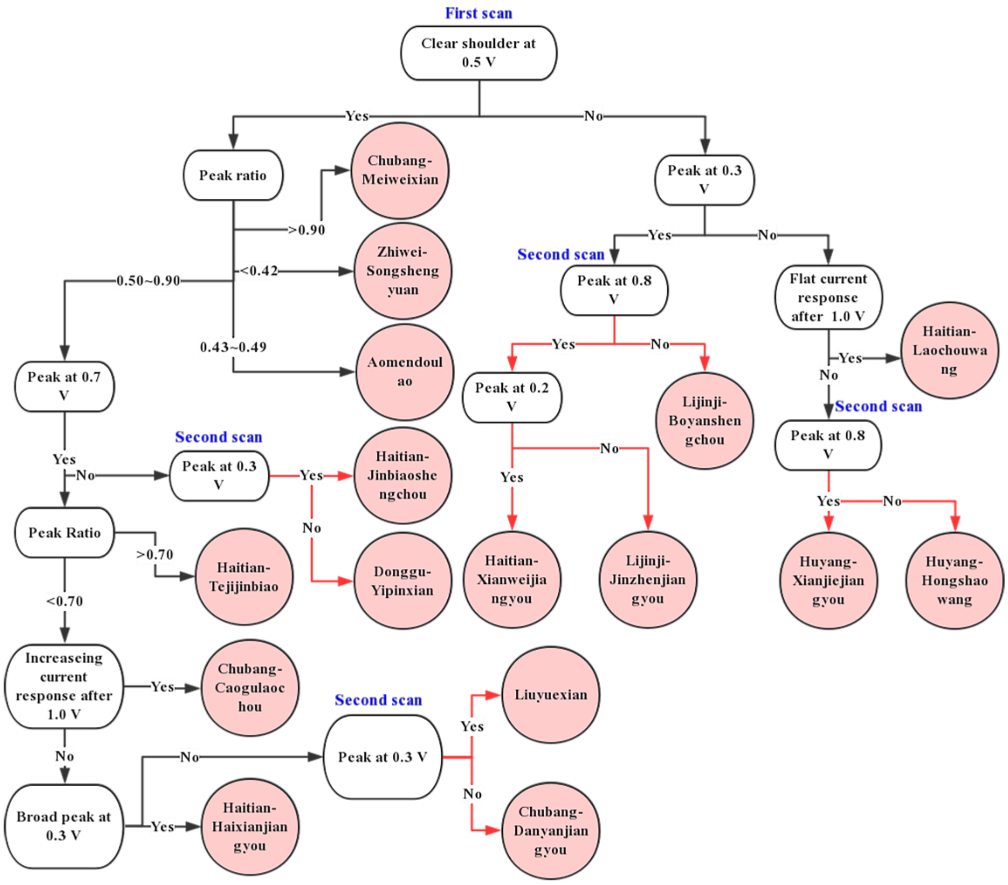

Figure 4. Cascade scheme for the electrochemical discrimination of soy sauce.

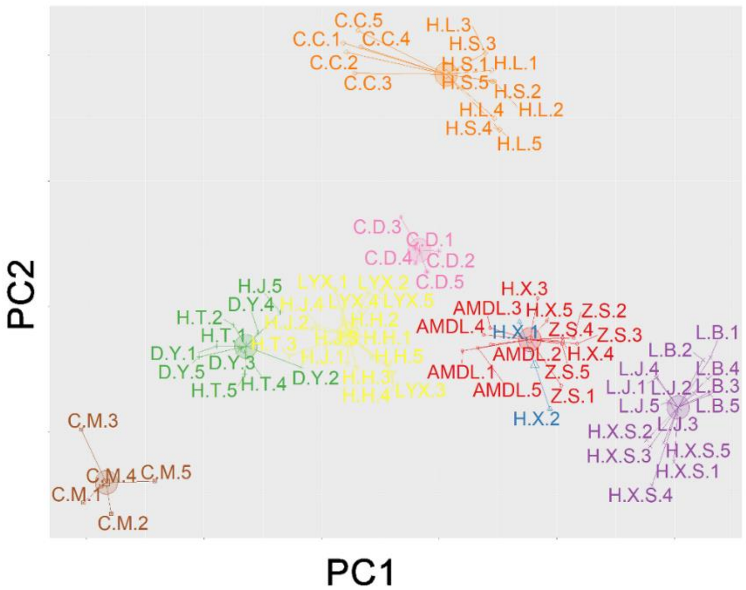

Figure 5. PCA diagrams of sixteen soy sauce obtained from normalized currents recorded by voltammetric scans.

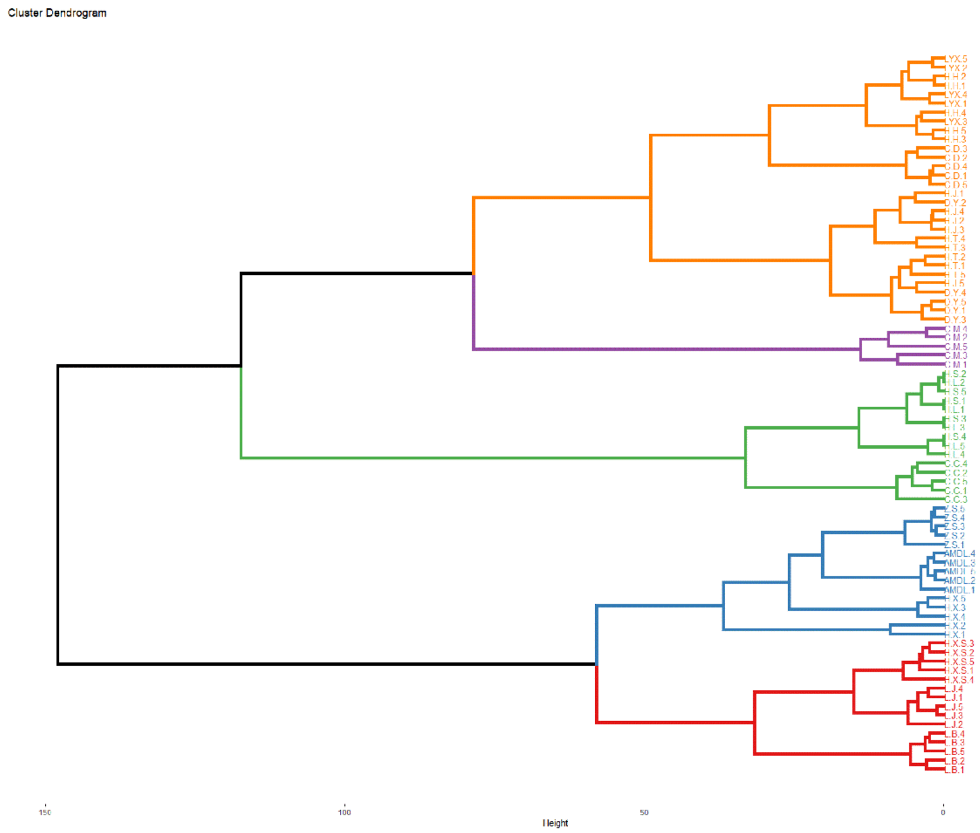

Figure 6. Clustering analysis sixteen soy sauce obtained from normalized currents recorded by voltammetric scans.

4. CONCLUSION

The direct insertion of a three-electrode system into soy sauce can be used for recording an electrochemical fingerprint due to the presence of electro-active compounds. The recorded fingerprint varies between soy sauces due to the different types and concentrations of electro-active compounds produced during the fermentation process. Therefore, the electrochemical fingerprint can be used as a barcode for identifying soy sauce authenticity. A cascade scheme was proposed in this work for the discrimination of sixteen soy sauces. Due to the high reproducibility of the fingerprint, cluster analysis may be a more practical and effective method for soy sauce identification.

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CONFLICT OF INTEREST
The authors declared that they have no conflicts of interest to this work.

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