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Mini Review

Analysis of Bean Products and Genetically Modified Soybean Using Electroanalytical Methods: A Mini Review

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Bean products are a common type of food in human daily diets. Analysis of bean products is not only related to the food industry but also to the dietary health of consumers. In this review, we summarize the results of electrochemical analysis and detection of some effective substances in bean products, including soybean isoflavones and biogenic amines. In addition, we describe in detail the detection of genetically modified soybean based on electrochemical sensors. This review will help food practitioners to understand the current research trends of electrochemical analysis of bean products and the future prospects in this field.

Keywords: Bean product; Analytical methods; Review; Electrochemistry; Food chemistry

1. INTRODUCTION

Bean products are processed from soybeans, adzuki beans, green beans, peas and broad beans. Most bean products are tofu and its byproducts, which are solidified from soybean milk [1-5]. The nutrition of bean products is mainly reflected in their rich protein content. Bean products contain essential amino acids similar to animal proteins. Bean products also contain minerals such as calcium, phosphorus, iron and vitamin Bl, vitamin B2 and cellulose. Bean products do not contain cholesterol; therefore, some people advocate that patients with obesity, atherosclerosis, hyperlipidemia, hypertension, coronary heart disease and other conditions should eat more beans and bean products [6-9]. In addition, with the improvement of people's living standards, consumption and health awareness, it is known that excessive consumption of processed meat products will increase the risk of colon cancer. Traditional meat substitutes have attracted much attention due to technological innovation [10-13]. This kind of product has the advantages of a controllable nutrient content and reducing the slaughter of

livestock and poultry. Vegetarian ham made from soy protein not only provides new ham products for consumers but is also conducive to the development of diet meat products and simulated cooked meat products that are suitable for patients with diabetes and cancer [7,14,15]. However, bean products are vulnerable to contamination of raw materials and in processing and storage. When the human body ingests toxic bean products, it can cause vomiting, poisoning and even death. Therefore, it is of great significance to establish a biosafety detection system for bean product analysis [16-19].

Food analysis is an important part of quality assurance systems in food processing and food nutrition evaluation [20-22]. Food, as the basic material for human survival and reproduction, is closely related to human health, daily life, economic development and social stability [23-25]. Food analysis and detection is an important part of food quality management. In recent years, with the development of agricultural technology and the modern food industry, the variety of food is increasing and people have begun to pay increasing attention to food safety. Therefore, scientific researchers have begun to develop new food analysis techniques [26-28]. Many high-sensitivity and high-resolution analytical instruments are used in food analysis. At present, chemical analysis (CA), enzyme-linked immunosorbent assay (ELISA), ultraviolet-visible spectrophotometry (UV), fluorescence analysis (FA), gas chromatography (GC), thin layer chromatography (TLC), high performance liquid chromatography (HPLC) and electrochemical analysis are the main methods used in food analysis [29-35]. Based on the electrochemical properties of substances in solution, electrochemical analysis is an instrumental method for qualitative and quantitative analyses of components. Electrochemical analysis has the characteristics of high sensitivity, high accuracy, good selectivity and easy automation and is suitable for on-site detection on various occasions [36-40].

The function of transgenic soybean is to introduce artificially isolated and modified genes into the existing soybean genome to modify the biological characteristics of soybeans [41-46]. There is no related gene linkage problem caused by traditional breeding through hybridization. In addition, the target genes in transgenic soybeans have a wider range of sources: they can be obtained from distant species or synthetic genes. This method increases the available genetic resources and avoids the breeding limitations caused by the narrow genetic background of soybean [47-50]. Therefore, transgenic soybean has become one of the most widely applied fields of transgenic technology. To meet the requirement of mandatory labelling, governments in various countries have invested considerable manpower and material resources into the field of genetically modified soybean detection and constantly improve detection technology. At present, the main detection methods of genetically modified soybeans are protein detection and nucleic acid detection [16,51-55]. Electrochemical gene chip technology is a new and highly effective molecular biology technology that has developed rapidly in recent years. In genetically modified soybean testing, the specific fragments of reporter genes, promoters and terminators that can currently be used are made into detection chips, which are hybridized with DNA of the products to be tested. After scanning, computer software is used for analysis to determine whether the samples to be tested are genetically modified products.

In this review, we summarize in detail the analysis research of electrochemical technology in bean product related foods. In this paper, according to the differences of the substance types, the detection of nutrient components and harmful components in bean products are summarized. We also summarize in detail the electrochemical detection of genetically modified soybeans. This review further

discusses the future directions of electrochemical analysis in bean products. We believe that this review is of great significance to food researchers, especially East Asian food scientists. At the same time, we believe that the relevant content can provide guidance for the electrochemical analysis of other foods.

2. SOYBEAN ISOFLAVONE ANALYSIS

Soybean isoflavones are a group of compounds with polyphenol structures isolated from soybean. Soybean isoflavones mainly include compounds with 3-benzopyrone as the nucleus [56-58]. Soybean isoflavones are secondary metabolites in the growth of soybean and are mainly distributed in the cotyledons and hypocotyls of soybean seeds [59-63]. A large number of medical experiments have proven that soybean isoflavones belong to the group of isoflavone phytoestrogens. Soybean isoflavones have both estrogenic and antiestrogenic effects and also have many physiological functions, such as regulating animal immune function, anti-tumour activity, anti-oxidation activity, preventing osteoporosis, antifungal activity, antiviral activity, anti-hemolysis and protecting the cardiovascular system. UV and HPLC are common methods for the determination of soybean isoflavones [64-66]. The ultraviolet absorption peak of soybean isoflavones is close to that of impurities. The background absorption of soybean isoflavones by ultraviolet spectrophotometry can be affected by impurities [67-69]. HPLC has high reproducibility and is readily automated, but its operation is complex and expensive. Some coexisting substances in food can pollute or damage the chromatographic column [70-73].

The content of soybean isoflavones can be detected using liquid chromatography with electrochemical detection. Klejdus and co-workers reported a combined method using liquid chromatography with electrochemical detection for the detection of soybean isoflavones [74]. First, the electrochemical profiles of daidzein and genistein were investigated on a carbon paste electrode. Then, the results of these two analytes were used for developing the liquid chromatography-electrochemical detection method. Soybean isoflavones can be separated using an Atlantis dC18 column by a solvent made from acetonitrile and acetate buffer solution. After parameter optimization, two linear calibration curves can be obtained for daidzein and genistein. The limits of detection of daidzein and genistein are 1.8 nM and 1.5 nM, respectively.

The detection of soybean isoflavones can also be performed using capillary electrophoresis combined with electrochemical detection. The contents of daidzein and genistein were used to reflect the content of soybean isoflavones. Peng and co-workers investigated the optimum conditions, such as the electrode potential, pH of buffer, separation voltage and injection time for detection [75]. The analysis showed that the responses were linear over three orders of magnitude for both substances. Escarpa and co-workers evaluated the accuracy of the electrochemical method for soybean isoflavone detection [76]. The secondary standard of the Drug Master File (SW/1211/03) has been to evaluate the accuracy. They established an isoflavonoid index based on the amperometric result at 1.0 V. Genistein is a precursor of antimicrobial toxins mainly found in legumes. Genistein is the simplest soybean isoflavone complex and an important nutrient molecule found in soybeans. Zhang et al. [77] studied the polarographic behaviour of genistein and found an irreversible reduction during the scan. Two electrons and one proton participated in the reduction process. Fogliatto and co-workers further performed

systematic research on genistein detection using square-wave voltammetry [78]. They also successfully applied the method for the determination of genistein in soy flours and soy-based supplements.

Currently, the main method for the electrochemical detection of soybean isoflavones is the use of other techniques to separate samples, followed by the use of electrochemical technology to detect them. These methods remain unable to detect food samples in the field. Therefore, future research should focus on the development of direct electrochemical technology, such as molecular imprinting technology, for the detection of soybean isoflavones.

3. BIOGENIC AMINES ANALYSIS

Biogenic amines are a group of low molecular weight organic compounds with biological activity and contain nitrogen [79-82]. Their primary production mechanisms are decarboxylation of amino acids and amination and transamination of aldehydes and ketones. Biogenic amines can be regarded substances formed by the partial or total substitution of hydrogen atoms in ammonia molecules by alkyl or aryl groups [83-85]. Biogenic amines are low molecular weight organic bases with aliphatic, heterocyclic or ester ring groups. These amines are mainly divided into monoamines, diamines and polyamines, including tryptamine, cadaverine, phenylethylamine, histamine, tyramine, putrescine, spermidine and spermine. Biogenic amines are ubiquitous in bean products [86-90].

Pineda and co-workers developed a biogenic amine detection method based on the integration of the current response from a Au electrode [91]. Cation-exchange liquid chromatography has been used for the separation of soybean extract samples before electrochemical determination. The limit of detection can reach 200 pM after optimization.

Carbon materials have been widely used for the determination of biogenic amines. For example, Huang and co-workers fabricated a molecularly imprinted biogenic amine sensor based on a multiwalled carbon nanotube-gold nanoparticle composite [92]. Tyramine has been selected as a target analyte for evaluating the performance of the sensor. The results indicate that the sensor could linearly detect tyramine from 108 nM to 10 μM, with a limit of detection of 57 nM. Similar work has been reported by Zhang et al. [93]. They simply used a graphene modified glassy carbon electrode for the determination of two types of biogenic amines. Figure 1 shows the graphene modified glassy carbon electrode for octopamine and tyramine determination. The linear detection ranges of octopamine and tyramine are 0.5 to 40 μM and 0.1 to 25 μM, respectively. In addition, electrochemiluminescence has also been used for biogenic polyamine detection based on SPE [94]. SPE was first modified by $Ru(bpy)_3^{2+}$-encapsulated silica nanoparticles.

The vast majority of biogenic amine detection research focuses on specific categories of substances, rather than evaluating all types of biogenic amines. Therefore, the development of an electrochemical analysis technology that can better reflect the total amount of biogenic amines will become the focus of future research.

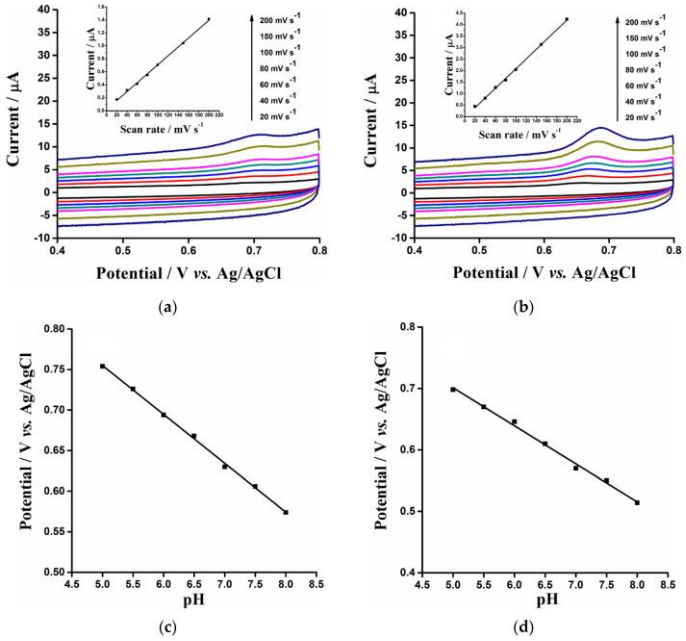

Figure 1. Electrochemical determination of octopamine and tyramine based on the graphene oxide modified glassy carbon electrode. (reprinted from Zhang et al. [93]). Copyright obtained from MDPI

4. GENE SEQUENCE ANALYSIS

Electrochemical gene sensors have attracted much attention in many fields, such as molecular biology, clinical medicine, food science, drug analysis and environmental monitoring, because of their sensitivity, rapidity, simple operation and ease of miniaturization [95-98]. The key technology of gene sensors is the modification of probe DNA. Because of their unique physical and chemical properties, nanomaterials will play an increasingly important role in the development of electrochemical DNA sensors.

The PCR product of the *Lectin* gene sequence can be detected using a DNA electrochemical sensor based on a chitosan-Fe_3O_4-graphene composite [99]. More specifically, the single-stranded DNA probe is first immobilized on the composite surface and then used for hybridization of target single-stranded DNA. The composite provides a high surface area and excellent biocompatibility during the test. Methylene blue has been used as indicator to evaluate the degree of hybridization. The results indicate that methylene blue can be used to reflect the concentration of single-stranded DNA from 1 pM to 1 μM. The soybean *Lectin* gene sequence has been successfully determined using the proposed electrochemical DNA sensor. Moura-Melo reported a screening method for the detection of genetically modified soybean based on the detection of cauliflower mosaic virus [100]. As shown in Figure 2, a thiolated capture probe and *p*-aminothiophenol gold surface have been used for determining layer PCR amplicons.

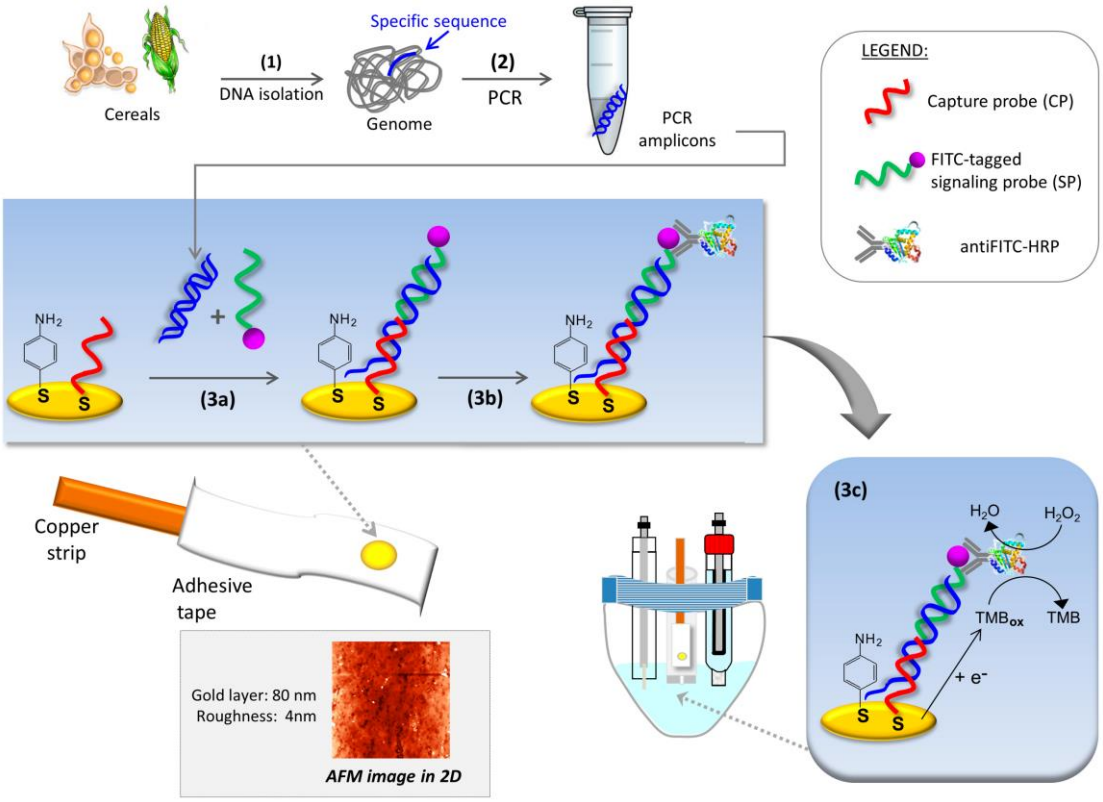

Figure 2. Schematic representation of electrochemical genosensor for detection of genetically modified soybean. (reprinted from Moura-Melo et al.[100]). Copyright obtained from MDPI

A similar method has been used for the determination of genetically modified soybeans. Manzanares-Palenzuela et al. [101] reported a multiplex electrochemical DNA platform that uses taxon- and event-specific DNA sequences. The DNA sequences are hybridized onto magnetic beads. The signalling probes are labelled with fluorescein isothiocyanate or digoxigenin. Both can be conjugated to peroxidase or alkaline phosphatase. The signals of these enzyme activities can be further recorded on an SPE and then converted to a qualitative result. The performance of this DNA sensor can linearly detect DNA concentrations between 2 and 250 pM, thus offering potential application for genetically modified food analysis. Partially reduced graphene oxide has also been used for electrode surface modification as well as for electrochemical determination of transgenic soybean gene sequences [102]. Moreover, graphene and TiO_2 composites can also be applied for electrode surface modification and then used for transgenic soybean gene sequence detection [103]. In addition, impedimetric DNA biosensors are another strategy for genetically modified soybean recognition [104]. In this case, the labelling process can be avoided during detection. Table 1 summarizes all the electrochemical based reports for genetically modified soybean determination.

Detection of genetically modified soybeans by electrochemistry is a popular research topic today, but effective immobilization and inactivation of DNA make the detection method more complex. The author believes that if we can develop a non-DNA based electrochemical detection method to judge whether soybeans are genetically modified, it will have great market prospects.

Table 1. Summary of electrochemical methods for genetically modified soybean determination

Detection substrate	Electrode modifier	Limit of detection	Reference
Glassy carbon electrode	-	190 fM	[101]
Gold electrode	-	-	[105]
Glassy carbon electrode	Platinum nanoparticles	1 nM	[106]
SPE	Acrylic microsphere–gold nanoparticle composite	77.9 fM	[107]
Carbon ionic liquid electrode	Partially reduced graphene oxide	0.29 pM	[102]
Gold electrode	-	225 ppm	[108]
Gold electrode	-	36 nM	[109]
Glassy carbon electrode	Gold nanoparticles decorated multiwalled carbon nanotube-reduced graphene oxide nanoribbons	33 aM	[104]
Electrochemiluminescence	-	0.1 pM	[110]
Gold electrode	Oligonucleotide	1.2 pM	[111]
Carbon paste electrode	Acrylic microsphere–gold nanoparticle	0.14 pM	[112]
Magnetoassays	-	0.123 ng DNA	[113]
Self-fabricated pad	-	-	[114]

5. CONCLUSIONS AND OUTLOOK

This review summarizes in detail the recent research progress in electrochemical analysis and detection technology in bean products. We focused on the electrochemical detection of soybean isoflavones and biogenic amines in bean products. In the detection of soybean isoflavones, the separation of samples is still an indispensable step. In future research, researchers should focus on the development of electrochemical analysis methods that only require a simple pretreatment to reduce the determination cost. In the detection of biogenic amines, most studies choose one or two substances as indicators to measure the total content of biogenic amines. In future studies, researchers should devote more efforts to establishing a technology for the overall evaluation of biogenic amines. Electrochemical detection of genetically modified soybeans is a hotspot in this field. Current research methods include the volt-ampere current method and impedance method. Efficient immobilization of DNA is very important, so different kinds of nanomaterials are used to modify the surfaces of electrodes. In future research, more new nanomaterials can be developed for DNA immobilization, and label-free detection methods can also be established.

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