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Research progress on preparation technology and detection methods of vanillin

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Abstract: Vanillin is an important spice, widely used in food, beverage, daily necessities, medicine and other fields. In this paper, the research progress of preparation techniques chemical synthesis, plant extraction and biotransformation of vanillin was reviewed. The detection methods of vanillin such as gas chromatography, liquid chromatography and electrochemical method were summarized, which provide reference for the development and research of vanillin in the future.

Keywords: vanillin, preparation technology, detection methods, research progress

1. Introduction

Vanillin, an organic compound with the molecular formula $C_8H_8O_3$, is the main ingredient in vanilla bean [1], originally from Mexico, as a broad-spectrum premium fragrance. Vanillin is widely present in nature, such as in Javanese lemongrass, benzoin, Peruvian balsam, clove sprouts, pods of vanilla and many other essential oils and plants. Widely used in fragrance, flavoring agents, new flavors, medicine, food, agriculture and other aspects, vanillin is currently the world's largest production of spices. Current vanillin in use includes synthetic vanillin and natural vanillin. Synthetic vanillin, the market price is lower [2], synthetic vanillin is not only a single fragrance, but also the synthesis process is so polluting that it cannot be accepted. Natural vanillin is mainly extracted from natural vanilla, but vanilla is grown in limited areas, yields are greatly affected by climate, labor intensity is high, and the resulting natural vanillin is extremely expensive, selling for up to US$ 4,000 per kilogram, about 300 times that of synthetic vanillin [2]. At present, the most potential biosynthesis method has the advantages of natural and inexpensive raw materials, clean and pollution-free production process, fast and efficient, and the use of biotechnology to produce natural vanillin has become a new channel worth promoting [3]. However, how to achieve the high yield required for industrial production, and simplify and economize the downstream product separation and purification process to achieve higher economic benefits, is still the main limiting factor for the high price of natural vanillin, which is also the bottleneck in the field of scientific research at this stage, and it is urgent to break through innovative research.

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2. Preparation technology of vanillin

2.1. Extraction method of vanillin

Natural extraction is another way to produce vanillin. It can be achieved by changing solvent to enable the extraction more efficiently. For example, conventional organic solvents can be replaced by innovative 'green solvents'. The solubility test took place when the natural deep eutectic solvents (NADES) were prepared with diverse concentrations ranging from 30 mg/mL to 620 mg/mL. Water, methanol and ethanol are the control experiments. The experiment was conducted at room temperature. The efficiency of extraction for NADES was then compared to that for ethanol and methanol by HPLC/DAD. We can use its retention time and its UV-spectra to determine the extraction ability. The efficiency of methanol turns out to be 3-4 times higher than ethanol, while the efficiency of the NADES is higher than either methanol or ethanol. However, as commonsense, methanol is toxic to human beings. In this way, we cannot use it in the food industry. The ability of extraction has to do with the viscosity. The lower the viscosity, the better the extraction. By adding a small amount of water, the viscosity will decrease tremendously. Thus, the amount of the water can definitely affect the extractability. The upside for NADES as a solvent is that they are not toxic, not volatile and increase the solubility of all phenolics. Although it has some downsides, it has a high level of safety. Many food products can use the NADES as a solvent directly [4] . The natural extraction can also be achieved by extracting crude extraction liquid through the method of high homogenate with vanilla beans. That's the pathway that we called monomers. We use monomers to polymerize and attract vanillin in the high homogenate. The advantage is that it is environmentally-friendly and it has high efficiency, fantastic quality, short production term and low cost [5].

2.2. Chemical synthesis of vanillin

Vanillin has been efficiently produced over the past few years by artificial chemical synthesis. 4 methods have been summarized below. In one method, new transition-display-based catalyst production studies are presented, their reaction conditions in cyanide with cobalt oxide metal catalysts using H_2O_2 as a new problem. The high conversion of isoeugenol compared to the fast conversion of the Fe catalyst provided a catalyst with a 61% vanillin selectivity at 63% conversion in 2 hours. Mechanical and chemical processes have been proven to prepare. The effective method of loading rate metal catalyst, the catalyst has high aldehyde for isoeugenol production enterprises [6] . Another method involves the hydrolysis of coniferoside with the inorganic acid and the enzymes as reagents. It then goes through the oxidation to become vanillin. However, this method has been abandoned in industrial production. The p-cresol method is very unique and it can be divided into two types. The first is to go through oxidation and then halogenation, while the second is to go through halogenation first and then oxidation. The oxidation first and then halogenation type is very simple. The yield of the first step is 91%. Moreover, it can enter the next step to synthesize directly. The overall yield can be reached to 85%, which is higher compared to halogenation first and then oxidation[7] . Flavin protein vanilla alcohol oxidase (VAO) acts on a variety of phenolic compounds and converts cresols and vanilla amines into high-yielding vanillin. VAO-mediated cresol conversion is performed in a two-step process in which the initially formed vanillin is further oxidized to vanillin. Catalysis is subject to presence. Catalyzed restriction of the formation of miscarriage complexes between enzyme-bound flavin and cresols. In addition, in the second step, the conversion of vanillin is inhibited by cresol competitive binding. VAO-catalyzed vanilla amine conversion is performed efficiently at alkaline pH. Vanillin is initially converted to vanilla amine intermediate, which is non-enzymatically dissolved into vanillin [8]. In sum, the p-cresol method is the most effective way to synthesize vanillin chemically. This is due to higher yield production and the simpler process of the approach.

2.3. Microbiological fermentation of vanillin

Currently, as the natural and healthy market affects the concept of consumption, the demand for vallinin grows rapidly. Synthesizing via microorganisms is one of the most crucial ways to produce

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vallinin. It is enacted in a natural way by countries around the world. As a result, investigating the methods to prepare natural vanillin through biological microorganisms has become a heated topic. (1) This involves the engineering method, which includes the metabolism of the polyethylene terephthalate (PET) through PC by *Ideonella sakaiensis*. A de novo pathway is able to transform TA (polyethylene terephthalate monomer) into vallinin. *E.coli MG1655* reduced aromatic aldehyde reduction (RARE) is the host of the whole reaction. Various types of processes can lead to significant growth in yield. For example, we can add trace elements to the aqueous solution, which results in a 15 fold increase in yield. We can enlarge the reaction headspace, which results in a 65 fold increase of the yield since TPADO is O_2 dependent. (2) Another engineering method comprises a degradation process. It makes use of the mutation of the gene code. The yield can be 80.9%. With the further constitutive and enhanced expression, the yield can be reached to 94.9%, which is very close to the maximum theoretical value. (3) The conventional curing method is developed mainly in Indonesia. The stored green vanilla beans can be scalded, autoclaved, open sunned, sweated, rack dried and conditioned. The number of microorganisms, species composition and enzymatic abilities will have huge differences if the curing location is different, which will cause inconsistency in flavors. (4) For the first method, the transformation can be 79%, while for the second method, the conversion can be 94.9%. Obviously, the second approach is more efficient than the first approach. The third approach doesn't contain an explanation of the yield. However, the process is far more complicated. Plus, the second approach doesn't need continuous extraction from the fermentation broth to get rid of the toxic product, which is fairly creative and safe.

3. Detection method of vanillin

3.1. Gas chromatographic method

Vanillin is a safe food additive, but high-dose intake of vanillin may cause liver and kidney damage, especially in infants and young children. Some production enterprises in order to have a better taste of the product, in the infant rice noodles added vanillin, whether the production enterprises can strictly in accordance with the requirements of the standard requirements of the development of the entire food industry, but also and the health of each of us is inseparable. According to relevant reports and literature, there are currently more liquid phase methods or liquid quality methods for the detection of vanillin in food, while there are fewer gas phase methods and more organic reagents used in liquid phase methods or liquid quality methods for processing samples are hexane or acetonitrile. Meng et al [9]. Using Agilent 7890B gas chromatography FID detector, using ethanol (compared to hexane or acetonitrile, safer) as the extract, ethyl hematerate as the internal standard, optimizing chromatographic conditions, realizing the separation of vanillin and impurities, the method is sensitive and accurate, the operation is simple, and it is a better method to quantitatively detect the vanillin content in rice flour.

3.2. Liquid chromatographic method

At present, the detection methods of vanillin mainly include gas chromatography [10], liquid chromatography [11], gas chromatography-tandem mass spectrometry [12], liquid chromatography-tandem mass spectrometry [13], capillary electrophoresis [14] and ultraviolet spectrophotometry [15]. Among them, the sample pretreatment process of gas chromatography, gas chromatography-tandem mass spectrometry and other methods is more complicated and the detection time is longer. Liquid chromatography, liquid chromatography-tandem mass spectrometry and other methods, although the pretreatment process is simple and the sensitivity is high, the equipment is relatively expensive, and a large number of organic solvents are required, and the method is only suitable for confirmatory analysis in the laboratory; Methods such as capillary electrophoresis, ultraviolet spectrophotometry and voltammetry are simple to operate, but they are susceptible to matrix influence and the reproducibility and accuracy of the detection results are poor. Zhang [16] report that the vanillin in food was detected by liquid chromatography, and after simple treatment, the sample was filtered by a

0.45 μm filter membrane, and the Waters high performance liquid chromatography (M6000 pump, U6k injector, M440 detector, column Hypersil ODS2 4.0 mm * 250 mm * 5 μm) was detected, the sample recovery rate was 94.99%, and the linear regression equation Y = (8.098e + 0.6) X was detected.

3.3. Electrochemical method

Vanillin is an electroactive group. Thus, it can be detected electrochemically. Compared to other methods of detection, electrochemistry detection methods have low cost and high accuracy. Nevertheless, it can cause the appearance of toxic substances due to the oxidation of vanillin. This could be overcome by modifying the tube. Metal-organic frameworks (MOFs) have large pore surface area and can adjust the pore size. During the experiment of testing, syntheses of the tetracarboxylic iron phthalocyanine (TcFePc), $[Fe_3O(OOCCH_3)_6OH]\cdot 2H_2O$ and were performed at CHI 760E electrochemical workstation. All of the reagents including trimellitic anhydride, urea, anhydrous $FeCl_3$, ammonium molybdate, trifluoroacetic acid (TFA), iron (III) nitrate nonahydrate ($Fe(NO_3)_3\cdot 9H_2O$), sodium acetate trihydrate ($NaOOCCH_3\cdot 3H_2O$), and N,N-dimethylformamide (DMF) from Shanghai.Prior to the modification, preparation of the electrode and the sample was performed. To get rid of adsorbed organic materials, 0.3 and 0.05 μm alumina slurries were used to polish the glassy carbon electrode (GCE). FePc MOF (1.5 mg) was sonicated in 1 mL DMF for 40 min. A suspension of FePc MOF (3 μL, 1.5 mg mL^{-1}) was dropped on the GCE under infrared light. The vanillin tablets were collected from China Pharmaceutical University Pharmaceutical Co., Ltd, and was grounded into powder (0.2 g vanillin). They were then dissolved into ethanol. The shape of FePc MOF is allowed to be visualized by using SEM and TEM. The electrical behavior can be studied by Electrochemical impedance spectroscopy (EIS). The effect of PH and scan rate can be investigated. As the PH gradually increased from 2-9, the precipitation of the proton had been indicated. The scan rate suggested that the oxidation is a process controlled by absorption. When the vanillin was detected, the increasing current indicated the increasing current.. After that, reproducibility of the sensor has been evaluated by DPV current. Response peak current of 2.02% indicates that it has a strong reproducibility. The stability has been measured by storing the modified electrode. 93% of the original current indicates a good storage stability. DPV and UV analysis has been applied to the real sample.

4. Conclusion

Vanillin is widely used and has good development prospects, especially the research and development of high value-added vanillin products, with huge market potential and considerable economic benefits. This paper summarizes the preparation method and detection technology of vanillin, and provides a theoretical reference for the development and research of vanillin in the future. In the future, the research direction of vanillin may include the following aspects: First, make full use of natural plant resources and give full play to the advantages of natural vanillin in terms of green, natural and safe; The second is to vigorously develop ethyl vanillin and improve economic benefits; The third is to optimize the synthetic process route, improve the quality and yield of vanillin, reduce consumption and reduce environmental pollution.

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