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REVIEWS

Solid-Phase Microextraction as a Promising Tool for the Determination of Volatile Organic Components in Vinegar

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Abstract—Vinegar is a widely used acidic condiment in the world with wide varieties and different flavors. Aroma can be used as an important factor to measure the quality of vinegar and affect consumer acceptance. Volatile organic compounds provide different aroma characteristics of vinegar and have an important impact on the sensory quality. Solid-phase microextraction is an effective sample pretreatment technology which is widely used to determine volatile and semi-volatile organic compounds in various matrices. In recent years, solid-phase microextraction has made rapid development in vinegar flavor analysis, quality control and production. This review focuses on the application of solid-phase microextraction in the determination of volatile organic compounds in vinegar in recent years.

Keywords: vinegar, flavor, solid-phase microextraction, volatile organic compounds, production

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Vinegar, an indispensable acidic condiment, has been widely used in almost all civilizations since ancient times. People usually add vinegar in the cooking process to improve the flavor and taste of dishes [1]. In addition to its role as a condiment, vinegar also has a variety of health benefits [2]. Research has shown that vinegar is rich in nutrients and a variety of bioactive components, such as phytosterols, tetramethylpyrazine, peptides, catechins, flavonoids and so on [3]. Therefore, high-quality vinegar can bring many benefits to human body, including antioxidant [4], lipid-lowering [5], stimulating appetite [6], promoting calcium absorption [7], accelerating fatigue recovery [8]. As a result, more and more food industries are willing to develop new vinegar products.

Vinegar can be divided into grain vinegar and fruit vinegar according to the raw materials for production [9]. Each country or region has its own unique vinegar products. In general, Europe, America, Africa and other countries and regions make use of apples, grapes and other fruits as raw materials to produce fruit vinegar, such as Italian balsam vinegar and Spanish sherry vinegar. However, East Asian countries and regions usually use rice, sorghum and other grains as raw materials, such as grain vinegar in China and Japan. The production strategy of vinegar can be divided into solid-state fermentation (**SSF**) and liquid fermentation (**LSF**) [10]. SSF technology is mainly used in Asia, especially in China. On the contrary, LSF, including surface static fermentation and submerged fermentation, is mainly used in the production of traditional vinegar and accelerated industrial production in European countries. Nevertheless, the specific fermentation processes are very diverse and complex, which makes vinegar a product with strong local characteristics and cultural heritage.

The quality and consumer acceptance of vinegar depend on various characteristics, the most important of which is vinegar flavor. Due to different brewing raw materials and production processes, the flavor of vinegar is diversified. The flavor substances may come from the formation of chemical reactions between raw materials, microbial metabolites and existing substances [9]. Volatile organic compounds (**VOCs**) provide different aroma characteristics of vinegar and have an important effect on the sensory quality of vinegar. VOCs in vinegar mainly include acids, alcohols, esters, ketones and aldehydes which are the main source of vinegar flavor [11]. They exist together in vinegar. When these substances interact in a certain proportion and content, they form the unique flavor of vinegar. Reports have shown that the variety and content of these VOCs can affect the quality of vinegar [12]. The common or representative VOCs in vinegar are shown in Fig. 1.

Organic acids are the main source of vinegar sour taste and the most important flavor substances which have an important impact on the overall sensory properties and quality of vinegar [13]. Esters which usually have typical fruit flavor characteristics are the main substances that constitute the flavor of vinegar. The esters in vinegar are an important sign to judge the

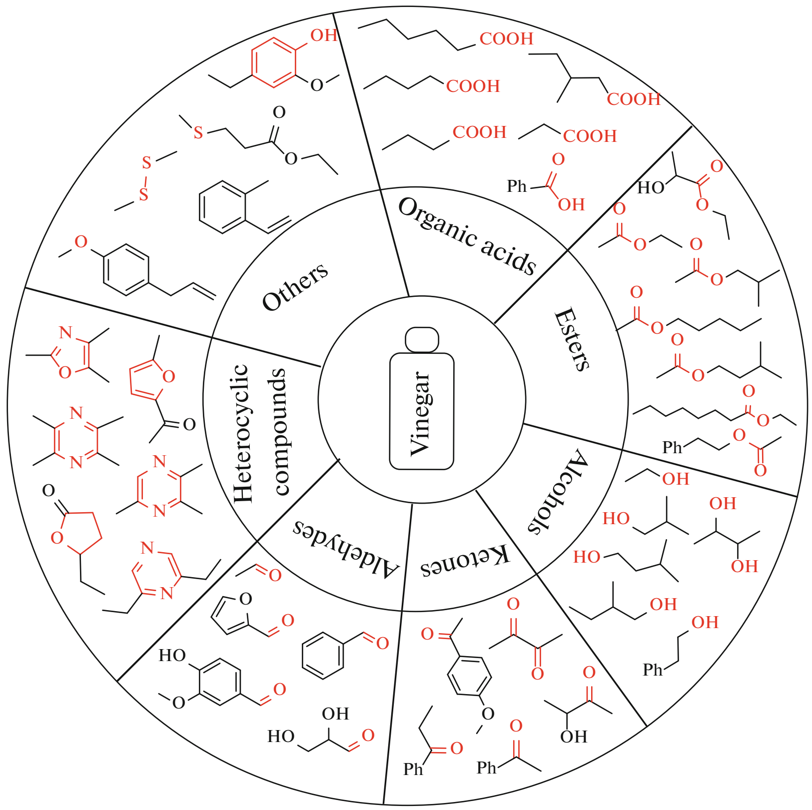

Fig. 1. Common or representative volatile organic compounds in vinegar.

quality of vinegar [14]. Alcohols are mainly produced in the fermentation process, and their types and quantities depend on the substrate composition and fermentation type. Alcohols usually have unique flavor characteristics [15]. Ketones in vinegar are mainly produced by microbial oxidation, amino acid degradation and thermal oxidative decomposition of unsaturated fatty acids. Generally speaking, the ketones have sweet floral and fruity flavor [16]. Aldehydes in vinegar are mainly produced by microbial fermentation or amino acid degradation. Aldehydes have floral smell which can improve the quality of vinegar [17]. Heterocyclic compounds in vinegar are mainly produced by microbial fermentation and generally have the flavor of nuts, coke and baking [18]. There are also other VOCs in vinegar which are usually less in content but have an important effect on the quality and flavor of vinegar. For example, phenolic compounds can play a fragrant and aromatic role which are especially important to the quality of vinegar. They can endow vinegar with various complex tastes and taste characteristics. At the same time, it has many biological functions, such as anti-oxidation, free radical elimination, anti-cancer and anti-inflammatory [19].

The concentration and extraction of VOCs are the basis for subsequent qualitative and quantitative analysis. The commonly used methods include solvent assisted flavor evaporation (SAFE), simultaneous distillation and extraction (SDE), solid-phase extraction (SPE), solid-phase microextraction (**SPME**), stir bar sorptive extraction (SBSE) and supercritical fluid extraction (SFE). Each method has its own advantages and disadvantages. Among these methods, SPME, as a highly sensitive, simple to operate, solvent-free and green sample pretreatment technology, has been successfully applied to the determination of VOCs, semi-VOCs and inorganic compounds in gaseous, liquid and solid state. Combined with gas chromatography-mass spectrometry (**GC-MS**), SPME has been widely used in the determination of VOCs in food [20].

SPME has three basic extraction modes: direct extraction, membrane protection extraction and headspace extraction [21]. In direct extraction, quartz fibers coated with the extraction stationary phase are inserted directly into the sample matrix, and target component is transferred directly from the sample matrix to the extraction stationary phase [22]. In laboratory operation, stirring method is often used for liquid samples to accelerate the diffusion of analytical components from the sample matrix to the edge of the extraction stationary phase. For gas samples, the natural convection of gas is enough to accelerate the equilibrium of analytical components between two phases. Membrane protective extraction is to avoid the damage of the extraction stationary phase. The protective membrane made of special materials provides a certain selectivity for the extraction process. Headspace extraction can be divided into two steps: first, the determined components diffuse and penetrate into the gas phase from the liquid phase. Then, the determined component is transferred from the gas phase to the extraction stationary phase. This model can avoid the pollution of the extraction stationary phase by high molecular weight substances and non-volatile substances in some sample matrices. Headspace solid-phase microextraction (**HS-SPME**) is a simple and sensitive extraction method, because it can extract volatile compounds from different food substrates without any side effects. Therefore, HS-SPME combined with GC-MS and gas chromatography olfaction (**GC-O**) are often used in flavor analysis.

This review focused on the application of SPME in the detection of VOCs in vinegar in recent years which is mainly divided into four aspects: determination of VOCs in vinegar by SPME; application of multivariate data analysis methods to distinguish different varieties of vinegar; exploring the dynamic variations in the VOCs of the vinegar subjected to different production processes; evaluating the effect of production conditions on the flavor of vinegar. It is expected to provide help for the researchers and manufacturers to produce the vinegar or other fermented foods with improved quality and consumer attractiveness.

Determination of volatile organic compounds in vinegar by solid-phase microextraction. The quality of vinegar is the primary factor for consumers to choose vinegar. Aroma components are an important factor affecting not only the quality of vinegar, but also the production process of vinegar [23, 24]. Under the influence of raw materials, fermentation methods, yeast, temperature and other factors, the metabolism produces various organic acids, esters, alcohols, ketones and so on, which makes the composition of volatile flavor components of vinegar very complex. Therefore, the determination of aroma components is of great significance for the quality control of vinegar. As a fast, sensitive and simple method, SPME has been widely used in flavor analysis. In recent years, important progress has been made in the study of VOCs in various vinegars by SPME.

Plioni et al. [25] applied headspace solid-phase microextraction combined with gas chromatography-mass spectrometry (**HS-SPME-GC-MS**) to analyze the volatile characteristics of vinegar prepared by free and immobilized cells of two kinds of cultured bacteria. The results showed that the vinegars produced by these two methods were rich in volatile substances (140 compounds), and the content of esters identified in the vinegar produced by immobilized cells was high. Hojjat et al. [26] quantified VOCs in vinegar produced from black Rosehip (Rosa pimpinellifolia L.) juice by SPME. The results showed that this vinegar was very prominent in VOCs, including a total of 28 VOCs. Its main components were 2-phenylethanol, acetic acid, octanoic acid, ethyl acetate, ethyl phenylacetate and 3-methyl-1-butanol. In addition, there were hexyl salicylate, 4-terpineol and dihydromethyljasmonic acid. Black rose fruit is a suitable raw material mainly used in vinegar production. Al-Dalali et al. [27] used SPME-GC-MS and GC-O to characterize the VOCs in three kinds of Chinese commercial vinegar (Zhenglong rice vinegar, Zhenjiang aromatic vinegar and Longmen smoked vinegar). Al-Dalali et al. [17] also investigated the VOCs of traditional Chinese rose vinegar by HS-SPME-GC-MS and GC-MS-O. The results have shown that aldehydes contribute greatly to the aroma of traditional rose vinegar, and hydroxyl acid is its main non-volatile flavor substance. Hattori et al. [28] used HS-SPME with gas chromatography-high temperature conversion or combustion-isotope ratio mass spectrometry (**HS-SPME-GC-TC/C-IRMS**) to determine the isotopic ratio of acetic acid in vinegar. Soon afterwards, Hattori et al. [29] determined the intramolecular carbon isotope distribution of acetic acid in 14 kinds of Japanese vinegar by improved HS-SPME-GC-TC/C-IRMS. Laura et al. [30] characterized the aroma of commercial sherry vinegar (PDO vinagre de Jerez) through HS-SPME-GC-O and optimized the conditions of HS-SPME to obtain a representative extract.

Similarly, SPME can also be used to detect bioactive VOCs in vinegar. For example, 2,3,5,6-tetramethylpyrazine (**TMP**) is a bioactive component related to alkaloids. It has been proven to have pharmacological effects in clinical application for more than 30 years and has played an important role in anti-cardiovascular diseases [31]. Xu et al. [32] used multiple HS-SPME coupled with GC-flame ionization detection (**FID**) to quantify TMP in various vinegar samples. The results showed that the uniqueness of the working curve of multiple HS-SPME-GC-FID method and the absence of matrix effect are helpful to quickly obtain comparable data of liquid samples with different matrices.

Application of data analysis methods to distinguish different varieties of vinegar. The VOCs of vinegar are rich in variety and quantity. Usually, subtle changes in these compounds affect the flavor of vinegar. Therefore, it is of great importance to study the relationship between the differences of VOCs in vinegar and the variety and quality of vinegar. This mainly depends on the multivariate statistical methods in metabolomics. The commonly used methods are: principal component analysis (**PCA**), partial least squares regression analysis (**PLS-DA**), cluster analysis (**CA**) and so on [33].

Liu et al. [34] used HS-SPME-GC-MS combined with multivariate analysis method, including PCA and partial minimum binary method, to determine the VOCs of 40 fermented vinegar samples produced by different raw materials, starter and processing technologies in different regions of China. Yu et al. [35]

Table 1. Selected reports using SPME coupled with statistical analysis methods to distinguish different varieties of vinegar

Vinegar	SPME fiber	Major statistical analysis method	Reference
Shanxi aged vinegar with different raw materials and aging times	60 µm PDMS/DVB	PCA	[24]
Different Chinese fermented vinegars	PDMS; CAR/PDMS; DVB/CAR/PDMS	PCA; PLS-DA	[34]
Chinese traditional aromatic vinegar	60 µm PDMS/DVB	PCA	[35]
Chinese vinegars	75 µm CAR/PDMS	PCA; CA	[36]
Balsamic vinegars of Modena of different maturation and aging	50/30 µm DVB/CAR/PDMS	PCA; CT	[37]
Geographical origin protected Chinese vinegars named Shanxi extra-aged vinegar and Zhenjiang vinegar	75 µm CAR/PDMS	PCA; FDA	[38]
Commercial cider vinegars with different acidities	75 µm CAR/PDMS	PCA	[39]
Typical Chinese commercial rice vinegars	50/30 µm DVB/CAR/PDMS	PLS-DA	[40]
Balsamic vinegars, namely, ''Aceto Balsamico Tradizionale di Modena''	100 µm PDMS	PCA	[41]

Abbreviations: PDMS is polydimethylsiloxane; PDMS/DVB is polydimethylsiloxane/divinylbenzene; CAR/PDMS is carboxen/polydimethylsiloxane; DVB/CAR/PDMS is divinylbenzene/carboxen/polydimethylsiloxane; CT is classification trees; PCA is principal component analysis; CA is cluster analysis; PLS-DA is partial least squares discrimination analysis; FDA is Fisher discriminant analysis.

used HS-SPME-GC-MS combined with PCA to determine the VOCs of 13 kinds of balsamic vinegar samples in Zhenjiang. Xiao et al. [36] studied the characteristic volatile components of Chinese vinegar by HS-SPME and GC-MS. Multivariate statistical techniques, such as PCA and CA, were used to characterize Chinese vinegar of different types, fermentation methods and producing areas. Cirlini et al. [37] used HS-SPME-GC-MS coupled with multivariate statistical techniques, such as PCA and classification trees **(CT)**, to classify Modena balsamic vinegar with different maturity and aging. This work represented the first attempt to classify Modena balsamic vinegar on the basis of maturity and aging. Zhu et al. [24] quantified the VOCs in Shanxi aged vinegar by SPME-GC-MS and verified its linearity, repeatability, reproducibility and accuracy. The difference and similarity between Shanxi aged vinegar samples was studied in combination with PCA. Selected reports using the SPME coupled with statistical analysis methods to distinguish different varieties of vinegar are shown in Table 1.

Exploring the dynamic variations in the volatile organic compounds of the vinegar subjected to different production processes. Vinegar flavor comes from microorganisms, enzymes and chemical transformation. During fermentation process, protein, fat and starch decompose to produce a series of volatile and non-volatile compounds: acids, alcohols, esters, ketones, aldehydes and sugars. Therefore, it is very important to study the dynamic changes of flavor substances in the fermentation process of vinegar to explore the formation mechanism of flavor substances and to control (monitor) and improve the quality of vinegar. With the development of SPME technique, there is increasing research to investigate food fermentation, concentrating on the dynamics of the VOCs.

Zhao et al. [42] used HS-SPME-GC-MS technology combined with aroma activity value method and aroma active component radar map to explore the effects of ultrasonic treatment on the physical and chemical properties, volatile components and aroma active components of Begonia vinegar. The results showed that the characteristic aroma of Begonia vinegar was in a descending order of esters > alcohols > others > acids, and ultrasonic technology has broad application prospects in shortening the aging time of fruit vinegar and improving the taste of fruit vinegar. Fang et al. [43] carried out the study on the succession law of bacterial community and its correlation with environmental factors and flavor compounds during the fermentation of Zhejiang rose vinegar. The dynamic changes of flavor substances during the fermentation of Zhejiang rose vinegar were studied by HS-SPME-GC-MS. The experimental results will help to understand the formation of flavor substances in Zhejiang rose vinegar. Chen et al. [44] explored the dynamic changes of VOCs in sugarcane vinegar in different production processes. VOCs were determined by SPME combined with gas chromatography. Zhang et al. [45] determined the volatile aroma compounds of Beijing rice vinegar at different fermentation stages by HS-SPME-GC-MS. PCA was used to distinguish specific aromatic compounds. The results have shown that the aroma components are different in each fermentation stage, and the standardization of aroma biomarkers of various types of vinegar was feasible. It can be used as an index or a prediction index for the identification of vinegar fermentation stage and sensory evaluation of vinegar, which provides the possibility for the identification and quality improvement of vinegar. Relying on HS-SPME-GC-MS technology, Song et al. [46] found that with the extension of fermentation time, the yield of acetic acid, phenylethyl acetate and isoamyl acetate in apple vinegar fermentation broth gradually increased. Al-Dalali et al. [47] assessed the aroma characteristics of traditional Zhenjiang balsamic vinegar and modern Zhenjiang balsamic vinegar at different aging stages by HS-SPME, GC-MS and GC-O. A total of 53 volatile compounds were identified.

Exploring the influence of production conditions on vinegar production by solid-phase microextraction. Vinegar flavor is greatly affected by brewing raw materials, production technology, region and other factors. Therefore, it is of great significance to explore the influence of production conditions on vinegar flavor. SPME, as a non-destructive detection method, can dynamically monitor the flavor changes of vinegar, which greatly helps to improve the quality of vinegar.

Al-Dalali et al. [48] used headspace solid-phase microextraction coupled with aroma extract dilution assay with GC-MS and GC-O to study the effects of different brewing processes, such as sun drying process (i.e., with or without), selecting different types of rice as raw materials (i.e., ordinary or glutinous rice) and adding seasoning materials (i.e., with or without spices or sugar), on the aroma of three kinds of Chinese vinegar. Ai et al. [49] evaluated the effect of Monascus on the main metabolites of Sichuan bran vinegar. The main metabolites in vinegar were studied by high performance liquid chromatography (**HPLC**) and HS-SPME-GC-MS. The results showed that the addition of Bacillus Arnebiae significantly promoted the accumulation of organic acids, aromatic esters and alcohols in vinegar, and their contents increased by 1.95, 2.30 and 3.55 times, respectively. The main components of organic acids, i.e., esters and alcohols, are acetic acid, lactic acid, phenylethyl acetate and β-phenylethanol. Perestrelo et al. [50] used SPME-GC-MS to evaluate the effect of impregnation process on the volatile characteristics of wine aromatic vinegar. Wang et al. [51] applied HS-SPME-GC-MS combined with PCA to evaluate the effect of ultrasonic treatment on the ripening of Zhenjiang vinegar. The results showed that the ultrasonically treated vinegar was equivalent to Zhenjiang naturally aged vinegar for 2–3 years. Zhu et al. [52] studied the effect of rheology on the release of eight main aroma components of aged vinegar by HS-SPME-GC-MS. The results showed that the rheological properties of sugars, salts, polyphenols, acids and macromolecules significantly affect the release of main aromatic compounds.

CONCLUSIONS

As a traditional acidic condiment, vinegar has a broad market prospect at home and abroad because of its unique flavor and many benefits to human health. At present, with the increasing demand for healthy diet, the research on vinegar flavor substances is of great practical significance to improve the quality of vinegar and develop new high-quality products. At the same time, solid-phase microextraction, as an attractive pretreatment technology for sample analysis, has the characteristics of high preconcentration factors, rapidity, less sample consumption, simple operation, no solvent consumption, automation and possibility of direct combination with modern instruments, such as gas chromatography. It is suitable for the determination of volatile and non-volatile substances and is more suitable for the development direction of modern analytical technology. It has a strong advantage in the determination of volatile components of vinegar. The effective combination of SPME and modern instrumental analysis technology will strongly promote the research process in the field of vinegar analysis.

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CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest.

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