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Comparison of four kinds of extraction techniques and kinetics of microwave-assisted extraction of vanillin from *Vanilla planifolia* Andrews

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ABSTRACT

Vanillin yield, microscopic structure, antioxidant activity and overall odour of vanilla extracts obtained by different treatments were investigated. MAE showed the strongest extraction power, shortest time and highest antioxidant activity. Maceration gave higher vanillin yields than UAE and PAE, similar antioxidant activity with UAE, but longer times than UAE and PAE. Overall odour intensity of different vanilla extracts obtained by UAE, PAE and MAE were similar, while higher than maceration extracts. Then, powered vanilla bean with a sample/solvent ratio of 4 g/100 mL was selected as the optimum condition for MAE. Next, compared with other three equations, two-site kinetic equation with lowest RMSD and highest R^2_{adj} was shown to be more suitable in describing the kinetics of vanillin extraction. By fitting the parameters C_{eq}, k_1, k_2, and f, a kinetics model was constructed to describe vanillin extraction in terms of irradiation power, ethanol concentration, and extraction time.

1. Introduction

Vanilla (*Vanilla planifolia* Andrews), a crop grown in tropical and subtropical areas, is one of the most widely used flavoring in confectionery, food products, beverages, ice cream, perfume, and pharmaceutical preparations in the world (Gu et al., 2012; Zidi, Tayeb, Boukhili, & Dhahbi, 2011). Vanillin (4-hydroxy-3-methoxybenzaldehyde), which was first isolated from vanilla in 1816, is the major flavor constituent of vanilla (Al-Naqeb, Ismail, Bagalkotkar, & Adamu, 2010; Perez-Silva, Gunate, Lepoutre, & Odoux, 2011). Currently, cured vanilla pods are the main source of natural vanillin. Aside from its appealing aroma, vanillin and vanilla extracts have also been reported to possess various health benefits, such as antioxidant, anti-mutagenic, hypolipidemic activity, and have considerable potential as food preservative and anticarcinogen (Al-Naqeb et al., 2010; Andrade et al., 1992; Jadhav, Rekha, Gogate, & Rathod, 2009; Sharma et al., 2006).

An increasing number of consumers prefer food from natural sources because of food safety concerns. However, natural vanillin, which has price varying from 1200 USD/kg to 4000 USD/kg, can only supply less than 1% of the total market demand (Kumar et al., 2010; Zidi et al., 2011).

Conventional methods for extracting bioactive compounds from foods and natural products, such as Soxhlet and maceration, usually require prolonged extraction time, large quantity of solvents, and heavy capital investment (Valdez-Flores & Canizares-Macias, 2007; Jadhav et al., 2009; Longare-Patron & Canizares-Macias, 2006). In recent years, some novel extraction techniques such as ultrasound-assisted extraction (UAE), pressure-assisted extraction (PAE) and microwave-assisted extraction (MAE) have been developed, as a result of their inherent advantages over conventional extraction methods, such as high extractive power, shorter time, less solvent, and lower cost.

Among these extraction methods, microwave-assisted extraction (MAE), an environmentally friendly process with economic advantages, is part of the Microwave Assisted Process (MAP) developed and patented by the Federal Department of the Environment in Canada (Alfaro, Belanger, Padilla, & Pare, 2003). MAE is based on the direct application of electromagnetic radiation on a material that has the ability to absorb electromagnetic energy and transform the energy into heat (Xie et al., 2010). Auxiliary energy enables extraction processes to shorten their analysis time, lower their energy consumption, require less amount of solvent, and produce higher yields compared with conventional extraction methods (Longare-Patron & Canizares-Macias, 2006; Xie et al.,

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2010). In recent years, MAE has been widely used to extract various biologically active compounds from different plant materials, such as the extraction of aloe-emodin from aloe and extraction of phenolics from beans (Beejmohun et al., 2007; Sturzoiu, Stroescu, Guzun, & Dobre, 2011).

Several papers have compared two or three extraction methods on the yield of vanillin extracted from vanilla beans. According to their results, new methods always gave higher vanillin yield or were less time-consuming than conventional methods (Jadhav et al., 2009; Longare-Patron & Canizares-Macias, 2006; Sharma et al., 2006). However, to the best of our knowledge, so far there is no report about the comparison of antioxidant activity and odour of vanilla extracts, and also little report about the extraction kinetics for vanillin.

In the first part of this research, the extracting efficiency of vanillin, antioxidant activity and odour of different vanilla extracts processed by maceration, UAE, PAE and MAE were systematically compared. In the second part, the effects of particle sizes and sample/solvent ratios for MAE on vanillin yield were investigated and the extraction conditions were optimised. Lastly, an extraction kinetic model describing the extraction process was successfully constructed.

2. Materials and methods

2.1. Materials and chemicals

Cured vanilla beans were obtained from the Spice and Beverage Research Institute (Hainan, China). The vanilla beans were chopped by hand or grounded to powder in an electric grinder (Yili, Zhejiang, China). The average particle size 0.630 mm was determined using a sieve analysis. Vanillin, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and ascorbic acid were sourced from Sigma Chemical Company (St. Louis, MO, USA). Methanol (high performance liquid chromatography (HPLC) grade) for HPLC analysis and ethanol for extraction analysis (analytical regeant grade) were purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Extraction methods

Four kinds of extraction methods were employed for the extraction of vanilla beans. The yield of vanillin extraction was calculated as follows:

$$C(\%) = \frac{y_v * 100}{y_b}, \quad (1)$$

where y_v (g) and y_b (g)are the weight of vanillin and vanilla beans, and C is the vanillin yield.

2.2.1. MAE

Chopped or powdered vanilla beans were mixed with 100 mL of water–ethanol or absolute ethanol solvent and were then heated by using a CW-2000 microwave device (Xintuo, Shanghai, China), which operated at a maximum power of 300 W and was topped by a vapour condenser. For the comparison of different extraction methods, powdered vanilla, a sample/solvent ratio of 4 g/100 mL, 70% (v/v) ethanol–water solution and a 150 W microwave irradiation power were applied to the samples. For the kinetic analysis, different microwave power varied from 50 W to 250 W, volume ratios of ethanol varied from 40% to 100%, and extraction time varied from 0.5 min to 15 min were used. The extraction process was composed of several cycles and each cycle included 25 s microwave treatment and 5 s interval.

2.2.2. UAE

Four grams powdered vanilla beans were mixed with 100 mL of 70% (v/v) ethanol–water solution. The blend was sonicated at room temperature (25 °C) by a SB-5200DT ultrasonic device (Xintuo, Shanghai, China) operating at the frequency of 40 kHz. The process time was varied from 15 min to 90 min.

2.2.3. PAE

Four grams powdered vanilla beans mixed with 100 mL of 70% (v/v) ethanol–water solution was pressurized at room temperature (25 °C) by using a Timatic Micro C pressure enhanced extractor (Timatic, Italy). Several cycles of 5 min compression time each one with a 5 min decompression time were applied for extraction. The process time was varied from 30 min to 180 min.

2.2.4. Maceration

Four grams powdered vanilla beans were placed in a 250 mL conical flask, to which 100 mL of 70% (v/v) ethanol–water solution was added. The flask with blends was continuously shaken in a shaking water bath (FOSS Analytical AB, Sweden) keeping temperature at 25 C. The process time was varied from 120 min to 720 min.

2.3. Determination of vanillin via HPLC

An HPLC (Agilent 1260 series HPLC, USA) equipped with a Zorbax Eclipse Plus C_{18} column (4.6 mm × 100 mm, 3.5 μm Agilent) was used to determine vanillin content. An isocratic elution using a mixture of 20% methanol and 80% acidified water, where 1000 mL of water was acidified with 5 mL of acetic acid, was added at a flow rate of 1.0 mL/min. The total injection volume for analysis was 5 μL. A UV detector at 280 nm wavelength was used, and the temperature of the column was maintained at 30 °C. The compounds were quantified by using the external standard technique. The samples were filtered through 0.45 μm membrane filters prior to analysis.

2.4. Determination of antioxidant activity

The radical scavenging activity assay for determination of antioxidant activity of the vanilla extracts described by Al-Naqeb et al. (2010) was used with a little modification for this research. Two milliliters of DPPH (0.06 mg/mL) with two milliliters diluted vanilla extracts were mixed well and left in the dark for 60 min. Then, the decrease in absorbance of the mixtures at 517 nm was measured by a UV–Visible spectrophotometer (specord 250, analytikjena, Germany). Antioxidant potential of vanilla extracts was quantified by the standard curve of ascorbic acid (R^2 = 0.9999). Finally, results were expressed in milligram (mg) ascorbic acid equivalent (AAE) per 1 g of vanilla sample.

2.5. E-nose analysis

The E-nose assay with modification of the method of McKellar, Rupasinghe, Lu, and Knight (2005) was conducted using the sensor array system (GEMMINI, Alpha M.O.S., France). Membrane filters of 0.45 μm used as the carriers for the odours were soaked in the vanilla extracts for 10 min and allowed to air dry for 30 min. Then, the membrane filters with odours of vanilla were placed in the sample vials and accumulated at 50 °C for 5 min. Headspace (1500 μL) carried by air (150 mL/min) was injected into E-nose. Discrimination function analysis (DFA) using the built-in software was applied for E-nose analysis.